THE ORIGIN AND POSSIBLE SIGNIFICANCE OF VARIATION OF LEAF STRUCTURE IN THE TWO NATIVE BRITISH OAK SPECIES, *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.

by

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The Blind Men and the Elephant

It was six men of Hindostan,
To learning much inclined,
Who went to see the elephant,
(Though all of them were blind);
That each by observation
Might satisfy his mind.

The first approached the elephant,
And happening to fall
Against his broad and sturdy side,
At once began to bawl,
"Bless me, it seems the elephant
Is very like a wall."

The second, feeling of his tusk,
Cried, "Ho! what have we here
So very round and smooth and sharp?
To me 'tis mighty clear
This wonder of an elephant
Is very like a spear."

The third approached the animal,
And happening to take
The squirming trunk within his hands,
Then boldly up and spake;
"I see," quoth he, "the elephant
Is very like a snake."

The fourth stretched out his eager hand
And felt about the knee,
"What most this mighty beast is like
Is mighty plain," quoth he;
"'Tis clear enough the elephant
Is very like a tree."

The fifth who chanced to touch the ear
Said, "Even the blindest man
Can tell what this resembles most;
Deny the fact who can,
This marvel of an elephant
Is very like a fan."

The sixth no sooner had begun
About the beast to grope
Than, seizing on the swinging tail
That fell within his scope,
"I see," cried he, "the elephant
Is very like a rope."

And so these men of Hindostan
Disputed loud and long,
Each of his own opinion
Exceeding stiff and strong,
Though each was partly in the right,
And all were in the wrong!

John Godfrey Saxe

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CHAPTER ONE

THE GENUS *Quercus*: AN INTRODUCTION

The genus *Quercus* is a large genus of shrubs and trees containing at least 450 species (Jones, 1959). It is a wide-ranging genus, being distributed throughout the temperate regions of the northern hemisphere, but extending into the tropical montane forest of Central America and southwards to Columbia and into Indomalaya. In Africa, the genus is confined to the Mediterranean Basin (Jones, 1959). Although wide-ranging, most species are restricted to the warmer temperate regions of America and Europe. Originally described as a Linnanean genus, the most recent treatments of the taxonomy are given by the monographs of Camus (1936-54) and Schwarz (1936-39) although the latter is concerned only with European species. Schwarz (1970) has prepared an up-to-date account of the European members of the genus for the Flora Europaea. He distinguishes four subgenera and twenty-seven species; the subgenera being differentiated on degree of deciduousness, endocarp characteristics and length of time taken to ripen the acorn (see Table 1.1).

In Britain, five oak species are frequently found - *Quercus ilex* L., the Holm Oak, *Quercus cerris* L., the Turkey Oak, *Quercus rubra* (*Q. borealis* Mich.), the American Red Oak, *Quercus robur* L. (*Q. pedunculata* Ehrh.), the Pedunculate Oak and *Quercus petraea* (Mattuschka) Liebl., the Sessile Oak. The last two species are considered indigenous to the British Isles, the remaining three have been naturalised - *Q. ilex* in southern England, *Q. cerris* in many parts of England and Wales, and *Q. rubra* throughout England (Jones, 1959). It is interesting to note that within the British oaks, there is at least one naturalised or indigenous representative of the four European subgenera (see Table 1.1).

Subgenus	Leaves	Fruit	Endocarp	British Species
Erythrobalanus (Spach) Örsted.	Deciduous (for European species only)	Fruit ripens in second year	Endocarp tomentose	*Quercus rubra*
Sclerophyllodrys O. Schwarz	Evergreen	Fruit ripens in first or second year	Endocarp tomentose	*Quercus ilex*
Cerris (Spach) Örsted.	Evergreen or Deciduous	Fruit usually ripening in second year	Endocarp glabrous	*Quercus cerris*
Quercus (Subgenus *Lepidobalanus* (Endl.) Örsted.)	Deciduous or Semi-evergreen	Fruit ripening in first year	Endocarp glabrous	*Quercus petraea*, *Quercus robur*

TABLE 1.1 DISTINGUISHING CHARACTERS OF THE FOUR EUROPEAN SUBGENERA OF *Quercus* L.

Taxonomic separation of the five British Quercus sp. is reasonably clear, apart from Q. robur and Q. petraea. The leaves of Q. ilex are lanceolate and small (3-7 cm.), those of Q. rubra are lobed, large (12-20 cm. x 10-15 cm.) and glabrous, those of Q. cerris being lobed, of medium size and pubescent. Q. petraea may rarely be confused with Q. cerris, but is frequently confused with Q. robur due to overlap of their character ranges so that their separation in the past has proved difficult.

Bauhin in 1623 was the first person to distinguish between the two types of oak with auricled leaves and stalked acorns (Q. robur) and with petiolate leaves and sessile acorns (Q. petraea), and before the end of the eighteenth century they were recognised as distinct species (Schwarz, 1935). Such separation did not prevent, however, misleading accounts of the species up until fairly recent times. Stewart and Corry (1888) in documenting the flora of north east Ireland record the distribution of Q. robur Linn. pointing out that the variety sessiliflora formed part of the old wood of Glenarm. The specific epithet sessiliflora is still used in continental work for Q. petraea. The London catalogue of British Plants (1895) lists only Quercus robur, but with three varietal forms - pedunculata (Ehrh.), intermedia (D. Don.) and sessiliflora (Salisb.). Hooker (1884) in a flora of the British Islands records the following under the heading Q. robur L.:

"The following varieties are very inconstant.

Q. sessiliflora, Salisb.; leaves petioled, peduncles very short
Q. pedunculata, Ehrh.; leaves sessile, peduncles long
Q. intermedia, D. Don.; leaves downy beneath, petioles and peduncles short"

This misconception was perpetuated two years later with the publication of the Handbook of the British Flora by Bentham and Hooker (1886) in which they described two forms of Quercus robur Linn. as the two races

of the species. The descriptions which followed clearly were descriptions of Q. robur and Q. petraea. The writings of Praeger (1909) in recording oak in Ireland reveal the confusion that existed at the turn of this century:

"Quercus Robur L. OAK - Divisions all. Frequent. Usually in the var. pedunculata Ehrh. Clearly indigenous in mountain districts, islands in lakes, and rough ground. To 750 feet on Mweelrea.
The range of segregates Q. pedunculata Ehrh. and Q. sessiliflora Salisb. has not yet been worked out. The present indications regarding their general distribution suggest that the Oaks of the metamorphic areas are the latter, while those of the limestone pavements are Q. pedunculata; but there are not yet a sufficient number of records to allow of this generalisation being stated definitely."

Modern accounts, eg. Camus (1936-54), Schwarz (1970), Jones (1959, 1968), Clapham et al. (1962) and Weimarck (1947a, b) separate the complex into two distinct species, Q. robur L. and Q. petraea (Matt.) Liebl., but misleading accounts of the species are still frequently published, eg. Dizerbo (1965) in constructing a key for the oaks of the Finistère keys out Q. sessiliflora Salisb., i.e. Q. petraea as a plant with glabrous adult leaves.

Intraspecific Variation within Q. robur and Q. petraea

Much of the taxonomic confusion in the delimitation of Q. petraea from Q. robur has resulted from the possible effects of hybridisation which will be discussed later in this chapter, but also from the large range of intraspecific variation observed within both species.

Many reports have noted large scale geographical variation within Q. robur, and these differences have frequently led to the establishment of subspecific, varietal or form taxa. Weimarck (1947a,b) recorded, for example, five forms of the species Q. robur subsp. pedunculata as: form brevipedunculata (Lasch) Schwarz, with short female catkins; form holophylla (Rehd.) Schwarz, with more or less entire leaves; form longipedunculata (Lasch) Schwarz, with long female catkins; form

petiolens DC., with cuneate-based leaves, lacking auricles and with a petiole 5-15 mm. in length and form mespilifolia (Wallr.) Weim. with a more or less entire leaf. Schwarz (1970) in the most recent and authoratative account recognises only three subspecies of Q. robur, subspecies robur described as having thin glabrous leaves with deep broad lobes and with an involucre about 12 mm. wide, subspecies brutia from southern Italy with longer lobes, deeper narrower sinuses and an involucre up to 23 mm. wide and subspecies broteroana with smaller leaf lobes and a large involucre from south-west Europe.

However, not all variation described in Q. robur has been assigned to formal taxonomic status, but it would appear that variation has been described for nearly every possible characteristic of the species. Shutyaev (1968) investigated the growth and frost susceptibility of a range of Q. robur seedlings from the Kursk province of Russia and found that growth through the season was greater in oaks of southern, south-western and western origin than those from the north and east, but that the seedlings from the south and west were more susceptible to frost damage and frost kill. These differences were ascribed to ecotypic differentiation. A similar study by Vlasov (1967) showed that the area of origin of acorns had a profound effect on growth, development and the ability of the seedlings to survive under steppe conditions. The acorns from local trees produced better seedlings under the steppe situation than acorns from low-lying forest areas. Geographical origin has also been shown to be important in the retention of leaves by oaks in winter. Krasnitskij (1968) studied seedlings of Q. robur from 31 areas in eastern Russia and was able to show that not only was leaf retention a heritable character, it was also closely associated with geographical origin - flood plain seedlings losing their leaves before highland seedlings. Leibundgut (1969) in an important study showed that in Q. robur, the

susceptibility of the trees to mildew attack (Microsphaera alphitoides) was dependent on provenance of origin - the trees showing an increase in susceptibility with provenance from west to east. Such gradients can also be detected in the anatomical structure of the leaves of Q. robur. Nescjarovič and Smirnova (1969) whilst studying leaves of Q. robur seedlings were able to show that a more xeromorphic leaf structure was found in seedlings grown from acorns from eastern, southern and south-western provinces of Russia than in plants grown from acorns collected in northern and north-eastern provinces. Such differences might well have influenced the results of Lavrinenko and Porva (1967) who showed that seedlings from different provenances survived to greater or lesser degrees when grown under uniform conditions.

Forms of Q. robur have also been recognised with differing bark characteristics. Ievlev (1972b) distinguished six different forms in a single nature reserve in south central Russia, and for forestry purposes, he also described the physical and mechanical properties of the wood of some of the forms. In the same nature reserve, Ievlev (1972a) also recognised several phenological and ecotypic groupings which he related to relief, soil types and site conditions. The usefulness of oak as a timber has led several forestry researchers to investigate the relationship between crooked stems of Q. robur and environmental parameters, eg. Kostov (1972) found that under conditions of warmer climate, the stem becomes strongly crooked, whilst in harsher, cooler conditions, the stem is mainly straight.

Q. robur also shows large variation in reproductive characters. Pletminceva (1967) recorded that Q. robur trees could be classified into early flowering and late flowering forms, and these in turn could be divided into the following ecotypes: 1) late flowering moisture loving oak 2) late flowering drought resistant mountain oak on poor dry salty

soil 3) early flowering drought resistant mountain oak on rich soil and 4) early flowering, salt resistant mountain oak on poor and salty soils. These groupings also contained 'heavy' and 'light' fruiting forms. Danilov (1967) found that acorn size in Q. robur was a heritable character, and two different types were distinguished; those with long and those with short acorns, acorn size varying within each shape category.

The presence of such variation within species does not always lead to the delimitation of taxonomic groupings, and recently attempts have been made to review the many subspecies and specific groupings related to Q. robur, eg. Schwarz (1970). Such a study was that of Menickij (1967) who by careful description of morphological and bioecological data of a range of supposed oak species - Q. longipes, Q. erucifolia, Q. haas, Q. curdict and Q. pedunculifolia - was able to conclude that they all comprised a single subspecies, Quercus robur ssp. pedunculiflora.

Q. petraea has been regarded by several authors as a less variable species than Q. robur (Jones, 1968) and it is true that fewer subspecies of Q. petraea have been described than of Q. robur. This might, however, reflect the taxonomic history of Q. petraea itself, as a subspecific ranking of Q. robur, rather than the inability of taxonomists to detect subdivisions of Q. petraea.

Garilov (1969) has reported the occurrence of several ecotypes of Q. petraea from the Stara Planina mountains which were distinguished by leaf shape and habitat preference. The lower altitude forms were indistinguishable from Q. polycarpa whilst the best adapted high altitude form resembled Q. dalechampii. These ecotypes were assessed for economic value (Garilov, 1970a, 1970b) and a large leaved, high altitude ecotype yielded the most timber. Q. iberica has recently been shown to be a rather indistinctly differentiated subspecies of Q. petraea from which

it can be distinguished by shorter leaf blades (Krasilnikov, 1966). Both the work of Garilov (1969, 1970a, 1970b) and Krasilnikov (1966) indicate the difficulties in delimiting Q. petraea from other species and differentiating the species itself at the subspecific level. Certainly, Q. polycarpa and Q. dalechampii are regarded by Schwarz (1970) as 'good' species even though they would appear to be indistinguishable from Q. petraea under certain environmental conditions. A variety of Q. petraea, var. pubescens Loud. has been noted by Davy (1933) as being much hairier than the typical Q. petraea with scattered bifid hairs on the undersurface of the lamina. This variety is very like Q. pubescens and found more to the west of Britain on wetter soils than normally associated with Q. petraea. Davy (1933) also describes a form of this variety, form longipedunculata Moss which has stalked acorns, a Q. robur characteristic.

The crooked and straight stems of Q. robur have also been noted by Csesznák (1966) in Q. petraea who determined that the erect form in Q. petraea is an indirect effect of inherited properties, particularly rapid growth, superior shade tolerance and resistance to pests.

The problems associated with intraspecific variation within Q. robur and Q. petraea are not unusual or atypical of oak species generally. As Jones (1968) points out: "All species of Quercus are variable". Among European oaks, patterns of variation similar to those described here for Q. robur and Q. petraea are common. Udra (1972) has recorded six forms of Q. mongolica which were distinguished mainly by acorn characters - large fruited, long-round, oval, small fruited and with a small calyx. De Rivas (1967) described biometrically leaf samples of Q. rotundifolia and concluded that it must be considered only a subspecies of Q. ilex. Q. rubra although a North American species has been planted extensively in Central Europe, and Flint (1972) has recorded that the degree of

cold hardiness of the twigs of this species is a function of the geographic origin of the tree, latitude being the most important factor, but longitude and elevation being important in the autumn.

Parallel evolution may also be an important evolutionary trend within the genus, producing strikingly similar leaf types in species that are distantly related. Some of these have been reported for European oaks and Tucker (1974) has recently reviewed the occurrence of such examples of parallel evolution in New World oaks.

Although a great deal of intraspecific variation occurs in both Q. robur and Q. petraea, the trend among taxonomists is to consider them as distinct entities with full specific status. Chapters 2 and 5 of this thesis discuss fully the characters used for the taxonomic separation of the two species.

Geographical and ecological separation of Q. robur and Q. petraea

Q. petraea and Q. robur, although native to Britain are wide ranging throughout Europe. The western limit of both species is set by Ireland and Britain in the north and Portugal in the south. The southern limits of both species are difficult to ascertain due to taxonomic confusion with related species, Q. robur with Q. pedunculiflora and Q. petraea with Q. pubescens. Q. robur occurs locally and generally in montane regions in the Mediterranean basin, but also occurs at low levels in coastal regions of Portugal, the Po Valley (Italy) and parts of the Adriatic coast and is montane in the eastern Pyrenees, Corsica, Sardinia, Calabria and the Balkan countries of Greece, Caucasus and Transcaucasia. Generally, Q. petraea fails to extend as far south as Q. robur, and where it does so, it is more montane. In parts of southern France and Italy, it is replaced by Q. pubescens (Jones, 1959). The northern limit is reached in the west in Caithness (Tansley, 1939)

in Orsk in the east and at 62°N in Norway, Sweden and Finland and 58°N in Livonia. Q. petraea fails to extend quite as far north. In the east, Q. robur reaches its limit in the Urals whilst Q. petraea reaches only to Poland, Rumania and Bulgaria. Q. petraea, therefore, is of more limited distribution than Q. robur, particularly in the east of its distribution range. Diagram 1.1 summarises the distribution of Q. robur and Q. petraea.

In Britain, although recorded from nearly every vice county (see Diagrams 1.2 and 1.3), such records take no account of planted stands. However, some generalisations may be made. In the north, both species become rarer, and this coupled with taxonomic difficulties (Cousens, 1962, 1963, 1965) and planted woodland make exact delimitation of the ranges difficult. Tansley (1939) recorded both species from Helmsdale in Sutherland. Tansley believed there to be no natural oakwood north of either Sutherland or Caithness and as Jones (1959) points out, the occasional record of oaks beyond Caithness are almost certainly of planted trees. The distribution and abundance of the species varies over the rest of the country. Q. robur is abundant and found in lowland England and eastern Scotland. Q. petraea is much more abundant in Wales, Devon, Cornwall, Ireland and the west of Scotland, and indeed in some of these areas may be the only species.

Altitudinal differences between the species are marginal; Q. petraea is usually regarded as the species more tolerant of higher altitudes and is recorded as being found 100-300 m higher than Q. robur. There would appear to be no climatic difference between the species; at the northern limit, the species are so close that it is impossible to find suitable stations to differentiate them clearly on climatic grounds (Jones, 1959).

Edaphically, two things would appear to dictate their different

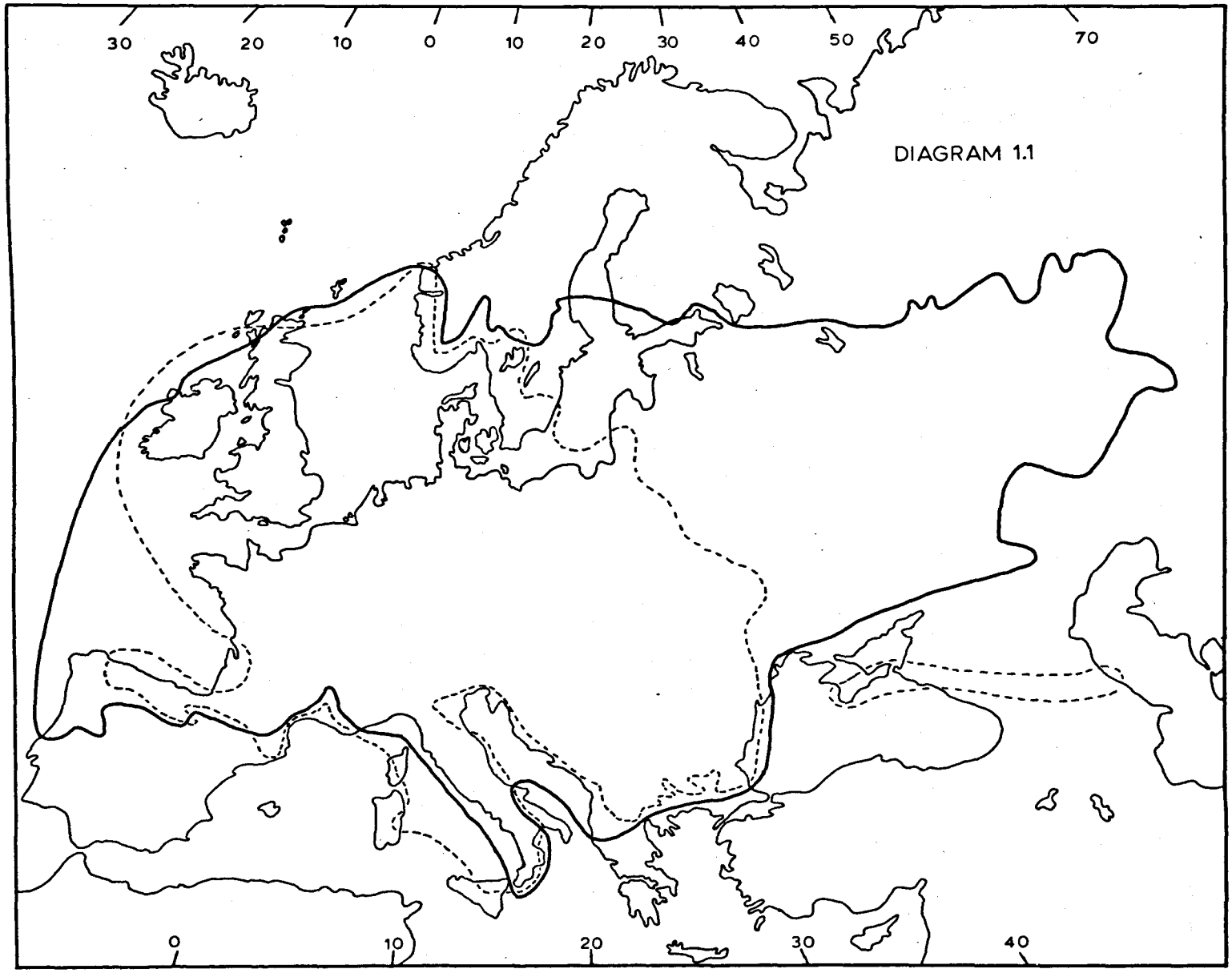


DIAGRAM 1.1 European distribution of Quercus robur L. and Quercus petraea (Matt.) Leibl.

—— Q. robur ------ Q. petraea

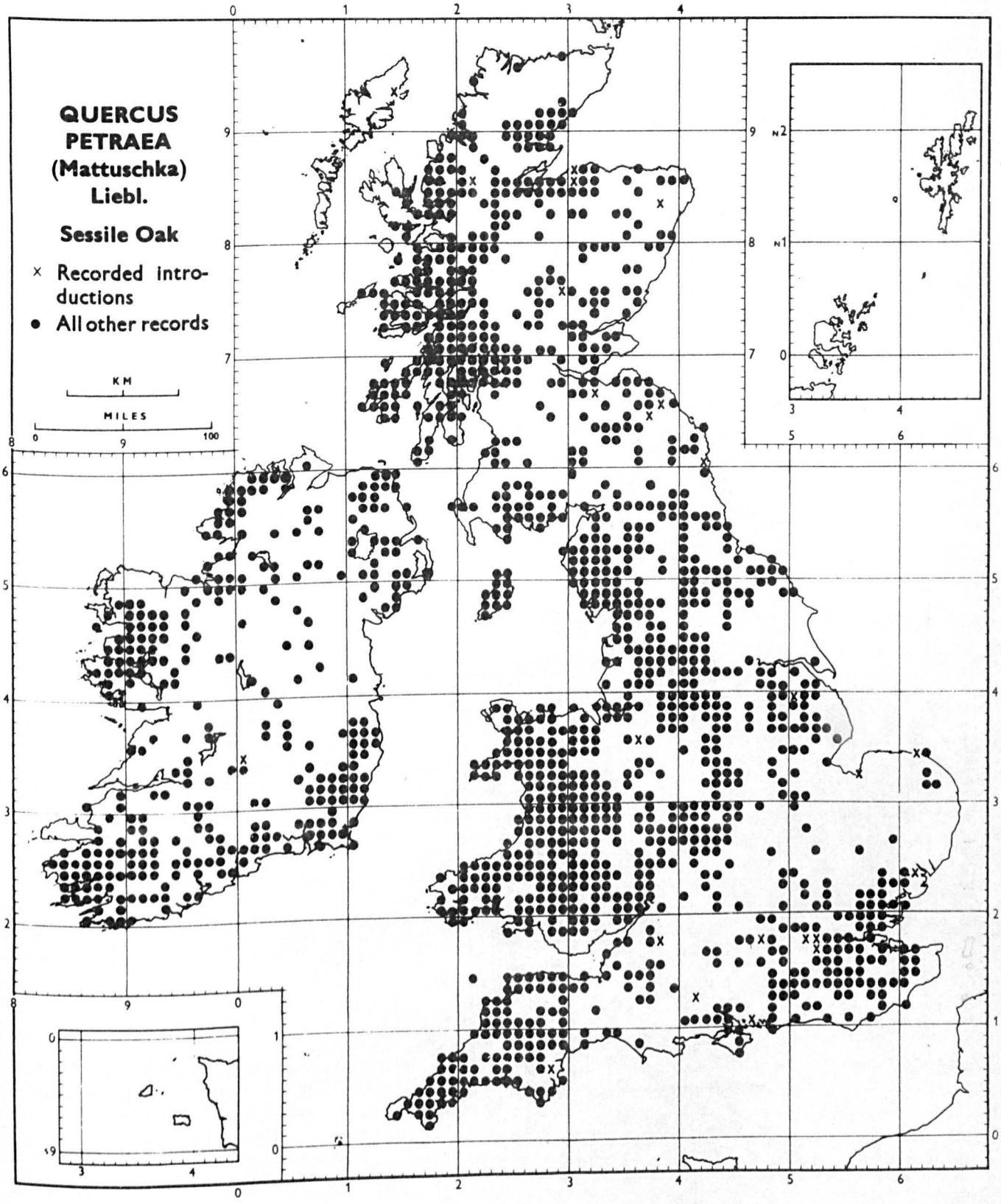


DIAGRAM 1.2 Distribution of Quercus petraea (Matt.) Liebl. in the British Islands (records up to October 1969; after Perring and Walters, 1962)

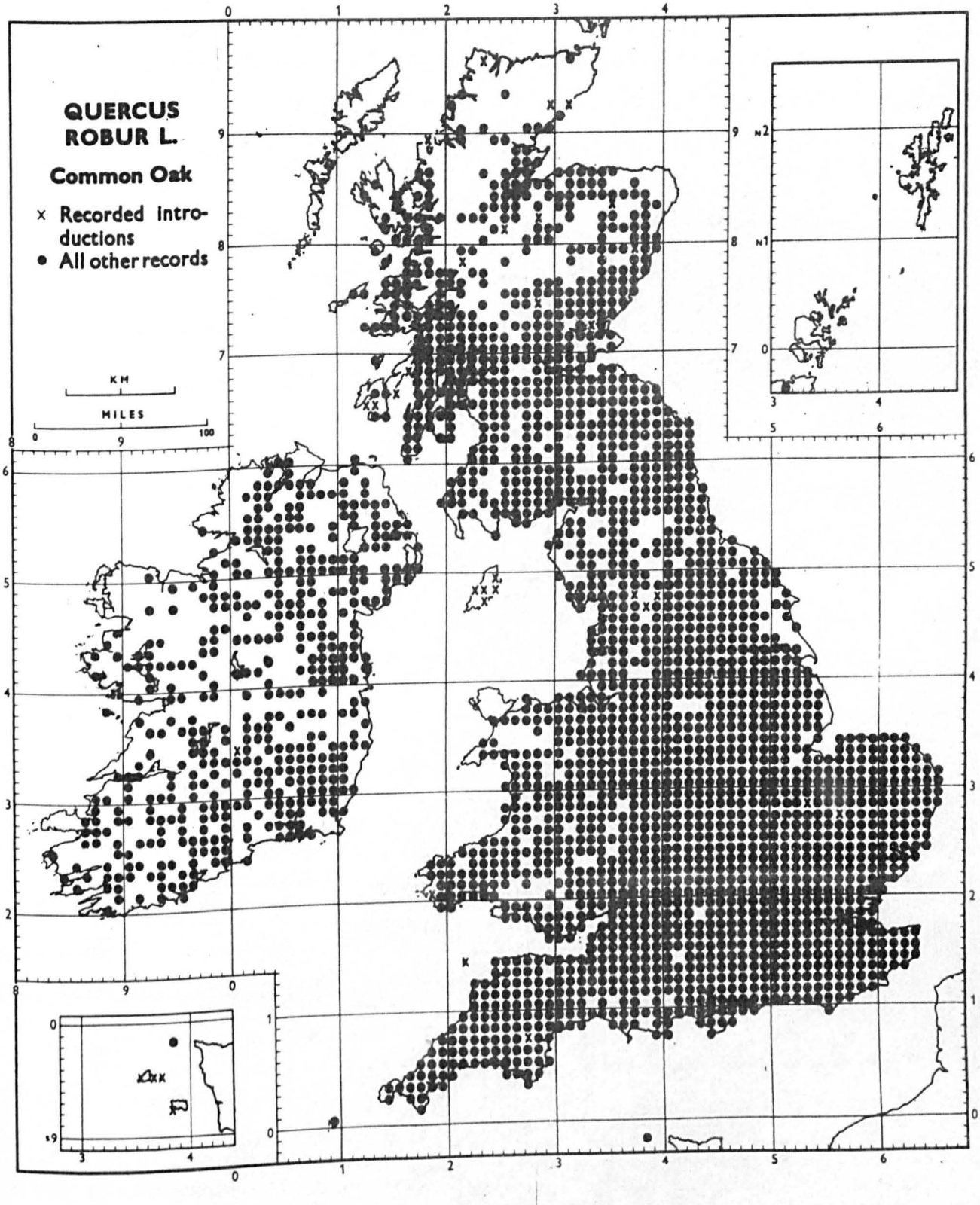


DIAGRAM 1.3 Distribution of Quercus robur L. in the British Islands (records up to October 1969; after Perring and Walters, 1962)

distributions:

1. A preference of Q. robur for basic, neutral rich soils and of Q. petraea for acid soils
2. A preference of Q. robur for moist, heavy soils and of Q. petraea for well drained soils (Jones, 1959)

Such preferences are reflected in the tolerance of the species to flooding, Q. robur being tolerant of both water-logging and flooding, Q. petraea being intolerant of both. These adaptive differences can be recognised on a small scale, where the species occur together, eg. Carlisle and Brown (1965), Moss (1914-20), Pearsall (1923).

Hybridisation within the genus

Species within the genus Quercus frequently hybridise with each other, and this has been a secondary source of variation apart from the natural variation shown by the species. Hybrids have been recognised from all sections of the genus and from all geographical areas covered by the genus.

Early examples of hybrids on the American continent are given by Vasey (1883) who described two hybrid oaks, Q. prinus x Q. alba and Q. alba x Q. stellata from Washington, D.C. and Britton (1882) who described a hybrid between Q. nigra and Q. phellos. At this time, approximately ten other hybrid oaks had been noted in the American flora mainly by Engelmann (1877). However, such ideas did not appear tenable to botanists of the time, and papers written on hybrid oaks at about this time took on a crusading spirit, eg. Ness (1918):

"In a little hybridising work, which I carried on with the Overcup Oak (Q. lyrata) as father and the Live Oak (Q. Virginiana) as mother, I have become impressed with the ease with which fertile hybrids may arise between species of oaks, even though the relationship be apparently quite distant."

The latest catalogue of hybrid American oaks is given by Palmer (1948) who describes 77 hybrid oaks and lists another 15, but this the author

admits is not a complete listing. Various experimental studies have been performed on American oaks of either a populational nature, eg. Stebbins et al. (1947) or analysis of the progeny of hybrid oaks, eg. Tucker and Bogert (1973).

During the last 25 years, there have been many accounts of hybrids between American oak species, and in particular, and more importantly, in the origin of species by hybridisation. One of the earliest papers was by Tucker (1952) in which he described the evolution of the oak species, Q. alvordiana from Q. turbinella and Q. Douglasii using large scale population collections. Tucker and Muller (1958) have discussed the possible derivation of Q. margaretta from Q. gambelii stock by hybridisation with other oak species, and Muller (1961) has speculated on the origin of Q. fusiformis as a hybrid of Q. brandegei and Q. virginiana. Tucker later described a highly complex situation in the species Q. undulata (Tucker, 1961a, 1961b, 1963, 1970 and 1971) in which seven species were believed to be involved. Q. undulata was considered as a species complex with Q. gambelii as the 'common' denominator which hybridised with different species - Q. arizonica, Q. turbinella, Q. havardii, Q. muehlenbergii, Q. mohriana and Q. grisea in different parts of its range.

The first record of a hybrid oak in America was by Michaux (1812) who described X Q. heterophylla, and it was only one year later that the first record of hybridisation between Q. robur and Q. petraea was reported (Camus, 1936-54). Indeed, the presence of hybrids or suspected hybrids in the flora of Britain probably gave the early taxonomist a complete range of types grading from Q. robur through intermediate forms to Q. petraea, and this led almost certainly to the misleading descriptions of the species and classification of them all under one specific name Q. robur, eg. Praeger (1909), Stewart and Corry (1888).

Some of the earlier descriptions did, however, recognise the intermediate forms as distinct entities and classified them as such, eg. The London Catalogue of British Plants (1895) lists Quercus robur intermedia (D.Don.) as a taxonomic grouping, Hooker (1884) lists Q. intermedia, D.Don. as a variety of Q. robur being delimited from it by having short petioles, short peduncles and 'downy' undersurfaces to the leaves.

Other authors have commented upon the ease with which the two British oak species form hybrids. Bentham and Hooker (1886), although recognising Q. robur and Q. petraea as two races of the species Q. robur, record that "where the two races occur together numerous intermediates are formed". Davy (1933) also believed that where the species grew together intermediates occur, produced as a result of hybridisation. He recorded them occurring from Perthshire south to Kent and Cornwall on dry, sandy and gravelly soils, particularly in valley bottoms in hilly districts. He also notes that they occurred in Continental Europe (Germany, France and Russia). Clapham et al.(1962) also note the occurrence of the hybrid on certain sands and point out that the hybrid is frequent where the two species occur together. Although Schwarz (1970) does not specifically mention hybrids between Q. petraea and Q. robur he does record that within each subgenus most species are interfertile and hybrids are therefore common when related species grow together.

In Europe, hybrids have been reported between the British species, eg. Høeg (1929), between British species and continental species, eg. Kalinina (1967), who recorded hybrids between Q. robur and Q. borealis and Q. mongolica, and between continental species. Andronikaskvili (1970) reported hybrids between Q. macranthera and Q. longipes, Q. imeretina and Q. hartwissiana. Ničota (1966) recorded a natural hybrid between

Q. cerris and Q. macedonica, whilst Barabits (1969) was able to show that variation in planted stands of Q. borealis in Hungary were due to seed being collected from different areas, where the acorns had been formed by hybridisation with species in the vicinity.

Introgressive hybridisation

The different types of variation discussed above, i.e. intraspecific variation caused by the natural variability of the species and derived from either intraspecific genetic variation or intraspecific phenotypic variation and the variation introduced into the species by hybridisation, has led many authors to attempt firstly descriptions of the variation observed within samples of oak species, i.e. population samples, and subsequently to attempt explanations of the origin of the variation they had previously described. Such researches suggested one important conclusion: that the majority of variation in oak populations in Britain, Europe and America was the result of introgressive hybridisation. This led Schwarz (1970) to observe that within the genus Quercus, hybridisation is so common that "much of the intraspecific variation is due to introgressive hybridisation".

Introgressive hybridisation or introgression was first described under this name by Anderson and Hubricht (1938) but much of the early work was carried out by DuRietz, eg. DuRietz (1930). His work was mainly concerned with the floras of New Zealand and Scandinavia and in particular on population studies of the genera Salix, Dracophyllum and Coprosma. He noted that in certain areas within each genus members of species pairs would hybridise, but further he noted that populations of the same parental species in areas of sympatric overlap converged as far as morphological characters were concerned. He attributed such convergence to hybridisation followed by backcrossing between the hybrids

and the spatially closest parent species. DuRietz (1930) described this process as one species becoming 'infected' with genes from another. A similar process had been noted in 1928 by Ostenfeld who suggested that a gradual infiltration of genetic material from one species to another could take place.

It was left to Anderson (Anderson and Hubricht, 1938; Anderson, 1949, 1953) to document fully the processes of introgression and to give the phenomenon a name. Anderson defined introgression as the repeated backcrossing of a natural hybrid to one or both parental populations. It results, therefore, in the transfer of genes from one species or semi-species to another across a breeding barrier.

The earliest descriptions of introgression within a particular genus were given by Anderson and Hubricht (1938) who documented their investigations into the genus Tradescantia. Other workers of the same period also produced documentation for the existence of introgression, eg. Wetmore and Delisle (1939) on Aster, Riley (1938) on Iris, and these resulted in the publication by Anderson of two definitive pieces of work on introgression - a book entitled 'Introgressive Hybridisation' published in 1949 and a paper written for the Biological Review in 1953. In these Anderson described the biometrical analysis of plant populations in order to determine the existence or otherwise of introgression. Such studies began with the collection of large scale population samples, these being chosen as extensive rather than intensive collections, a distinction later to be advocated in genecological sampling methods, eg. Wilkins (1959), Harberd (1961). These samples were then scored for a range of characters, so as to provide a description of each individual in the sample. In order to carry out this task expeditiously, Anderson (1949, 1953) used the Hybrid Index method of scoring in which character expressions typical of one species were designated one score, character

expressions typical of another species were designated another score, and intermediate expressions were positioned between the two scores accordingly. Hairiness might therefore be scored as present and abundant 2, absent 0 and sparsely present as 1. Quantitative characters could be converted to a hybrid index score by splitting the range of the quantitative character up into classes in much the same way as a histogram is divided into classes. Provided that the typical expression of each character for each species is designated one value, i.e. all expressions of character of one species score zero, and all expressions of the same characters of the other species score, let us say 4, by totalling up the score for each character of an individual, that individual can be classified as belonging to one species or the other or some way in between.

The use of the Hybrid Index permitted two important analyses to be carried out on population samples - the frequency histogram and the pictorialised scatter diagram or PSD. Since each individual could be assigned to a position between two species, the frequency of each summed hybrid index could be determined and used to construct a frequency histogram of such summed hybrid indices. Anderson (1949) argues that the shape and position of such a histogram will give clues as to the possible taxonomic status of the population. Pure species populations would show normal distributions, with no intermediate forms, whilst introgressed populations would show the possession of intermediate forms giving the normal distribution a distinct tail and skewed distribution. This gross analysis can be supplemented by a PSD. This analysis consists of the construction using two quantitative characters of a two-way scatter diagram in which the axes of the diagram are the two quantitative characters. Each individual can then be assigned a position on the scatter diagram using its values for the two quantitative characters. Other

characters can be represented on the diagram by attaching rays of differing length and ornamentation to each dot. These pictorialised dots represent therefore the total measured variation of the plant and have been termed metroglyphs (Anderson, 1957). By studying such diagrams it may be possible to recognise that different characters are loosely associated, though not completely correlated with each other and this loose, non-random association of various characters is regarded by Anderson (1949) as critical evidence of introgressive hybridisation.

This description of early attempts at describing introgression in plant populations was not, however, how the investigation was normally carried out in practice. Generally, populations could be assigned to one species, but the other species responsible for the hybridisation was either totally unknown or one of a range of possible species. In such cases, the population under study was scored using extreme hybrid index values for character expressions typical of the species of the population, and using opposite extremes or intermediate values for atypical character expressions. In the construction of the PSD, use could then be made of the process of extrapolated correlates in which the characters of the species responsible for the hybridisation could be deduced from the range of variation shown by the population, eg. Anderson (1953), was able to extrapolate in this way from an Oxytropis albiflorus population to a description of a plant which on consultation of a flora was found to be Oxytropis Lambertii, and which was later found at slightly lower elevations from the original Oxytropis albiflorus population.

The techniques used by Anderson have been discussed in detail since not only were they the first attempts at analysing complex taxonomic data of this sort, but they have persisted until the present for analysis and description of populational variation, eg. Wigston (1971). Indeed,

the range of reported examples of introgression is now extremely wide. Agnew (1968a) has shown that introgressive hybrids occur between Juncus effusus and J. conglomeratus, but in this case backcrossing only appears to take place with J. effusus, leading to gene flow from J. conglomeratus into J. effusus. Elkington (1968) has reported introgressive hybridisation to be taking place between two species of Betula in north-west Iceland, Betula nana L. and Betula pubescens Ehrh., using the techniques of the hybrid index and scatter diagram methods of Anderson. Kenworthy et al. (1972) have described similar introgression between the same two species using hybrid index methods in Sutherland. Fassett and Calhoun (1952) have detected introgression taking place between Typha latifolia and T. angustifolia in Wisconsin, U.S.A. and Makinen (1965) has shown introgression to have taken place between Carex digitata and C. pediformis spp. rhizodes.

It is important to point out, however, that the original use of the term introgression has since been distorted to include many examples of hybrid swarms, which Heiser (1973) does not regard as examples of introgression in the Anderson sense. Cases of hybridisation which deviate from the three phases of introgression, i.e.

1. The initial formation of F_1 hybrids
2. Their backcrossing to one or other of the parental species
3. Natural selection of certain favourable recombitant types,

to produce a complete series of transitions from one species through to the other should rightly be called 'miscibility' and not introgression (Dansereau and van Steenis, cited by Davis and Heywood, 1963).

The techniques for the study of introgression have not been improved since Anderson first proposed the scatter diagram with one exception, the use of chemical characters. An excellent example of the use of such characters is given in an account of introgression in Picea by Hanover

and Wilkinson (1970). Using chromatographic separation of the phenolic compounds of the foliage of Picea sitchensis, P. glauca and P. engelmannii they were able to show that some phenolic substances were species specific and that these provided evidence of some introgression between P. sitchensis and P. glauca.

The existence of introgressive hybridisation has been challenged, however; several authors, among them Barber and Jackson (1957), Grant (1966, 1967), believing that the variation described in populations as resulting from introgression could be the result of other phenomena. Heiser (1973) has replied to such criticisms, but it is not proposed here to discuss the arguments for or against - these will be considered in the concluding discussion of this thesis as they specifically apply to Quercus.

Introgression within the genus Quercus

As noted earlier, Schwarz (1970) observed that much of the intra-specific variation within oaks is due to introgressive hybridisation, and the literature would tend to support this view, since many examples exist.

De Rivas (1968) has described a biometrical study of Q. canariensis Willd. and shown that populations of the species show evidence of introgression from Q. faginea, Q. pubescens and Q. mas. A similar study by Sutilov (1968), using hybrid indices, pictorialised scatter diagrams, and extrapolated correlates has shown varying degrees of introgression taking place between Q. robur and Q. pubescens where the two species were sympatric. In the same work he also noted a parallel situation between Q. robur and Q. hartwissiana, both examples leading to difficulties in demarcating the species.

In the American flora, although much work exists on the detection of hybrids, few examples have been noted on introgression. Cooperrider (1957) used Anderson-type techniques to study introgression between Q. marilandica and Q. velutina and the same techniques were used by Maze (1968) in detecting hybridisation between Q. macrocarpa and Q. gambelii. This latter study is important since it is one of the few instances of introgression between currently allopatric oak species. Maze (1968) surmises that the introgression took place during past sympatric association between the species. An interesting piece of circumstantial evidence in this work was the occurrence of an obligate parasitic wasp found on Q. gambelii in the Rocky Mountains and on Q. macrocarpa in the Black Hills, suggesting also a period of past sympatricity. Ledig et al.(1969) has described introgression between Q. alba and Q. prinus but using the technique of discriminant analysis, a statistical tool for the analysis of complex taxonomic data.

Introgression between Q. robur and Q. petraea

Three major studies have been completed on introgression in the British oaks; Cousens (1961, 1962, 1963 and 1965), Carlisle and Brown (1965) and a study contemporaneously carried out with the present investigation, Wigston (1971). Two other studies on the Continent have also recently been published; Becker (1972) and Belous (1972).

The early work of Cousens (1961, 1963) was based on oak populations found in Scotland, where other previous workers had experienced difficulty in recognising oaks as belonging to one species or to the other, eg. Finlayson cited by Cousens (1961). Preliminary results suggested that Q. petraea woodland predominated in Scotland, and 'good' Q. robur was found in all Q. robur dominated woodland, and this led to the publication in 1962 by Cousens of notes on the identification of

Scottish oaks. Such optimism was short-lived, however, since in the following year Cousens published the results of his full scale investigation into variation of Scottish oaks in which he observed that more than half of the fertile material collected could not be diagnosed with any degree of confidence as belonging to either species. Although initially measuring nine characters - leaf length, petiole length, auricle type, abaxial stellate pubescence, lobe number, lobe depth, peduncle length, peduncle diameter and peduncle pubescence, only six were used to finally analyse the populations, those that were considered to completely separate the species petiole length (as expressed as a percentage of leaf length) and peduncle length, the primary characters, and auricle type, stellate abaxial pubescence, peduncle pubescence and peduncle diameter, the secondary characters. Cousens (1963) did not construct a Hybrid Index as such but classified each specimen with respect to how it differed from the theoretical species type or TST. A specimen showing all four secondary characters within the diagnostic range for one species was defined as belonging to the TST, and consequently a specimen showing one character in the indeterminate range was classified as showing one 'degree of difference' from the TST. Using four secondary characters, there were, of course, nine such character combination classes; I being the theoretical Q. petraea combination, IX being the theoretical Q. robur combination, II being with one degree of difference from Q. petraea and so on, V being a class containing those specimens indeterminate for all four secondary characters. Scatter diagrams were produced using the primary characters as the axes, and each point on the scatter being assigned to one of the character combination classes.

The use of such techniques led to the conclusion that the variational patterns observed within population samples are "what would be expected

from widespread and massive introgression" (Cousens, 1963). There were problems in this study, mainly the apparent lack of 'good' species material within Scotland, with which to make valid comparisons. This prompted the publication two years later of an enlarged survey (Cousens, 1965) in which attempts were made to obtain 'pure' populations of Q. robur and Q. petraea for comparison with Scottish populations. Samples of Q. petraea were collected from Eire, and Q. robur from Yugoslavia and parts of south-east England, all of which showed some evidence of introgression.

On the scatter diagrams of this second study, Cousens derived an 'introgression path' by calculating mean values for the two primary characters for each of the nine combination classes, so that on the scatter diagram, it was possible to construct a line or pathway connecting the two TSTs and passing through each combination class. Consideration of the effect of introgression on such population statistics led Cousens (1965) to suggest the following stages through which introgression may pass and may be recognised:

"Stage 1 - a few hybrids are established and backcrossing has begun - the proportion of TST's will be high; their mean values will lie at the very end of the Introgression Path but there will be a distinct 'tail' stretching out into the intermediate zone between the two species concentration centres.

Stage 2 - hybridisation is continuing and introgression is well advanced - the TST Primary Character means will have moved down the Introgression Path and there will be a substantial proportion of the specimens contributing to the 'tail' which will also reach into the intermediate zone.

Stage 3 - hybridisation has ceased (between the species) and continuing backcrosses are resulting in a gradual assimilation of the 'alien' genes - the TST Primary Character means will have moved a little further down the Introgression Path and the 'tail' will have contracted.

Stage 4 - the process of assimilation is complete - there will be no obvious introgressive trend and its origin will only be apparent if there are data from non-introgressed populations for comparison."

Using this sequence, the Scottish populations were regarded as being in Stage 2, whilst the Irish Q. petraea populations were in a late Stage 3,

and in consequence, the Irish populations were thought to be the result of a past introgression which had been almost entirely assimilated into the Q. petraea gene pool.

Carlisle and Brown (1965) recorded the taxonomic status of a mixed oak wood, Roudsea Wood, Lancashire using the techniques of the Hybrid Index frequency histogram and the Pictorialised scatter diagram. The results suggested that hybridisation and introgression had occurred between the species, and more importantly, that the species showed separate soil preferences in the soil mosaic found in the woodland. Q. petraea was found to predominate on the slate sites whilst both species grew on the limestone and peat areas. In assessing the usefulness in the techniques they used, Carlisle and Brown (1965) believed that the Hybrid Index over-estimated the morphological intermediacy of the samples, whilst the PSD under-estimated the Q. robur component. The methodology used by Carlisle and Brown (1965) although naive and less testing than the theoretical analysis of Cousens (1965) and of more limited geographical distribution than Cousens (1963, 1965) it did have the advantage of using a much wider range of characters (23 in all, although this was later reduced to 20), covering all aspects of the morphological biology of the trees, from crown characters, bark characters and leaves to reproductive characters. Certainly, for numerical taxonomic studies, at least 60 characters has been suggested as a minimum although as Sneath and Sokal (1973) point out, this is a problem still to be solved.

Wigston (1971) has approached the problem of variation in oaks in two ways, by the use of pictorialised scatter diagrams and the use of discriminant function analysis. This combination proved useful in detecting introgressed populations in the south-west part of England. Only five leaf characters were used for the investigation, leaf shape,

lobe regularity, lobe number, form of the basal part of the lamina and abaxial pubescence. Intraspecific variation in the two species appeared to be approximately equal, but interspecific variation of various populations was indicative of introgression. Some of these populations were apparently undergoing active introgression, whilst others had stabilised in an intermediate taxonomic state, and these appeared to be correlated with either woodland management (active introgression) or the aureolic margins of the Dartmoor granite (stabilised intermediates). Mixed populations like those of Carlisle and Brown (1965) were shown to have an edaphic separation of the species. Wigston (1971) also tentatively recognised a general southwesterly trend in taxonomic variation from Q. robur, through intermediates (both individuals and populations) to Q. petraea.

Two more recent studies have been those of Becker (1972) and Belous (1972). Belous (1972) used the hybrid index method of Anderson (1949) with seven characters on twelve population samples in the Ukraine, and was able to show that introgression was actively occurring. Becker (1972) has used petiole length, presence of auricles, leaf shape and peduncle length to separate the species, but his unsuccessful attempts to separate the species completely without the presence of large numbers of intermediate forms led him to the conclusion that hybridisation must be occurring between the species, and that this hybridisation was in the form of introgression.

An alternative to introgression

The widescale introgression reported by Cousens (1963, 1965), Carlisle and Brown (1965) and Wigston (1971) between Q. robur and Q. petraea within the British Isles has been criticised by several authors. Jones (1968) notes:

"It is scarcely possible to find populations of either species that are so isolated from other species of Quercus that the possibility of their being influenced by introgression can be excluded, and which would therefore give us an objective measure of the intraspecific variation. Thus how much of the variation that we find is attributed to introgression between the two species and how much is attributed to normal intraspecific variation is fundamentally a matter of personal opinion; we can certainly attribute much to introgression if we wish."

Jones (1968) goes on to argue that his view of oak species is a broad definition due to a) the considerable genetical variation that is normal in populations of trees such as oak which are more or less self-sterile and reproduce only sexually and b) it is more useful practically if names indicate groups of individuals that resemble each other in general appearance and behaviour rather than individuals that resemble each other in some hypothetical ancestry. Although admirable sentiments, the natural inquisitiveness of the scientist should lead him to seek a solution to a problem rather than 'sweeping it under the carpet' as Jones (1968) would have him do.

In support of the view of Jones (1968), several research workers have failed to detect hybrids in British oak populations. Yapp (1961) only recorded a few trees of problematic status whilst surveying oaks in the Ullapool area of Scotland, contrary to the results of Cousens (1963). Hadfield (1960) whilst studying oak populations, came to the rather paradoxical conclusion that the populations studied showed enormous variation in both size and shape of lamina and that consequently hybrids were regarded as being rare. Gathy (1970) studying a random collection of oaks from a long established mixed forest in Belgium identified eighteen trees of Q. petraea, nine trees of Q. robur and only three of a possible hybrid origin. Gathy (1970) concluded that natural hybridisation is therefore rare and introgression rather slight.

Jones' main criticism (1959) of the work recognising widespread introgression is that it is based on an imperfect understanding of the specific characters of oaks, but this view also appears to be supported

by genetical evidence. This will be fully reviewed in Section 4 of this thesis, suffice it to say here that hybrids between Q. robur and Q. petraea have never been raised above the seedling stage from artificial crossings, suggesting that the widespread introgression reported has no genetical basis. The comments of Cousens (1963) are, however, of importance here. He argued that since the species are so very variable, then interfertility between the species will probably also vary, so that conclusions concerning the crossibility of two oak species can only be drawn after attempts have been made to cross individuals from all ranges of the spectrum between the species. Stebbins (1950) also points out that the oak is such a long lived species that over the period of its lifetime there is a good possibility of producing large numbers of hybrid seed even though it may only be of low interfertility with other species.

Two other pieces of evidence, not considered by Jones, are relevant here. Firstly, as mentioned before, the criticisms of introgression as a biologically valid phenomenon and secondly the existence of subspecific variation within both Q. petraea and Q. robur. Much of the subspecific variation described for these two species has come from continental work, and indeed Jones (1959, 1968) does not mention the occurrence of subspecific rankings except for those noted by Moss (1914-1920), i.e. two varieties of Q. petraea var. longipedunculata and var. sphaerocarpa. So many of the described subspecies, varieties and forms of the two species bear such a close relationship to what might be expected of a hybrid, that such variation must be considered as a factor in the recognition of hybrid oaks. The variety of Q. petraea var. longipedunculata recorded in Britain is a variety having a character normally regarded as typical of Q. robur, i.e. a long peduncle. The much hairier form of Q. petraea, var. pubescens Loud. has been recorded

more from the west of Britain on rather wet soils (Davy, 1933), and this must have important implications in recognising hybrids in British populations. Two forms of Q. robur, form cristata and filicifolia supposedly occurring in Britain have either long petioles or tapered bases and are downy with stellate hairs, and have been regarded by Hadfield (1960) as being of hybrid origin. Jones (1959) notes a form of Q. petraea that has very deeply lobed leaves, a Q. robur character, and these he regards as being either hybrids or ecotypes. Such variation has led to past ideas on hybridisation being changed through the efforts of research, eg. Krasilnikov and Abakarova (1970) suggested that Q. pedunculiflora is not a hybrid of Q. pubescens x Q. robur but is a subspecies of Q. robur. Thus the taxonomy of the genus Quercus is in a state of flux.

The arguments both for and against the role of hybridisation as a factor in the variation in British oaks has recently been reviewed by Gardiner (1970) who described the situation quite rightly as a 'hybrid controversy'.

This thesis is presented in the spirit of an integrated approach to the problems of the taxonomy of Quercus robur and Quercus petraea, and in particular to the origin and possible significance of the variation found within and between oak populations in England and Wales. The thesis will in consequence examine the following:

1. The patterns of morphological and anatomical variation observed within individual trees of the two species, in an attempt to define the influence of environmental parameters on taxonomic characters normally used to delimit the species. Subsidiary work on leaf development and seedling morphology and anatomy is discussed in relation to its bearing on the central issue of the influence of the environment on taxonomic characters (Section 2).

2. The analysis of oak populations from Wales, the Midlands and East Anglia using the methods of cluster analysis, discriminant function analysis and principal component analysis, to determine the possible extent of hybridisation, and the influence of external parameters that might promote it (Section 3).

3. The reproductive biology of the two species in particular artificial hybridisation, and the pollen viability of the two species, and suspected hybrids. Observations are also presented on the karyotype and on the morphological structure of the pollen (Section 4).

4. The comparative physiology of the two species from the point of view of the growth analysis of their seedlings, and the leaf resistances to water vapour loss of the adult and seedling leaves in order to establish a fundamental difference between the species that had been suggested by the results of Section 2 (Section 5).

5. Simulation models of introgression in oak 'populations' to attempt answers to questions not easily investigated by an observational or experimental approach (Section 6).

The final section presents a concluding discussion in which the specific findings of this thesis are discussed in relation to the wider fields of hybridisation, introgression and taxonomy.

SECTION TWO

THE INDIVIDUAL

CHAPTER TWO

THE OAK CANOPY I

Introduction

Survival for a plant species depends largely on the ability of the species to adapt to new and changing environmental conditions. Wide-ranging plant species frequently show marked patterns of variation which are assumed to be the response of the species to marked environmental changes over the geographical area which the species occupies. Such variation may be at the molecular, physiological, anatomical or morphological level and may be the result of genecological differentiation, eg. the differentiation of Geranium sanguineum described by Lewis (1969) or plastic modifications of the phenotype, eg. the effect of light intensity on the leaf shape of Ipoemea caerulea described by Njoku (1956). The literature on both types of variation in relation to many environmental variables is extensive and it is not proposed here to give a complete coverage of such variational patterns, but to concentrate on those of importance in understanding variation in oak.

In tree species, the canopy structure is such as to produce environmentally different conditions at different levels in the canopy. Consequently, within a canopy, there are gradients, not only of light, but also wind speed, humidity and temperature (Hanson, 1917). Since carbon dioxide diffuses from the soil (Montieth et al., 1964) and is absorbed by the different layers of foliage, a carbon dioxide gradient normally exists in tree canopies. Leaves frequently show modifications from the 'normal' type to take account of these gradients, and these modifications are assumed to be adaptive in nature. The modified leaves are termed 'sun' and 'shade' leaves and these show changes consistent with plants from sun and shade habitats. Differences between plants grown in high light

conditions have been documented by Hanson (1917); Pearsall and Hanby (1926); Maximov (1931); Shields (1950); Parker (1956); Lewis (1972); Daubenmire (1974), etc. These differences, morphological, anatomical and physiological, are detailed in Table 2.1 which compares the differences observed in sun and shade plants of the same species.

Although the morphological and anatomical characters of leaves are frequently recorded as being of adaptive significance and therefore of survival value, little consideration has been given to establishing this important relationship. Of direct relevance here is the establishment of the importance of leaf morphology and anatomy in influencing the physiological behaviour of the leaf. Lewis (1972) has recently reviewed the physiological significance of variation in leaf structure, particularly with regard to xericity and light intensity.

Little information exists in respect of sun and shade differences in the leaves of Q. robur and Q. petraea, although, as noted in Chapter 1, Nescjarovič and Smirnova (1969) have described a more xeromorphic leaf in Q. robur seedlings from southern areas of Europe. Brenner (1902) found that leaves of Q. robur in humid atmospheres had shallower lobes than leaves in drier habitats. The response of Q. petraea to humidity changes was recorded as much lower. Jones (1959) records no differences between leaves on the same tree other than those between lammas shoots and spring shoots. Cousens (1963) recorded some differences in leaf length, and petiole length between different aspects of the lower canopy of an isolated Q. robur tree, an isolated Q. petraea tree and a suspected hybrid. Some differences were also noted between the top and lower parts of the crown. Cousens (1963) concluded from his researches that to minimise variation in population samples, particularly with regard to the leaf dimensions, the south-east aspect should be used for sampling. Carlisle and Brown (1965) removed leaves

TABLE 2.1 MODIFICATION OF PLANT STRUCTURE UNDER FULL SUNLIGHT AND SHADE CONDITIONS (after Daubenmire, 1974)

Full Sunlight	Shade
Morphological features:	
1. Thick stems, well developed xylem and supporting tissues	Thinner stems, poorly developed xylem and supporting tissues
2. Less leaf area/plant	More leaf area/plant
3. Short internodes	Long internodes
4. Prolific branching	Poorly branched
5. Weakly developed endodermis	Well developed endodermis
6. Small cells in leaf blades resulting in	Larger cells in leaf blades
a) Smaller, thicker leaf blades or blade segments	Larger, thinner leaf blades or blade segments
b) Stomata small and close together	Stomata larger and spread apart
c) Small vein islets	Large vein islets
d) More hairs per unit area	Fewer hairs per unit area
7. Leaves deeply lobed	Leaves shallowly lobed
8. Thick cuticle and cell walls	Thinner cuticle and cell walls
9. Chloroplasts fewer and smaller	Chloroplasts larger and more numerous
10. Better developed palisade	Poorer developed palisade
11. Weakly developed spongy mesophyll	Better developed spongy mesophyll
12. Small intercellular spaces	Large intercellular spaces
13. Ratio of internal : external leaf surface large	Ratio of internal : external leaf surface small
14. Lateral walls of epidermal cells straight	Lateral walls of epidermal cells wavy
15. Leaf blades not flat, less compound and oriented at other than right angles to the path of incident light	Leaf blades flat, more compound, oriented at right angles to the path of incident light

Full Sunlight	Shade
16. Low ratio of total leaf area to vascular tissue of stem	High ratio of total leaf area to vascular tissue of stem
17. Roots long, more numerous, more branched and with a higher root/shoot ratio	Roots short, fewer, less branched and with a low root/shoot ratio
18. Greater fresh weight and dry weight of both roots and shoots	Root and shoot, fresh weight and dry weight low
Physiological features:	
1. Lower chlorophyll content giving a greenish yellow colour	High chlorophyll content
2. High photosynthetic rate per unit area in bright light	Lower photosynthetic rate per unit area in bright light
3. High respiration rate and consequent high compensation point	Lower respiration rate and consequent low compensation point
4. Low percentage of water on a dry weight basis	High percentage of water on a dry weight basis
5. More rapid transpiration	Less rapid transpiration
6. Higher salt and sugar content giving a more negative osmotic potential. Can withstand 20-30% loss of water content without wilting	Lower salt and sugar content giving a less negative osmotic potential. Can withstand 1-5% loss of water content without wilting
7. Decrease in acidity of cell sap	Increase in acidity of cell sap
8. High carbohydrate/N ratio	Low carbohydrate/N ratio
9. Low K, Ca and P content	High K, Ca and P content
10. Greater vigour of flowering and fruiting	Less vigorous in flowering and fruiting
11. Earlier appearance of flowers, but later maturation of leaves	Later appearance of flowers, earlier maturation of leaves
12. More calories per gram dry weight of seeds	Fewer calories per gram dry weight of seeds
13. Greater resistance to temperature and drought	Poorer resistance to temperature and drought

from the upper half of the south side of the crown when sampling Roudsea Wood for oak leaves, but failed to record if this was to reduce variation due to shading. Wigston (1971) used leaf litter collections for population samples, a sampling technique to be avoided due not only to the influence of possible sun and shade differences in the canopy which cannot be accounted for by such a method, but also because of the influence of lammas growth, which as Jones (1959) points out is very different from spring growth. We are, therefore, remarkably ignorant about the influences of sun and shade on altering leaf morphology and anatomy in British oaks. Since the detection of hybrids requires a knowledge of the natural range of variation of the individual and of the species, it would seem that before the variation in a population can be fully assessed, evidence must be presented on the variation found within individuals, i.e. the plastic modifications of the phenotype. This chapter examines such modifications of oak leaves.

Taxonomic characters and the differentiation of *Quercus robur* and *Quercus petraea*

Confusion in the delimitation of *Quercus robur* and *Quercus petraea* is due primarily to the lack of any diagnostic character for the species, i.e. a character that is easily recognisable, with a narrow range of expression and which clearly differentiates between the two species (Davis and Heywood, 1963). In all characters commonly used to differentiate the species, there is great variability, and, in many instances, overlapping of the ranges of the characters for the species occurs. This has led to very misleading descriptions of the species being published, eg. many Continental floras describe the leaf of *Q. petraea* as glabrous and this has been perpetuated in recent accounts of the species - Fournier and Pardé (1952); Dizerbo (1965). The

misunderstanding of the characters of Quercus, and the misleading accounts of their occurrence, have possibly led to erroneous descriptions of the distribution of the species both in Britain, eg. Harris (1927) and on the Continent, eg. Glørson (1944). Although in Britain, little confusion exists in recognising oaks as Q. petraea/ Q. robur (with the possible exception of Q. cerris which may be confused with Q. petraea), the presence of many more species, particularly closely related and morphologically similar ones, has led to misleading accounts of characters and geographical distribution in Continental work.

The most comprehensive assessment of distinguishing characters is given by Jones (1959). Table 2.2 lists the characters recorded by Jones, and their expression in the two species. Several investigations have utilised the characters suggested by Jones (1959), and have modified them or given quantitative expression to some qualitative characters. Some of the more important of these are summarised in Table 2.3.

In assessing the variation of leaf characters in oak canopies, eleven morphological and six anatomical characters have been measured. These are discussed below - (see also Diagram 2.1).

Lamina length (LL):

Lamina length, as measured from the tip of the lamina to the base, is a notoriously variable character, but Q. petraea has been noted as having a longer lamina than Q. robur (Jones, 1959).

Petiole length (PL):

An important character in distinguishing the species, but it can be difficult to measure. It is usually defined as the length of the petiole from the point of attachment to the twig to the base of the lamina. In oaks, particularly in Q. petraea, the lamina may join the

TABLE 2.2 A COMPARISON OF CHARACTERS USED IN DIFFERENTIATING *Quercus robur* AND *Quercus petraea*

Character	*Quercus robur*	*Quercus petraea*
A. Habit:		
1. Stem persistence	Tendency for main trunk to disappear in crown	Tendency for main trunk to persist in crown
2. Branches *	Irregular branching	Branching more regular and straighter
3. Angle between branches *	Wide	Narrow
4. Twigs *	Decrease in size from main boughs to twigs abrupt, leading to short slender twigs	Gradual decrease in size, leading to longer, stouter twigs
5. Crown	Open crown, foliage in clusters	Dense crown, foliage uniformly distributed
B. Bark:		
1. Depth	Thick	Thinner
2. Fissuring	Deeply fissured into elongate blocks, but not scaling	Shallower fissures, forming short more or less rectangular blocks which often tend to exfoliate
C. Terminal Buds:		
1. Size	Small	Large
2. Shape	Obtuse	Acute
D. Leaves of spring shoots:		
1. Shape *	Obovate, widest well above the middle of the lamina, and narrow at the base	Ovate, widest at or just above the middle of the lamina, and tending to be rounded at the base
2. Lobe regularity *	Irregular	Regular
3. Lobe depth *	Deep sinuses between the lobes	Shallow sinuses
4. Number of lobe pairs *	3-5 (-6)	5-6-8
5. Venation	Some veins run to the sinuses between the lobes	No veins to sinuses

Character	Quercus robur	Quercus petraea
6. Base of lamina		
a) Shape	Narrow, cordate	Broad, often cordate, the cuneate base often described is less usual
b) Auricles *	Strong	Weakly auricled
7. Petiole *	Very short (2-3-7 mm.), lamina often nearly sessile	Long (13-25 mm.)
8. Hairiness *	Glabrous except sometimes for a few inconspicuous simple hairs on the lower surface of the lamina or mid-rib	Long clustered hairs on the lower surface along the mid-rib. Small stellate hairs on the lower lamina surface
9. Size and texture	Small, pale matt green and thin	Larger, more coriaceous, dark green and glossy above
E. Acorns:		
1. Colour *	Pale fawn	Uniform dark brown
2. Stripes *	Olive green longitudinal stripes visible in fresh mature acorns	Stripes absent
3. Shape	Very variable in shape, larger, longer and more oblong	Variable in size and shape, generally smaller and rounder
4. Cupule	Scales closely imbricated, flat	Scales looser, tumid
F. Fruiting peduncle:		
1. Peduncle length	2-9 cm. long	Absent or up to 3-4 cm. long
2. Peduncle diameter	Slender	Stout
3. Peduncle pubescence	Glabrous	With clustered hairs

Table derived from the work of Jones (1959) with the exception of character E4.

* Characters recognised by Jones (1959) as more important in distinguishing the species.

Character	Jones (1968)[1]	Cousens (1962, 1963)	Carlisle and Brown (1965)	Clapham et al. (1962)	Butcher (1961)	Schwarz (1970)
A 1	-	-	X	Q. petraea branches higher than Q. robur	-	-
2	X[+]	-	-	-	-	-
3	-	-	Q. robur > 45° Q. petraea < 45°	-	Q. robur horizontal branches Q. petraea spreading branches	-
4	X[+]	-	-	Q. robur twigs glabrous, grey brown in colour	-	-
5	-	-	X	Crown of Q. robur broad, that of Q. petraea narrow	Q. robur, broad crown, Q. petraea narrow crown	-
B 1	-	-	X	-	-	-
2	X[2]	-	X	Q. robur fissured bark	-	-
C 1	X[+]	X	Q. robur < 5 mm. long, Q. petraea > 5 mm. long	X	-	-
2	X[+]	X	X	X	-	-
D 1	-	X	X	Q. robur obovate - oblong	Q. robur - obovate, Q. petraea - obovate	Q. robur - obovate, Q. petraea obovate

Character	Jones (1968)[1]	Cousens (1962, 1963)	Carlisle and Brown (1965)	Clapham et al. (1962)	Butcher (1961)	Schwarz (1970)
D 2	Q. robur - lobes and veins spreading at a wide angle, Q. petraea regular lobes on either side, lobes and veins at a narrower angle to mid-rib +	X	X	X	-	-
3	X +	X	Q. robur > 50% of one half leaf width, Q. petraea < 50% of one half leaf width	-	-	-
4	Q. robur 3-4 + Q. petraea 5-6	X	Q. robur < 5 Q. petraea > 5	X	-	Q. robur 5-7 pairs, Q. petraea 5-8 pairs
5	Q. robur - frequent veins to sinuses, Q. petraea - such veins rare +	X	X	-	-	X
6 a	-	X	X	X	X	-
b	X *	X	X	X	X	-
7	Q. robur 2-6% lamina length Q. petraea 9-15%* lamina length	X	X	Q. robur to 5 (-10) mm.	X	Q. robur < 5 mm. Q. petraea 18-25 mm.

Character	Jones (1968)[1]	Cousens (1962, 1963)	Carlisle and Brown (1965)	Clapham et al. (1962)	Butcher (1961)	Schwarz (1970)
D 8	X *	X	X	X	X	X
9	-	-	-	Q. robur, dull green, Q. petraea shiny	-	Q. petraea 7-12 cm.
E 1	X *	X	X	-	-	-
2	X *	X	X	-	-	-
3	-	-	X	-	Q. robur 22 mm. long, Q. petraea 25 mm. long	-
4	X [2]	-	X	X (Q. robur cup 15-20 mm. diameter)	Q. robur and Q. petraea 15 mm. diameter, with small ovate scales	X
F 1	Q. robur 15-60 mm.* Q. petraea 0-4 mm.	X	Q. robur > 30 mm. Q. petraea < 20 mm.	Q. robur 20-80 mm. Q. petraea 0-10 mm.	-	-
2	Q. robur <1.5 mm. diameter, Q. petraea >*1.5 mm. diameter	X	-	-	-	-

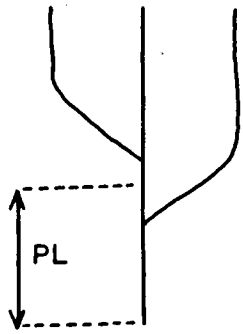
Character	Jones (1968)[1]	Cousens (1962, 1963)	Carlisle and Brown (1965)	Clapham et al. (1962)	Butcher (1961)	Schwarz (1970)
F 3	X *	Q. robur - glabrous, Q. petraea some pubescence	X	-	-	-

TABLE 2.3 CHARACTERS USED BY DIFFERENT AUTHORS TO SEPARATE Q. robur from Q. petraea

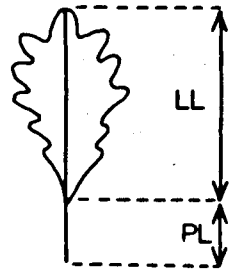
1 Jones (1968) distinguishes between:

Characters of greatest taxonomic value	*	X :	description of character conforms closely to that offered by Jones (1959)
Characters of lesser value	+		
Useful, yet less reliable, characters	2	- :	character not recorded by author(s)

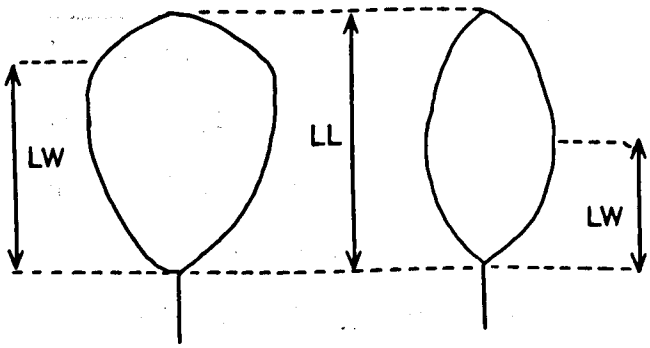
DIAGRAM 2.1



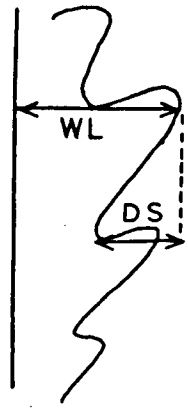
PETIOLE LENGTH IN DIFFICULT CASES



PETIOLE RATIO (PTR) = $\frac{LL + PL}{PL}$

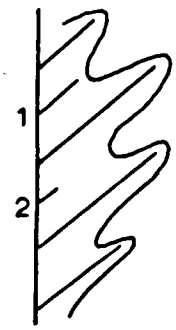


OBVERSITY = $\frac{LL}{LW}$ = $\frac{\text{LAMINA LENGTH}}{\text{LAMINA LENGTH TO WIDEST PART.}}$



LOBE DEPTH RATIO (LDR) = $\frac{WL}{DS}$

VENATION



1 - INTERCALARY VEIN DEEMED PRESENT

2 - INTERCALARY VEIN DEEMED ABSENT

DIAGRAM 2.1 Some Quercus L. characters and their measurement

petiole at different points on either side, so that the actual base of the lamina is staggered. The difference between each side may be as much as 5 mm., a considerable amount when the petiole itself may be only 5 mm. long. Throughout this investigation, if leaves have such a staggered base to the lamina, the petiole was deemed to join the lamina at the mid-point between the base of the lamina on the two sides (see Diagram 2.1).

Petiole ratio (PTR):

A ratio defined as: $\frac{\text{Lamina + petiole length}}{\text{Petiole length}}$

Cousens (1963) assessed a similar ratio (petiole percentage) as:

$$\frac{\text{Length of petiole}}{\text{Length of lamina + petiole}} \times 100$$

(Cousens (1961) in defining petiole percentage does not in fact clearly define the denominator of this ratio, the denominator being described as total leaf length, which could mean total lamina length or as interpreted here as length of lamina and petiole.)

Leaf area (AR):

Q. robur leaves are generally regarded as being smaller than those of Q. petraea, eg. Ellenberg (1939), but little definitive work exists. Leaf area has been measured by the method of printing the leaf outline onto ammonia-developed Diazo paper, then cutting out and weighing the prints and calculating areas from the weights of paper of known areas (Newton and Blackman, 1970).

Venation (V):

Intercalary veins (Scharwz, 1970) are present in many oak species and consist of veins running to the sinuses between the leaf lobes. Obviously, in assessing such a character, it is important to consider the relative number of sinuses with such veins. A ratio was, therefore,

calculated as:

$$\frac{\text{Number of veins to sinuses}}{\text{Total number of sinuses}} \times 100$$

Decisions on what constituted an intercalary vein were difficult and in consequence the following definition was used: An intercalary vein was deemed to be present if a vein ran more than half-way to the sinus and was a vein of equal or nearly equal size to those running to the tips of the lobes. This is practice proved a useful definition, but Wigston (1971) found difficulty scoring this character, and later abandoned it.

Number of lobe pairs (LN):

Recorded by Jones (1959, 1968) as the number of lobe pairs, but difficulties arise in cases where the number of lobes on each side of the leaf varies. Consequently, the number of lobes on each leaf was counted but, for comparative purposes, the result is expressed as the average number of lobes per side. Following Cousens (1961), only lobes possessing a vein running from the mid-rib to the leaf margin of the lobe were counted, so that subsidiary lobes or small indentations of the leaf margin at the basal part of the lamina were not counted.

Obversity or leaf shape (OB):

Obversity can be determined as the position of the widest part of the lamina. Diagram 2.1 illustrates the measurement of this character. The character score was calculated as:

$$\frac{\text{Lamina length}}{\text{Length of lamina, from base of lamina to the widest part of lamina}}$$

Wigston (1971) has used a co-ordinate system of fitting an elipse to the outline of the leaf margin to estimate leaf shape, but it is felt that, since leaf margin shape is so variable particularly between different halves of the same leaf, an exact measure is unwarranted and could be misleading.

Index	Description
2	Leaf has several simple hairs but stellate hairs are very few and found mainly in the vicinity of the mid-rib.
1	Stellate hairs virtually absent, but the leaf still has some simple hairs.
0	Leaf completely glabrous except for the odd one or two simple hairs especially around the mid-rib. Stellate hairs completely absent.

Hairiness was assessed at x20 under a binocular microscope.

Basal shape of lamina and auricle development (BS and AD):

Although Cousens (1962) regards the basal shape of the lamina as the most important single diagnostic character, he draws attention to the difficulties in scoring either basal shape or auricle development separately due to the interaction that occurs between them. Wigston (1971) has scored both characters together using the descriptions given by Cousens (1962) - see Diagram 2.2:

Q. robur: 'strong auricles' - lamina margins strongly reflexed producing characteristic 'points' where the lamina joins the petiole and at the ends of the circular indentation at the base of the lamina; the latter reach and often overlap the petiole on the abaxial surface.

Intermediate: 'medium auricles' - (a) lamina margins strongly reflexed, but point B is 'above' A and/or does not reach or overlap the petiole, and (b) margins weakly reflexed but points produced.

Q. petraea: 'weak auricles' or absent - the leaf base may be cordate with some reflexion of the lamina, but points are never produced.

Although useful in differentiating between the types, the present author feels that the basal shape should be considered separately from the development of the auricle if only to take account of the leaves where extreme expression of one character is observed, but not extreme expression of the other. For example, it is possible to observe leaves

Lobe depth ratio (LDR):

Lobe depth is normally measured as the ratio of the width of the lobe to the depth of the sinus immediately below, eg. Silliman and Leisner (1958). A similar measurement has been used by Tucker (1963) and Maze (1968). Cousens (1961) used measurements across the total width of the leaf from lobe tip to lobe tip for the lobe width and from sinus base to sinus base for the estimate of sinus depth. In this study, the ratio

$$\frac{\text{Width of lobe}}{\text{Depth of sinus}}$$

has been used, for that lobe at or immediately below the widest part of the lamina. Melville (1960a, 1960b) has attempted to fit sine wave curves to the leaf margins of oaks, and this might well prove useful in the future for assessing lobe depth.

Leaf hairiness (HR):

Both simple and stellate hairs are present on the abaxial surface, and although in the population studies discussed in Chapter 5 they are treated as separate characters, for the purpose of this investigation, they are treated as a single character. The character was assessed on a five point scale from 0 (characteristic of Q. robur) through to 4 (characteristic of Q. petraea). The work of Cousens (1961, 1962 and 1963); Jones (1959, 1968); Carlisle and Brown (1965) and Davy (1933) have been used to construct the following five point index:

Index	Description
4	Stellate hairs abundant, such that they produce a completely dense cover to the undersurface of the leaf. There are usually 3 or 4 stellate hairs/'laminar island' (area enclosed by the veinlets). The veinlets, lateral veins and mid-rib may also bear stellate and/or simple hairs.
3	Stellate hairs not as abundant as above. There is usually only 1 per lamina island.

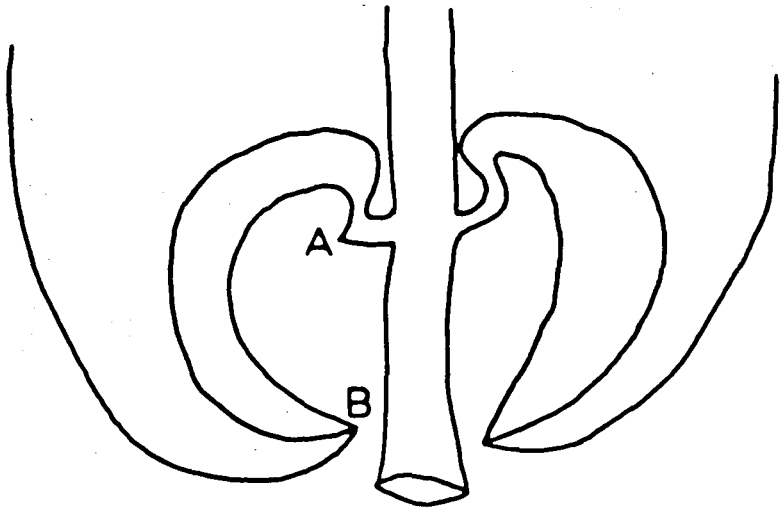


DIAGRAM 2.2

DIAGRAM 2.2 Basal shape of lamina and auricle development - see Text for explanation (redrawn from Cousens, 1962)

DIAGRAM 2.3

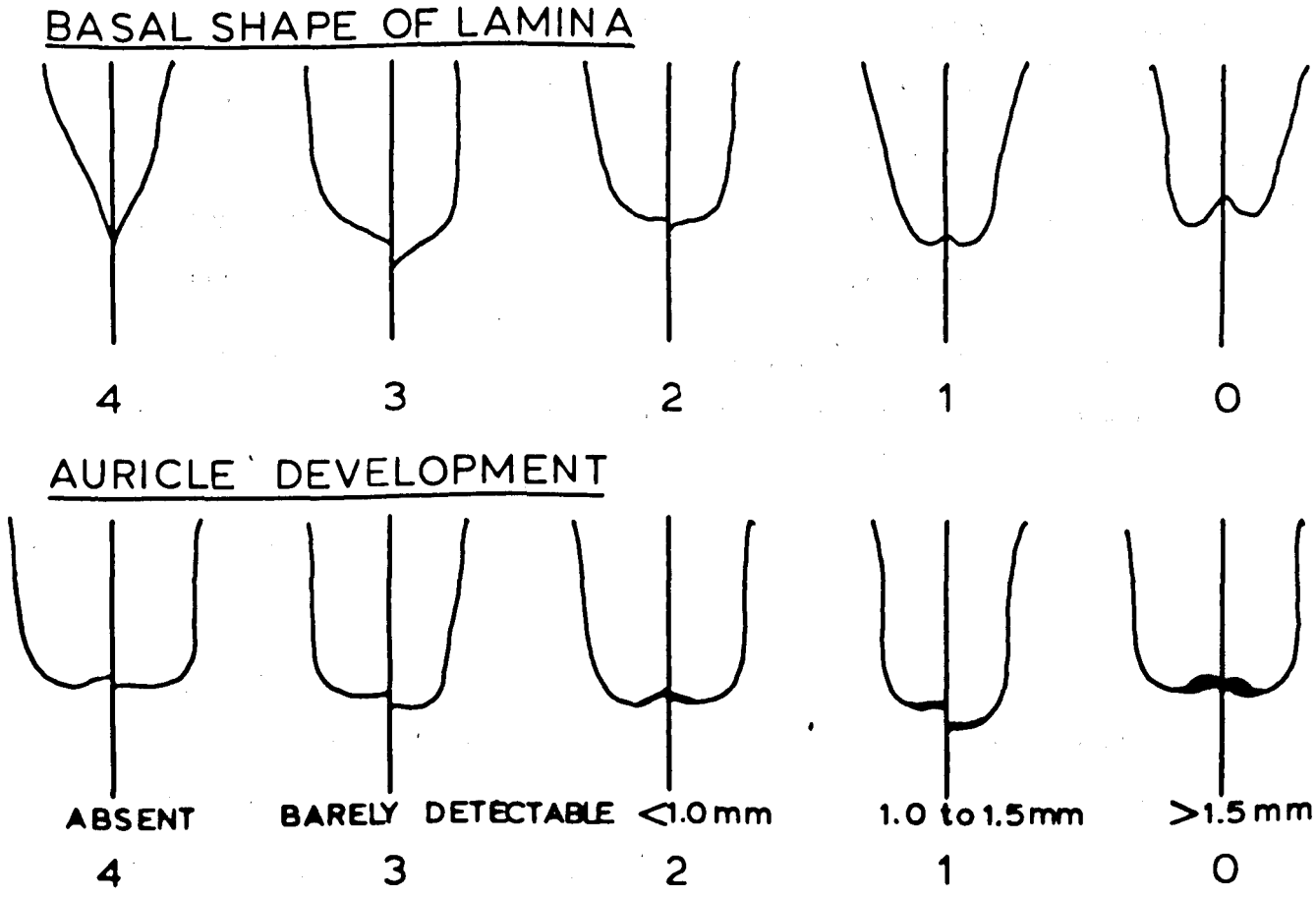


DIAGRAM 2.3 Scoring scheme for auricle development and basal shape of the lamina

which conform to the general basal shape shown in the Q. robur type in Diagram 2.2, but all evidence of an auricle is absent. For this reason, a series of types were set up to illustrate the range of basal shapes found in British oaks. These are shown in Diagram 2.3. For the purposes of this chapter, it is not important whether or not these conform to one species or another, since only the range of variation is being studied, not absolute differences between the species. A similar range of types has been set up for auricle development. The differences between these depend on the amount of tissue that is actually reflexed. These too are depicted in Diagram 2.3.

Anatomical characters have never been used to differentiate Q. robur and Q. petraea, but since the objective of this investigation was to study the range of variation of leaf characters on individual trees, assessment of anatomical characters might prove useful in explaining any patterns found in morphological characters and helpful in suggesting possible connections between the leaves and their position in the canopy. The following anatomical characters were measured: total lamina thickness (TTh), palisade thickness (PTh), spongy mesophyll thickness (MTh), epidermal thickness (ETh) - both upper and lower together, stomatal density (SD) and the number of cell layers in the palisade tissue (CL).

Sampling the oak canopy

As originally conceived, the investigation into leaf variation within the canopy structure was to have compared woodlands of differing tree densities. It proved only possible to sample two types of woodland which will be described as 'open' and 'closed'. The open woodland consisted of trees spatially separated from each other such that there

was at least one canopy diameter between the edges of the canopies of adjacent trees. This in practice was easy to recognise. The closed woodland consisted of trees with overlapping canopies to the extent that approximately 30% of the canopy was overlapped by the canopy of adjacent trees. The sampling was completed over two years, 1969 and 1970, leaves being collected in early August, sufficiently early to avoid possible lammas growth and late enough for leaf expansion to be completed, but unfortunately due to move of Universities, the sites also had to be changed. The following woodlands were used:

Uffmoor Wood - (Grid Reference SO 950 815)

A predominantly Q. robur woodland mixed with birch, ash and beech, with a canopy that was described as 'closed'. Sampling of Q. robur trees in this woodland was carried out during 1969.

Wyre Forest - (Grid Reference SO 745 762)

A predominantly Q. petraea woodland that has been floristically described by Salisbury (1925). Although a variable woodland in terms of both tree density and natural/planted aspects, in several areas conditions approaching those required of the 'closed' type of canopy could be found. Sampling of Q. petraea trees in this woodland was carried out during 1969.

Hetchell Wood - (Grid Reference SE 438 443)

Hetchell Wood lies to the north-east of Leeds. In the surrounding countryside, there are many small woodlands containing oak. This area provided both Q. robur and Q. petraea trees under 'open' canopy conditions. Sampling was carried out during 1970.

Five trees were selected to represent each species and canopy type, i.e. closed Q. robur, open Q. robur, closed Q. petraea, open Q. petraea. On each tree, a small branch was removed from the north side and south side in both full sunlight and deep shade conditions.

Generally, the sun branches were at the edge of the canopy whilst the shade branches were approximately 150 cm. inside the canopy. This sampling was repeated at three canopy heights - 3, 8 and 13 metres - using a combination of ladders and a 4 metre pruning pole. These heights are designated the bottom, middle and upper layers of the canopy. Trees of approximately 15 metres were chosen for sampling, so that the upper samples were in the top part of the canopy, where the diameter of the crown was still reasonably wide and not tapering. Ten leaves were removed from each branch. Five were pressed in herbarium sheets, oven dried and used for examination of morphological characters. Each of the other five leaves was split down the mid-rib, half-fixed under vacuum in F.A.A. (5% formalin, 5% glacial acetic acid, 90% alcohol) for 48 hours and then stored until required in 70% alcohol; the other half was cleared in a 7.5% solution of sodium hypochlorite, washed in distilled water, taken through an alcohol series to absolute alcohol and stored in a mixture of 75% glycerol and 25% absolute alcohol.

In order to assess the effects of pressing and fixing on leaf characters, a number of samples of leaves were measured fresh and after pressing or fixation. The results are presented in Table 2.4. Over a range of leaf characters, both morphological and anatomical, there appeared to be little difference between the percentage change recorded in either species, and it is concluded from this that, although pressing and fixation affect leaf dimensions, the species are affected to the same amount thus preserving any differences apparent before treatment.

The pressed leaf material was assessed for the following leaf characters - basal shape of the lamina (BS), auricle development (AD), hairiness (HR), lobe number (LN), leaf venation (V), leaf area (AR), lamina length (LL), petiole length (PL), petiole ratio (PTR),

Character	Q. robur			Q. petraea		
	Fresh	Pressed/Fixed	% Change	Fresh	Pressed/Fixed	% Change
Leaf length (mm.)	99.8	95.6	- 4.2	123.0	112.7	- 3.9
Petiole length (mm.)	6.56	6.34	- 3.4	21.50	20.79	- 3.3
Lobe depth ratio	2.73	2.56	- 6.2	3.63	3.42	- 5.8
Leaf area (sq. cm.)	33.4	30.4	- 8.9	44.3	39.6	-10.6
Total lamina thickness (μ)	71.8	68.4	- 4.8	76.9	72.6	- 5.6
Stomatal density (per sq. mm.)	237.0	220.0	- 7.4	183.0	167.0	- 8.7
Palisade mesophyll thickness (μ)	31.9	30.4	- 4.8	31.2	29.6	- 5.2

TABLE 2.4 THE EFFECT OF PRESSING AND FIXING ON SOME LEAF CHARACTERS OF Quercus L.

obversity (OB) and lobe depth ratio (LDR). The cleared leaves were scored for stomatal density (SD) at x400. The fixed leaves were hand-sectioned and measurements made of leaf anatomy on the unstained leaf sections - total leaf thickness (TTh), palisade thickness (PTh), spongy mesophyll thickness (MTh), total epidermal thickness (ETh), and the number of cell layers in the palisade (CL). Both position on the leaf (Whitehead and Luti, 1962) and the presence of vein material can affect the anatomical leaf characters. For these reasons, all measurements were made on sections through the middle of the lamina (as measured from edge of the lamina to the mid-rib and from the tip to the base) and in sections devoid of lateral vein material. Counts of stomata were also prepared on parts of the lamina closest to the middle that contained no underlying vascular tissue. Five measurements were made on each leaf section and five counts of stomatal density completed on each cleared leaf so that mean values for each anatomical character could be calculated for each leaf.

It had originally been decided to study peduncle and acorn characters as well as leaf material, but this proved impossible. The very poor acorn production in 1970 (see Chapter 8) coupled with difficulties in detecting acorns whilst sampling in the middle and upper canopy resulted in very poor collections of fruit material. The results from these were inconclusive due almost certainly to small sample sizes and possibly to some variation in sampling time, and have therefore not been included here.

Light intensity measurements were made at each canopy site for the different trees in the different woodlands. These were carried out using a dome solarimeter, on the ecologist's standard 'overcast' day (Fairbairn, 1954), alternate readings being taken in position and in the open, away from the shading influence of the trees. The

results are expressed as the quantity of light at the position as a percentage of the light in the open.

Results and analyses

Three extra characters have been derived from the character measurements described in the previous section:

$$\text{Palisade ratio (PR)} - \frac{\text{Palisade thickness}}{\text{Total lamina thickness}} = \frac{\text{PTh}}{\text{TTh}}$$

$$\text{Mesophyll ratio (MR)} - \frac{\text{Spongy mesophyll thickness}}{\text{Total lamina thickness}} = \frac{\text{MTh}}{\text{TTh}}$$

$$\text{Epidermal ratio (ER)} - \frac{\text{Total epidermal thickness}}{\text{Total lamina thickness}} = \frac{\text{ETh}}{\text{TTh}}$$

The means and standard deviations for each of the twenty characters for the different canopy sites are given in Appendix 1. Results for the five trees representative of each canopy type were generally similar (analyses of variance not presented here showed no significant differences between the five trees of each canopy type for the twenty different characters) and for the tables in Appendix 1, the results have been pooled. The results for each character have been subjected to a three-way analysis of variance in which the main effects are canopy sites, i.e. aspect, sun and shade; canopy level, i.e. upper, middle and lower levels; and species, i.e. Q. robur and Q. petraea for the two types of forest (open and closed canopies). A least significant difference of means has been calculated for each character and is presented along with the analyses of variance in Appendix 1. The measurements of light intensity are recorded in Table 2.5 and Figure 2.1 shows the changes of light intensity with canopy height.

	Closed canopy		Open canopy	
	Q. robur	Q. petraea	Q. robur	Q. petraea
SUSu	76	78	87	91
SMSu	32	36	64	54
SLSu	24	25	56	48
SUSh	40	32	50	43
SMSh	20	18	26	25
SLSh	18	17	23	20
NUSu	48	38	59	55
NMSu	22	24	39	34
NLSu	13	14	27	26
NUSh	25	19	29	27
NMSh	14	7	16	12
NLSh	9	6	15	10

TABLE 2.5 LIGHT INTENSITY IN DIFFERENT OAK CANOPIES MEASURED AS A PERCENTAGE OF LIGHT INTENSITY UNDER OPEN CONDITIONS

Key:
S - South aspect
N - North aspect
Su - Sun position
Sh - Shade position
U - Upper canopy level
M - Middle canopy level
L - Lower canopy level

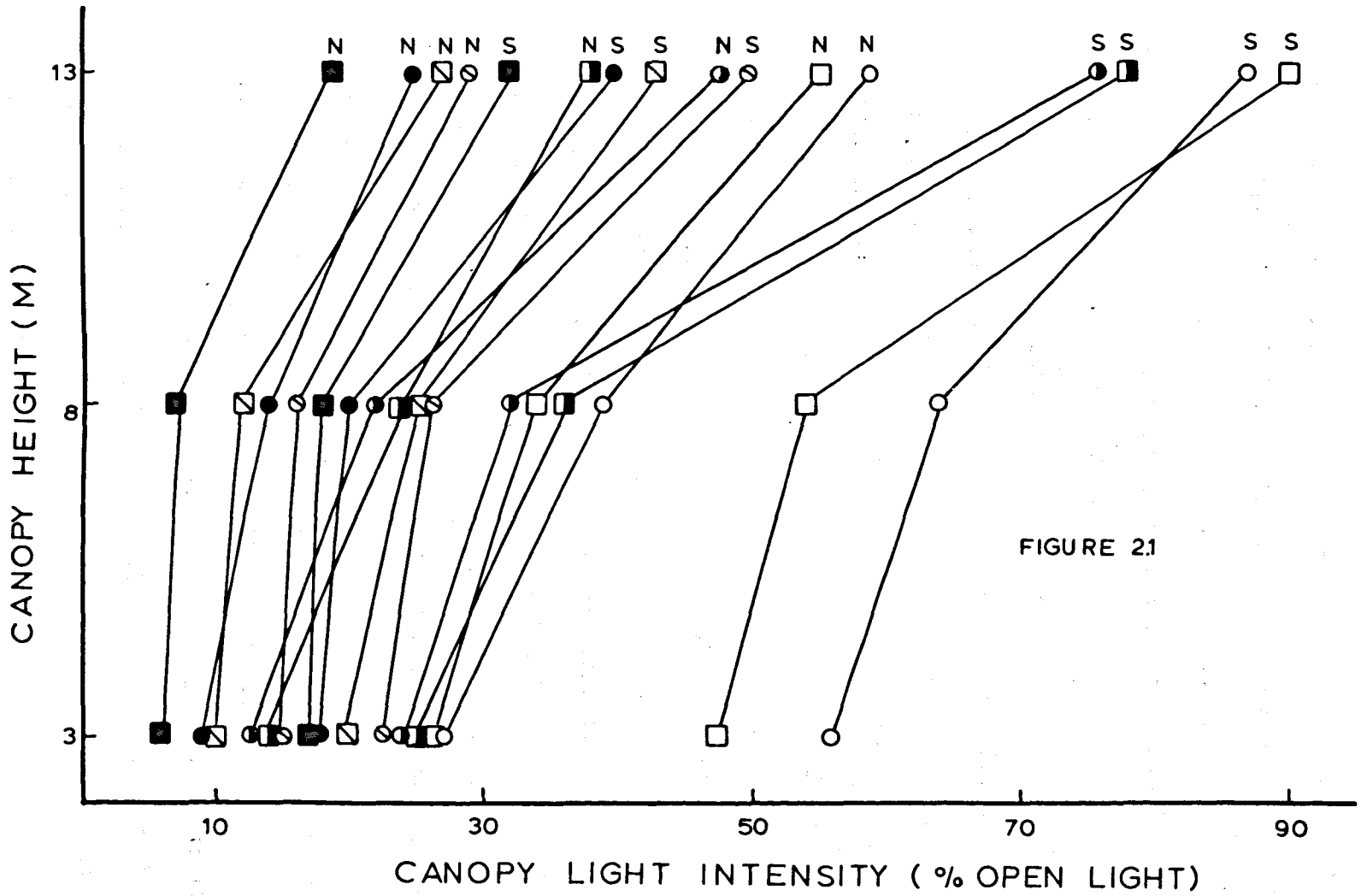


FIGURE 2.1

FIGURE 2.1 Light intensity expressed as a percentage of light intensity under open conditions at different canopy heights and different canopy sites
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◨ PSC; ■ PShC
N = north aspect, S = south aspect of canopy)

Discussion

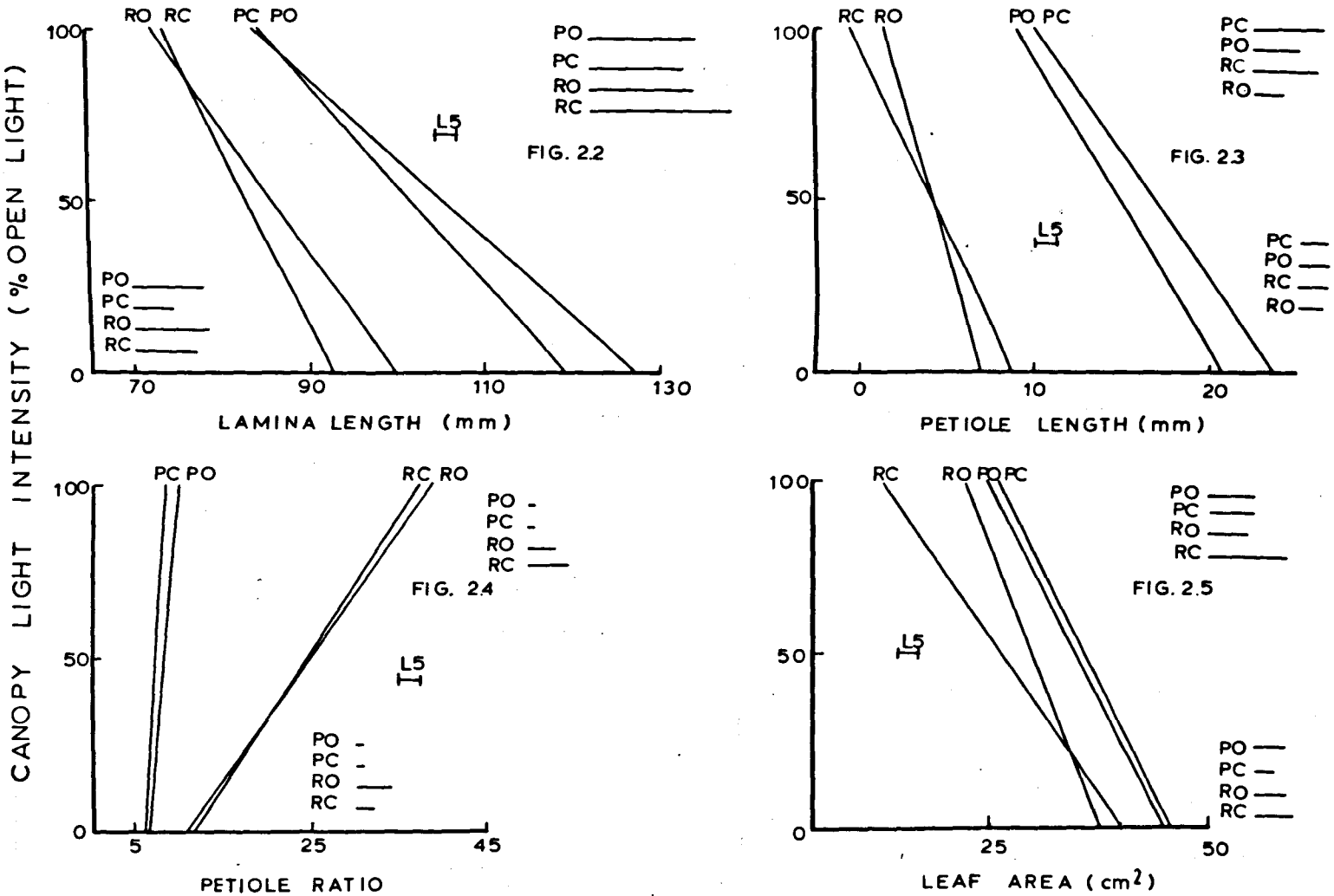
The characters measured in this investigation can be considered in two ways - as varying with canopy height or varying with light intensity. The latter is more instructive since the same canopy height will produce different light intensities depending on the species and openness of the canopy (see Table 2.5). Consequently, this discussion will firstly consider the variation of the measured characters with light intensity.

Figures 2.2 to 2.21 show the response of all characters to changes of light intensity. In Figures 2.2 to 2.21 individual points have not been plotted, only the calculated linear regression lines for each species and each canopy type. This data has been tested for the fitting of quadratic, cubic and quartic lines using a computer program developed jointly with Dr. T. V. Callaghan, University of Manchester, but few characters gave significant curvilinear fits, and in those which were significant, the linear regression gave the better fit. Characters MTh and ER failed to give significant linear regressions, but for comparative purposes, the calculated regression lines have been drawn in Figures 2.15 and 2.21. Figures 2.2 to 2.21 also include the calculated standard errors to the lines at 100% light intensity and 0% light intensity.

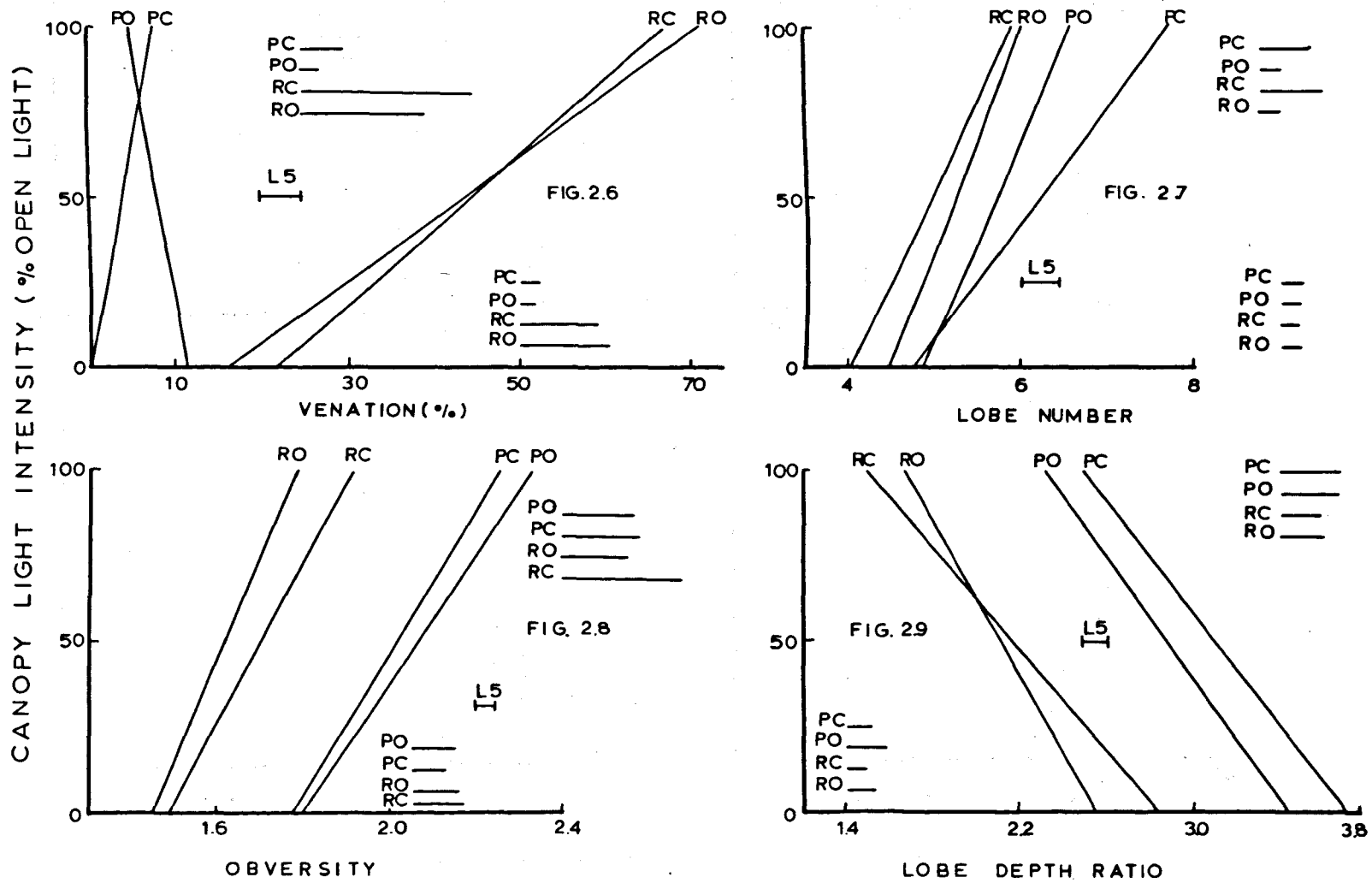
The twenty characters can be divided into four groups with regard to their response to changes in light intensity:

a) Light neutral - these are characters which appear to remain constant with changing light intensity and show no significant correlation with light intensity. Both MTh and ER can be described as light neutral characters.

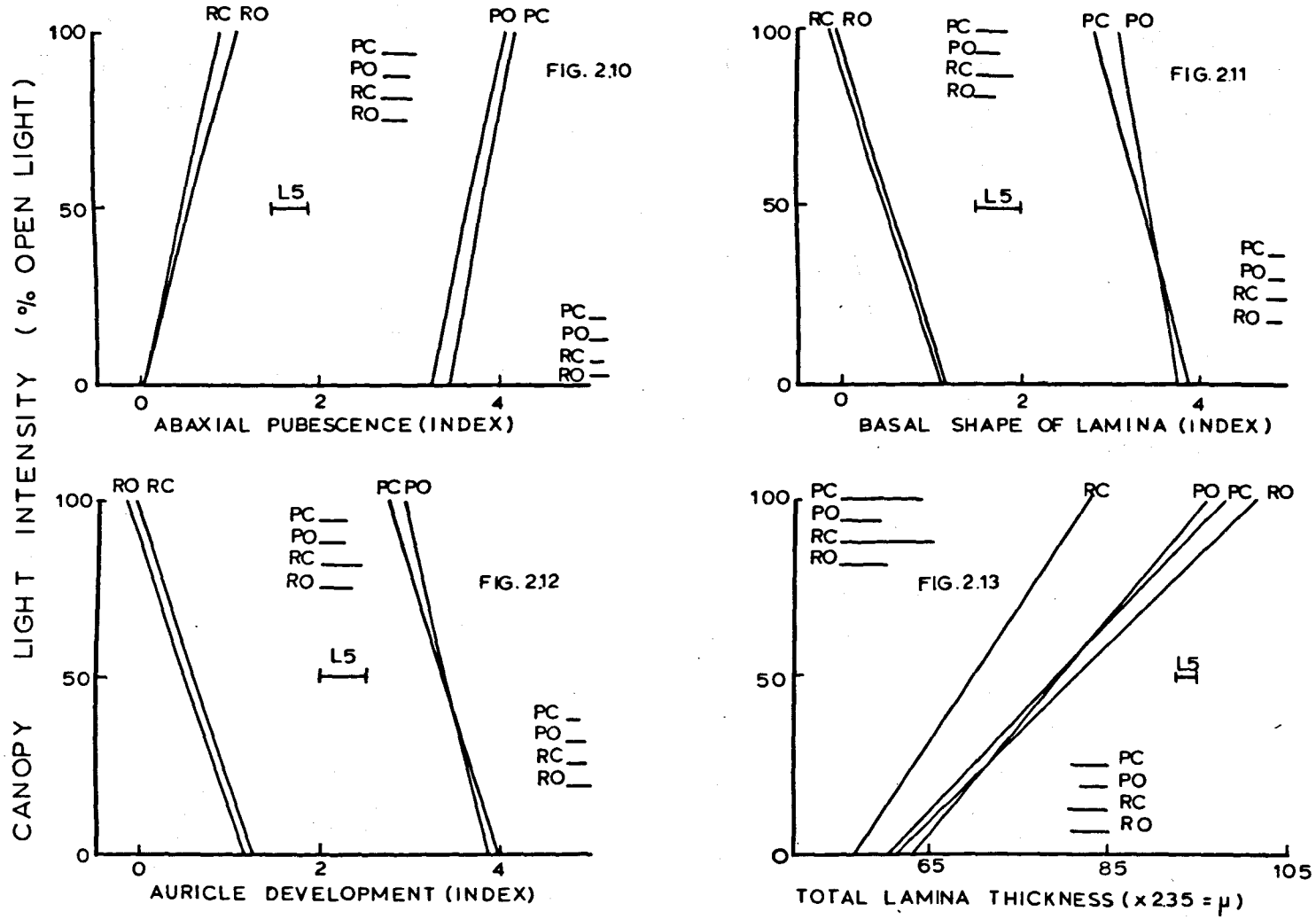
b) Light positive, continuum - these are characters which follow the light gradient in a linear fashion, but which fail to differentiate



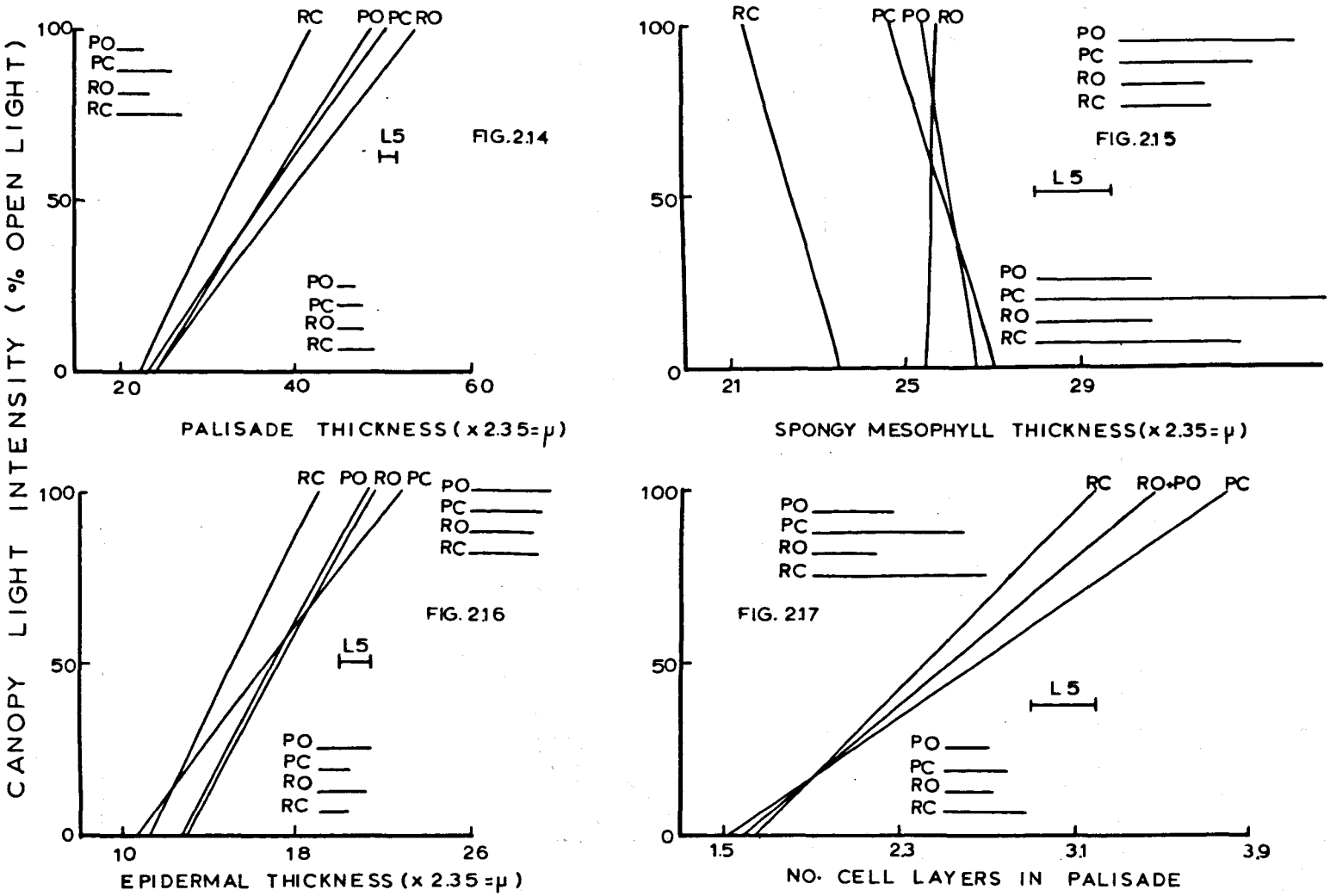
FIGURES 2.2-2.5 Variation in leaf characters with canopy light intensity; lamina length, petiole length, petiole ratio and leaf area respectively. The graphs show the calculated linear regressions for the four woodland types (open and closed, Q. robur and Q. petraea) together with the standard errors to the lines at 100% and 0% light intensities and the least significant difference of means (L5).



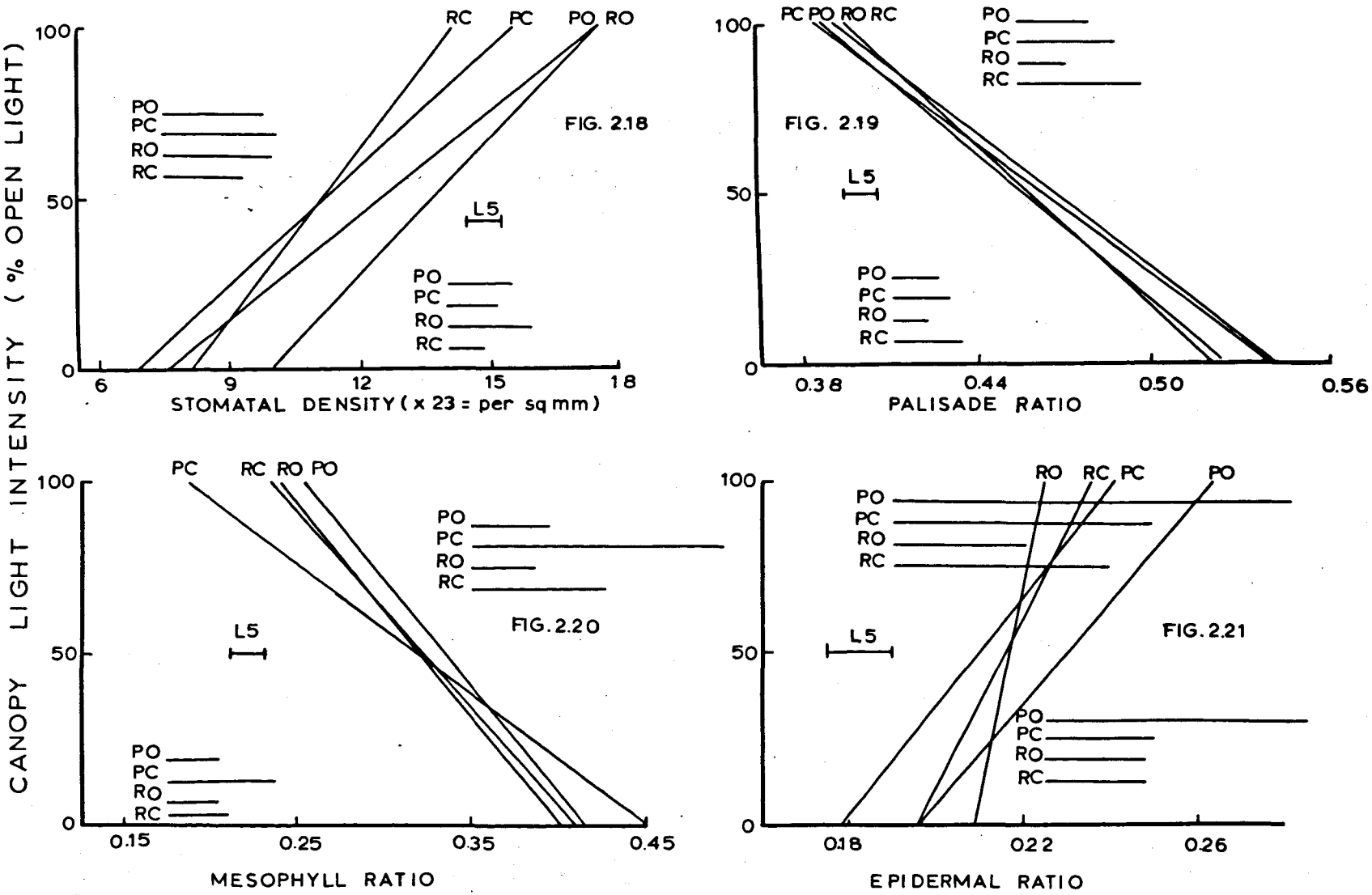
FIGURES 2.6-2.9 Variation in leaf characters with canopy light intensity; venation, lobe number, obversity and lobe depth ratio respectively. The graphs show the calculated linear regressions for the four woodland types (open and closed, Q. robur and Q. petraea) together with the standard errors to the lines at 100% and 0% light intensities and the least significant difference of means (L5).



FIGURES 2.10-2.13 Variation in leaf characters with canopy light intensity; abaxial pubescence, basal shape of the lamina, auricle development and total lamina thickness respectively. The graphs show the calculated linear regressions for the four woodland types (open and closed, Q. robur and Q. petraea) together with the standard errors to the lines at 100% and 0% light intensities and the least significant difference of means (L5).



FIGURES 2.14-2.17 Variation in leaf characters with canopy light intensity; palisade thickness, spongy mesophyll thickness, epidermal thickness and number of palisade cell layers respectively. The graphs show the calculated linear regressions for the four woodland types (open and closed, Q. robur and Q. petraea) together with the standard errors to the lines at 100% and 0% light intensities and the least significant difference of means (L5).



FIGURES 2.18-2.21 Variation in leaf characters with canopy light intensity; stomatal density, palisade ratio, spongy mesophyll ratio and epidermal ratio respectively. The graphs show the calculated linear regressions for the four woodland types (open and closed, Q. robur and Q. petraea) together with the standard errors to the lines at 100% and 0% light intensities and the least significant difference of means (L5).

clearly between the species, all measurements lying close to the regression line. Characters showing such a response are TTh, ETh, PTh, CL, SD, MR and PR.

c) Light positive differential - characters showing this variation are OB, PTR, LDR, HR, PL, AD and BS. Although showing variation with light intensity in a linear fashion, there is nevertheless little overlap between the species, so that at all light intensities, the leaves of both species remain distinct.

d) Light positive partial differential - four characters, LL, AR, V and LN show this type of variation. For these characters, the species show the same responses to changing light intensity, but at different rates so that at one light intensity, the species show the same expression of the character, but at a different light intensity, differences can be seen in the expression of the character. The two clearest examples of this type of character can be seen in V (Figure 2.6) and LL (Figure 2.2). In LL, at 100% light, although Q. petraea shows the longest LL, the standard errors are large, and the ranges overlap the LL of Q. robur. At 0% light intensity, the species are clearly separated. V follows the same pattern, except here, it is at low light levels at which overlap occurs, the species being well separated at high light intensities. LN and AR are not as clear, but it is thought that both probably belong in this group rather than in the light positive continuum group. At high light intensities, there is overlap of the ranges of AR as seen in Figure 2.5. At lower light intensities, the amount of variation in each species is less, and the species are clearly separated. In LN (Figure 2.7), at high light intensities, the species are separated due partly to the low variation observed in the 'open' canopy stands. At lower light intensities, a small degree of overlap occurs particularly between the Q. petraea

woodlands and the open Q. robur woodland.

Such a 'classification' of characters is very useful in taxonomic studies, since one can distinguish those characters that always differentiate the species, i.e. 'good' characters - the light positive differential group, those characters which may differentiate the species, i.e. the light positive partial differential group, and those characters which fail to differentiate the species, i.e. the light neutral and light positive continuum groups, taxonomically useless characters.

Let us now examine more closely the variation exhibited by the characters.

Characters traditionally regarded as being useful in distinguishing the species are AD, HR, BS, PL and PTR. AD and also BS show little variation with respect to light intensity (Figures 2.11 and 2.12), although for both characters, in both species, the leaves at lower light intensities and consequently at the lower canopy levels are more 'Q. petraea-like', the difference in both species being approximately one unit on the 0-4 scale between the 100% and 0% light intensities. Leaf hairiness, HR, follows a similar trend, with very little, but significant change with light intensity, except in this instance, the lower light intensity leaves are less hairy and therefore more 'Q. robur-like'. PL varies considerably from the high to lower light intensities, particularly in Q. petraea (mean 11.06 mm. at open south upper sun and mean 25.06 mm. at closed north lower shade) but also in Q. robur (mean 2.07 mm. at open south upper sun and mean 9.80 mm. at closed north lower shade). However, the integrity of the species is maintained (see Figure 2.3). When corrected for leaf length, i.e. PTR, the variation in Q. petraea is much less apparent (see Figure 2.4), whilst Q. robur varies greatly between the higher and lower

light intensities.

The other morphological characters, OB, LL, AR, LN, V and LDR vary in similar fashion. Q. robur leaves are generally shorter, smaller, with fewer deeper lobes, more intercalary veins and more obovate in shape. Although both species show variation in V, only Q. robur leaves show large changes in V with decreasing light intensity.

Anatomically, the Q. robur leaves are thicker in open canopy, sun situations, but have much thinner leaves in closed canopy, shade situations than Q. petraea. A parallel difference can be noted in the character PTh and possibly in MTh. When these characters are expressed as ratios of the total lamina thickness, PR and MR, it can be seen from Appendix 1 that the PR of Q. robur is always greater than that of Q. petraea for comparable sites and that the MR of Q. robur is always less than that of Q. petraea for comparable sites. ETh, although declining with lowered light intensities, shows few consistent differences between the species and this is also true for the character when expressed as ER. SD decreases with decrease in light intensity, and as Figure 2.18 shows, the stomatal density is highest generally in open situations. Such changes of SD may well result, however, from the decreased leaf area since the leaves in more open situations are smaller (see Figure 2.22). The number of layers of cells in the palisade tissue - CL - decreases with lowered light intensity, but it would appear that changes in palisade thickness are due almost entirely to changes in the number of cell layers (average length of palisade cells at the lower level of the canopy 37.6 μ, average length at the upper level of the canopy 33.6 μ).

Table 2.1 lists the characters typical of sun leaves and shade leaves, and it is interesting to compare the expression of characters

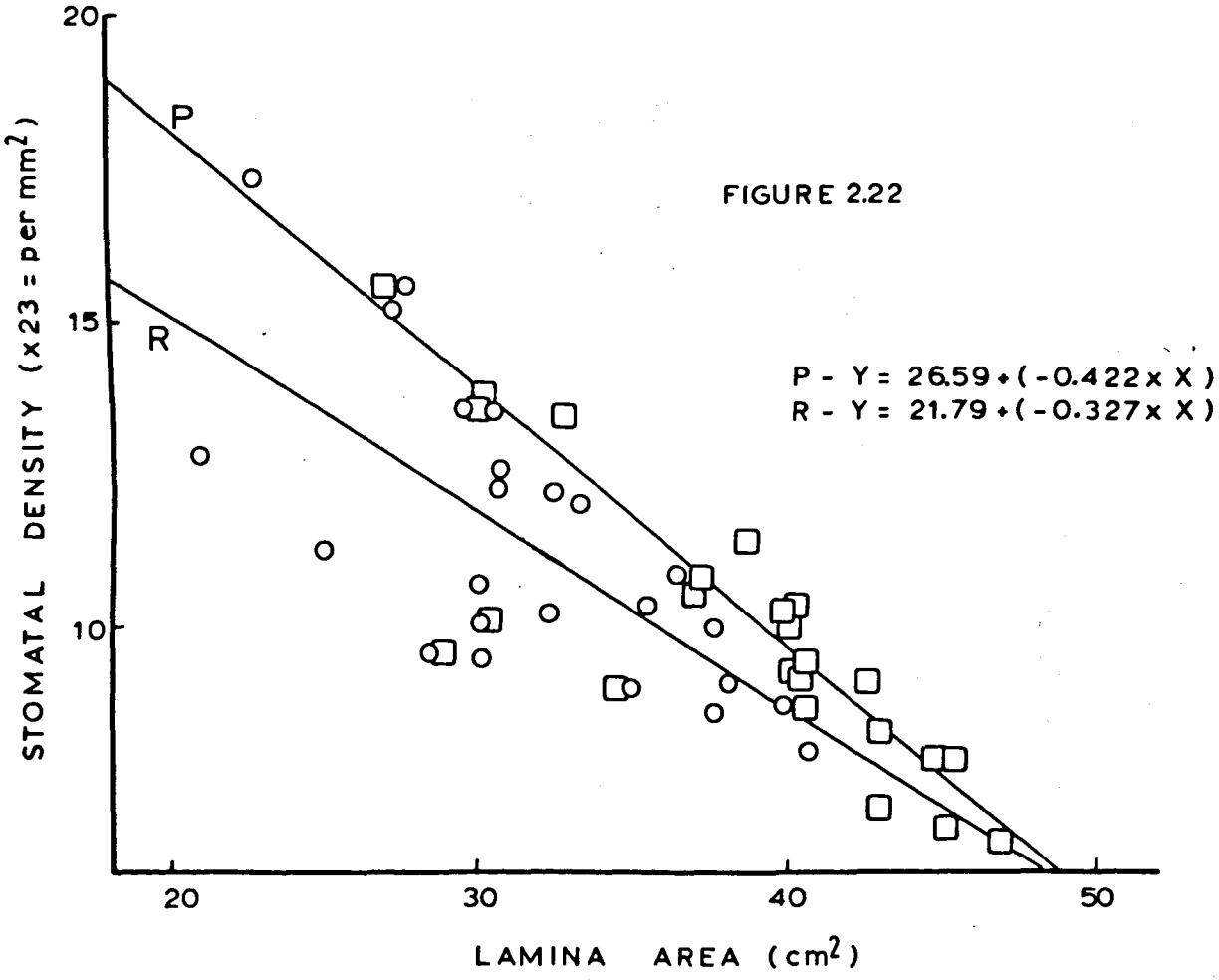


FIGURE 2.22 Correlation between stomatal density and leaf area for leaves at different canopy sites
(O Q. robur; □ Q. petraea)

noted here for British oaks with Table 2.1. Sun leaves have the following character expressions: smaller, thicker leaf blades, higher stomatal densities, more hairs, deeper lobed, better developed palisade, weakly developed spongy mesophyll, and well developed xylem. Q. robur certainly conforms very closely to this description, the leaves of Q. petraea conforming more to the shade leaf description. The leaves of Q. robur (in comparison to Q. petraea) are smaller both in terms of leaf length and leaf area, are thicker (at high light intensities), generally have higher stomatal densities, much deeper lobes, a thicker palisade layer (at high light intensities), a poorly developed spongy mesophyll (except at very high light intensities), and the presence of large numbers of intercalary veins particularly at the higher light intensities must serve to increase the amount of xylem in the leaf. Only leaf hairiness fails to conform to the sun leaf description, but it must be remembered that Q. robur leaves are generally described as glabrous. It is important to note, however, that at higher light intensities, the amount of leaf hairiness is increased. It should be expected, therefore, that Q. robur should show some of the physiological attributes expected of a sun leaf. Some aspects of this are explored in Chapters 9 and 10.

Light intensity has been measured under different oak canopies in some earlier studies. Fairbairn (1954) reported preliminary studies of light intensity under sessile and pedunculate oak. He was interested in comparison of different methods of light intensity measurement. When measuring light along lines in forests, the percentage of light under Q. robur compared to that in the open was 24%, whilst under Q. petraea it was 17%. Measurements at breast height under individual trees gave 8% and 7% for Q. robur and Q. petraea respectively when compared to measurements in the open.

Consequently, light intensity was lower under Q. petraea trees and canopies. Similar results have been described in this investigation (see Table 2.5). Ovington and Madgwick (1955) give similar results but for Q. petraea only.

It should be noted, however, that light intensity is not the only environmental variable to change through the canopy structure (see 'introduction' to this chapter) and the differences described here, although attributed to differences in light intensity, are almost certainly due to a combination of light and other factors (Isanogle, 1944). For example, wind speed is an important variable in canopy structure (Cionco, 1965) and it has been shown to have an important effect on leaf anatomy, eg. Whitehead and Luti (1962) - Zea mays, and Whitehead (1962) - Helianthus annuus, and also on leaf transpiration, eg. Woolley (1961) and Hygen (1954).

The differences recorded here for variation in leaf characters have been noted in other tree species. Wylie (1949) was able to show that in an isolated crown of Acer platanoides, the leaf lamina was thicker on the south aspect as also were total epidermal thickness, spongy mesophyll thickness and palisade thickness. Results for Quercus would agree for total lamina thickness, palisade thickness, total epidermal thickness, but not spongy mesophyll thickness. It should be noted that Daubenmire (1974) regarded a well developed spongy mesophyll as characteristic of shade leaves and is thus at variance with the results of Wylie (1949). Wylie (1951) later widened his survey to include ten species of dicotyledonous deciduous trees, including two American oak species, Quercus macrocarpa and Q. velutina. The blade thickness was much greater on the southern periphery of the crown compared with the northern periphery, and this in turn was much greater than the interior of the crown (eg. Q. velutina: South periphery 190 μ, North periphery 166 μ, Interior crown, 98 μ). Epidermal thicknesses (both upper and lower),

spongy mesophyll and palisade thickness all showed similar differences. Veins on the leaves from the southern periphery were also much closer together than the other two sites. These differences in venation are good evidence for regarding Q. robur as having a sun leaf. Isanogle (1944) has used artificial shading conditions to study the effect of shading on leaf development. Leaves developed from unshaded buds were different from leaves from shaded buds on the opposite side of the stem by being generally thicker with thicker epidermis, palisade and spongy mesophyll. The palisade thickness was not, however, due to more cell layers as is the case in this study but due simply to more elongate cells. Korstian (1925); McDougall and Penfound (1928); Penfound (1931); Cormack and Gorham (1953); Cormack (1955) and Jackson (1967) have shown similar results in a series of plant species ranging from conifers through forest herbs and shrubs to angiosperm forest trees.

There have been some attempts to relate anatomical leaf structure to physiological behaviour. Marsh (1941) found that sun leaves had between 1.1% and 11.6% lower water content than shade leaves of the same species. Osmotic pressures of sun leaves were 2.1-5.4 atmospheres higher than those of the shade leaves. Marsh (1941) concluded that as transpiration from sun leaves is more rapid, and photosynthate was as abundant in shade leaves as in sun leaves, this accounted for a higher concentration of osmotically active substances found in the sun leaves. Pieters (1960) has investigated the relationship between the rate of photosynthesis and the thickness of the mesophyll of sun and shade leaves of Acer pseudoplatanus. Using leaves of different thicknesses, there was a clear positive correlation between maximum rate of photosynthesis and thickness of the palisade tissue. In a second experiment, he grew seedlings under three light intensities (100%, 80% and 50% of daylight). Although there were some differences between the treatments, the 100%

treatment having a generally higher maximum rate of photosynthesis, there was little difference in the palisade thickness of the leaves. The results of the present investigation would suggest that shade conditions occur below at least 50% light intensity (see Table 2.5) and consequently, true shade leaves probably did not develop in Pieters' experiment. McClendon (1962) has also tried to relate the thickness of deciduous leaves to their maximum photosynthetic rate in 23 species including Q. robur. The thickness in this instance was measured as thickness density, i.e. gms/cm^2, and a linear relationship was found between this and the maximum photosynthetic rate. Ansari and Loomis (1959) investigating leaf temperatures have shown that thinner and in consequence shade leaves heat up much faster in sun conditions than thicker sun leaves.

Fekete and Szujkó-Lacza (1973) have recently published the results of an investigation that parallels very closely the methods presented here. They were interested in the relationship of leaf anatomy and photosynthetic activity to environmental factors in the oak, Quercus pubescens. At present, only the results of the anatomical work have been published; the results of the photosynthetic work might well add to our understanding of leaf variation in oaks. Larcher (1960) has measured transpiration and photosynthesis of Q. pubescens and Q. ilex but no differences have been recorded between sun and shade leaves.

Some workers have studied differences between the lobing of sun and shade leaves. Talbert and Holch (1957) have recorded the lobing of sun and shade leaves of a wide range of species including Q. gunnisonii, Q. stellata, Q. macrocarpa, Q. velutina, Q. gambelii, Lactuca canadensis and Viburnum trilobum. In all, the study considered 10 herbs, 10 shrubs and 17 trees. Fifty-seven per cent of the perimeters of the shade leaves were larger than those of the sun leaves,

and 98% of sun leaves were more deeply lobed than the shade leaves. The sun leaves were hairier with more prominent veins and shorter petioles. In the present investigation, these differences were also noted.

Attempts have been made by Vogel (1968, 1970) to relate the shape of leaves to their convective cooling potential. Using leaf-shaped copper plates with thermistors, he measured the convective cooling when the leaf was at 15°C above ambient temperature and in wind speeds of <1, 10 and 30 cm/sec. and found that under all conditions, models of the sun leaf of Q. alba dissipated more heat per unit area than the shade leaf model of the same species.

It would seem, therefore, that two general conclusions can be reached from the results presented here:

1. Variation of leaf morphology and anatomy in oak canopies is great, and any sampling of oak populations must carefully take account of such variation, and attempt to minimise it. The fact that such variation exists must also be accounted for when assessing variation exhibited by population samples.
2. The anatomical and morphological differences observed probably have underlying physiological significances and if, as has been reported in the literature, the hybrid tree is morphologically intermediate between the two species, then it might also show physiological differences from either parent which would have subsequent implications in the habitat preferences of the hybrid.

CHAPTER THREE

THE OAK CANOPY 2

Introduction

The researches presented in Chapter 2 suggest that plastic modifications of the phenotype of oak are quite substantial, but this does not represent the whole picture. The differences between 'sun' and 'shade' leaves represent only the range of modification observed (although not possible) but not the basic leaf type of which sun and shade leaves are modifications. This basic leaf type will be described as the 'neutral' leaf - it is, of course, conceivable that the sun leaf or indeed the shade leaf may be the 'neutral' leaf. The concept of the neutral leaf is an interesting one, but one that is difficult to define. In the purest sense, the neutral leaf probably represents the leaf as defined by the genetic system of the plant, i.e. in the equation $P = G+E+I$ where P = phenotype, G = genotype, E = environment, I = interaction between genotype and environment, the neutral leaf would be that phenotypic leaf P when $E = 0$ and $I = 0$. However, this is not a good practical definition, since it is not amenable to experimentation. The leaf in the overwintering bud could be regarded as the neutral leaf, although it must be remembered that the overwintering bud is subject to external environmental influences that may be important in determining the final form of the leaf. Differences in the structure of the neutral leaf of the two species would represent real differences between the species, not differences that may be determined by environmental factors as in the results produced in the previous chapter.

There is some evidence that the external environment has little effect on overwintering buds. Cormack and Gorham (1953) give reasons for believing that the differences of leaf expression between sun and

shade leaves are not predetermined by the environment of the bud during its formation the previous season. Their work was completed on two shrub species, Menziesia glabella and Lonicera glaucescens. Hansen (1961) regarded the sun and shade leaves of Hedera helix to be the result of the illumination under which the leaves unfolded. In Fegus sylvatica, however, Hansen (1959) showed that although the structure of the internal tissue of the leaf, particularly the palisade tissue, depends on the light intensity during unfolding, the leaf size and structure were determined by the light intensity experienced by the bud before unfolding of the leaves. Anderson (1955) investigating Cornus florida L. and Viburnum prunifolium L. showed that the embryonic leaves of both sun and shade plants were basically alike in cell structure and this too lends support to the possible non-involvement of environmental factors in deciding the unexpanded leaf structure of the overwintering bud. Isanogle (1944) reported that in Cornus florida rubra and in Acer platanoides the number of cell layers in the embryonic leaves were six and five respectively, and this was not dependent on the position of the tree from which the buds were removed. She concluded from this that at least as far as the number of cell layers was concerned, differences of environment had no influence. Mitchell and Soper (1958) found that different regimes of light and temperature had little effect on the dimensions of the stem apex, and that this in turn had little influence on final leaf size of both Lolium perenne and Paspalum dilatatum. Some work does point to the contrary however. Bötticher and Behling (1939) reported that leaves from 'sun' buds of trees were more numerous, with a higher fresh weight, higher dry weight and ash content than leaves from 'shade' buds. Similarly Wieckowska (1970) believed that in the leaves of Ulmus laevis, factors influencing leaf shape were operative at the bud stage and during leaf primordia formation. Later work by

Wieckowska (1972) on Fagus sylvatica showed that venation appeared in leaf primordia in late July (when presumably they would be influencing sun or shade conditions) and that if primordial formation was induced earlier, both leaf shape and venation were irregular. Cameron (1970) was able to show that in a range of characters, morphological, anatomical and physiological, all were influenced by light intensity in juvenile and intermediate leaves.

Anderson (1955) notes that both the time and manner in which the characteristic differences between sun and shade leaves appear are not well known. If, as seems possible, the external environment has little effect on the morphology and anatomy of unexpanded leaves in over-wintering buds, then the study of the unexpanded and expanding leaves will give good evidence of the structure of the 'neutral' leaf. This chapter examines the expansion of oak leaves in an attempt to show when and how the differences between sun and shade leaves appear, but also to attempt a description of the neutral leaf of the two oak species.

Sampling and characters

The woodlands used for following the expansion of leaves were the same as those used in the previous chapter, i.e. Uffmoor Wood for 'closed' canopy Q. robur, Wyre Forest for 'closed' canopy Q. petraea and Hetchell Wood and surrounding area for 'open' canopy Q. robur and Q. petraea. Of paramount importance in this investigation was to obtain trees at the same stage of leaf expansion, which entailed close monitoring of forests to determine when trees came into leaf. The range of variation in such times can be quite great. Jones (1959) records a difference of two or three weeks between individuals of the same population coming into leaf and notes that where populations are mixed, there are few differences in time of leaf of the two species. During

late March and April, a number of trees were observed for bud swelling, and finally five of each species in each woodland type were chosen as the experimental trees. Assessment of all canopy sites for all characters would have been a quite considerable undertaking, and consequently only two sites were chosen on each canopy - the north lower shade and the south lower sun positions. This enabled sampling to be carried out expeditiously since sampling was at the lower canopy levels, but from such stations, large differences could be expected. The trees chosen were generally not the ones later used for extensive canopy sampling as the sampling for leaf development and canopy differences might have placed too heavy a burden on the trees since sampling is of necessity destructive. The unfortunate difference between years 1969 and 1970 noted earlier in Chapter 2 also applies, but careful measurements of bud swelling during March and early April probably ensured that the 1970 samples were chronologically matched to the 1969 samples.

From each canopy site, twenty leaves were removed. Ten leaves were pressed and ten leaves were halved, one half being fixed in FAA, the other being cleared in a sodium hypochlorite solution as described in Chapter 2. Sampling was carried out every six days until ten samples had been completed, i.e. the last sample was 54 days after the first and occurred sometime in the middle of June. By the time of the last sample, all characters at all sites had stabilised.

The pressed leaf material was assessed for morphological leaf characters - AD, BS, HR, LN, AR, LDR, LL, PL, PTR, OB and V, the cleared leaf material for SD and the fixed leaf material for TTh, MTh, PTh, ETh and CL as described in Chapter 2. PR, MR and ER have been calculated from these results also as described in Chapter 2.

Light intensity measurements were carried out on each sampling date, at each site using a dome solarimeter. Light measurements were

completed outside the canopy so that all canopy light measurements could be expressed as a percentage of open conditions.

Results

The results are presented in Appendix 2, which lists the means and standard deviations for the canopy sites and different forests for the twenty characters. The results for each character have been subjected to a three-way analysis of variance in which the main effects are light conditions, i.e. open, closed, sun and shade; species (Q. robur and Q. petraea); and time (the ten sampling dates). A least significant difference of means has been calculated from the analyses of variance for each character. The variation of light intensity with time is graphed in Figure 3.1, and the change in some character states over time is shown in Figures 3.2 to 3.10.

Discussion

Figure 3.1 records the change in light intensity over the seven weeks of the experiment. As expected, the light intensity at the beginning of the sampling period was much higher for all stations than the light intensity measurements made after the canopy had closed (in Chapter 2). As the canopy closed, so light intensity dropped until it reached a steady level which represents the state of the forests at which canopy cover is complete. Nevertheless, even at the first sampling, light intensities were very different, reflecting the shade conditions caused simply by twig and branch density. In sun open canopy conditions, the light intensity reached a steady state after the fourth sampling period but it is noticeable that under more shade conditions, the time taken to reach the steady state is much longer, eg. closed, shade Q. robur takes some 42 days (i.e. at the 7th sample)

FIGURE 3.1

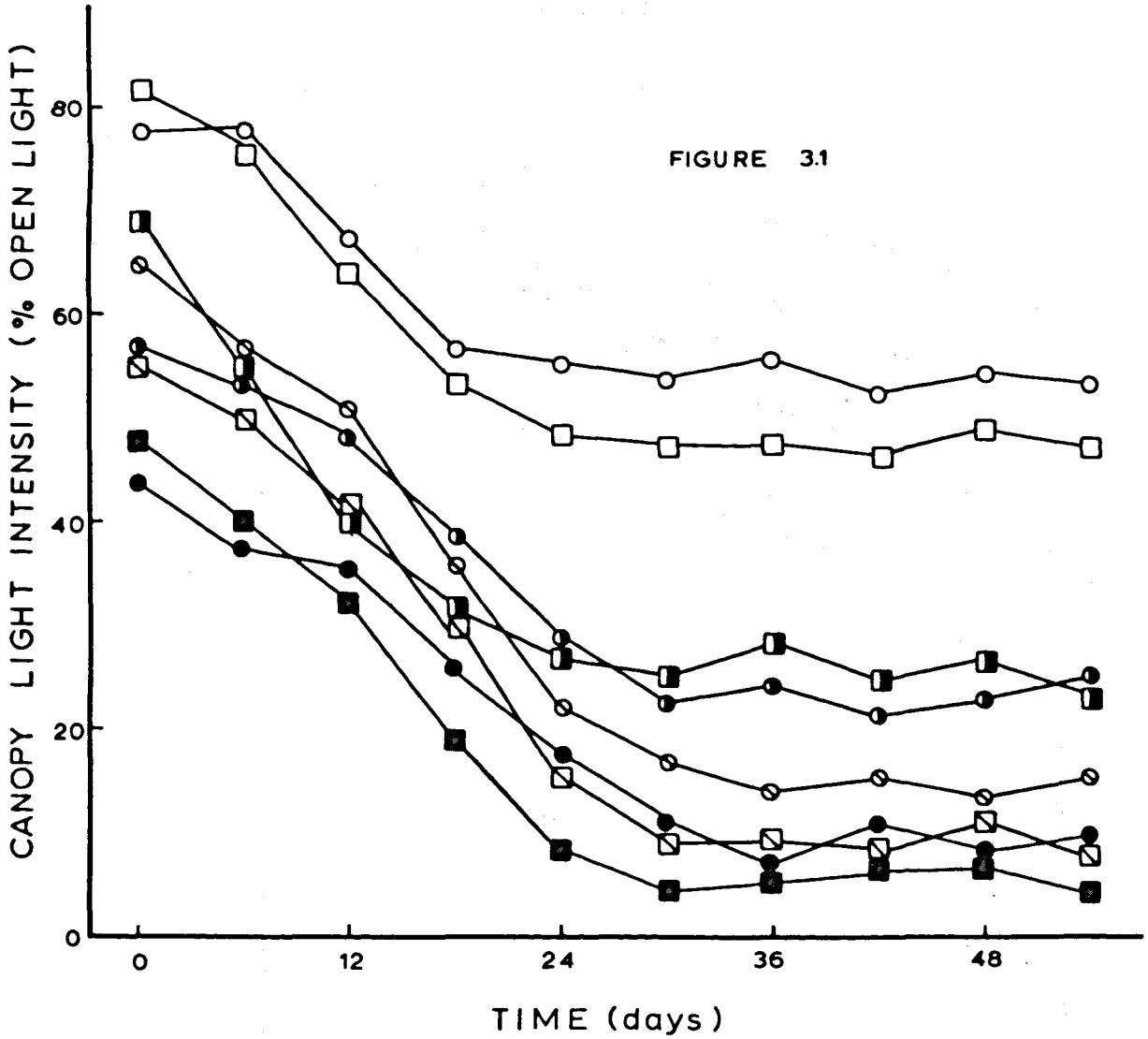


FIGURE 3.1 Changes during leaf expansion: Light intensity, expressed as a percentage of light intensity under open conditions
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□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

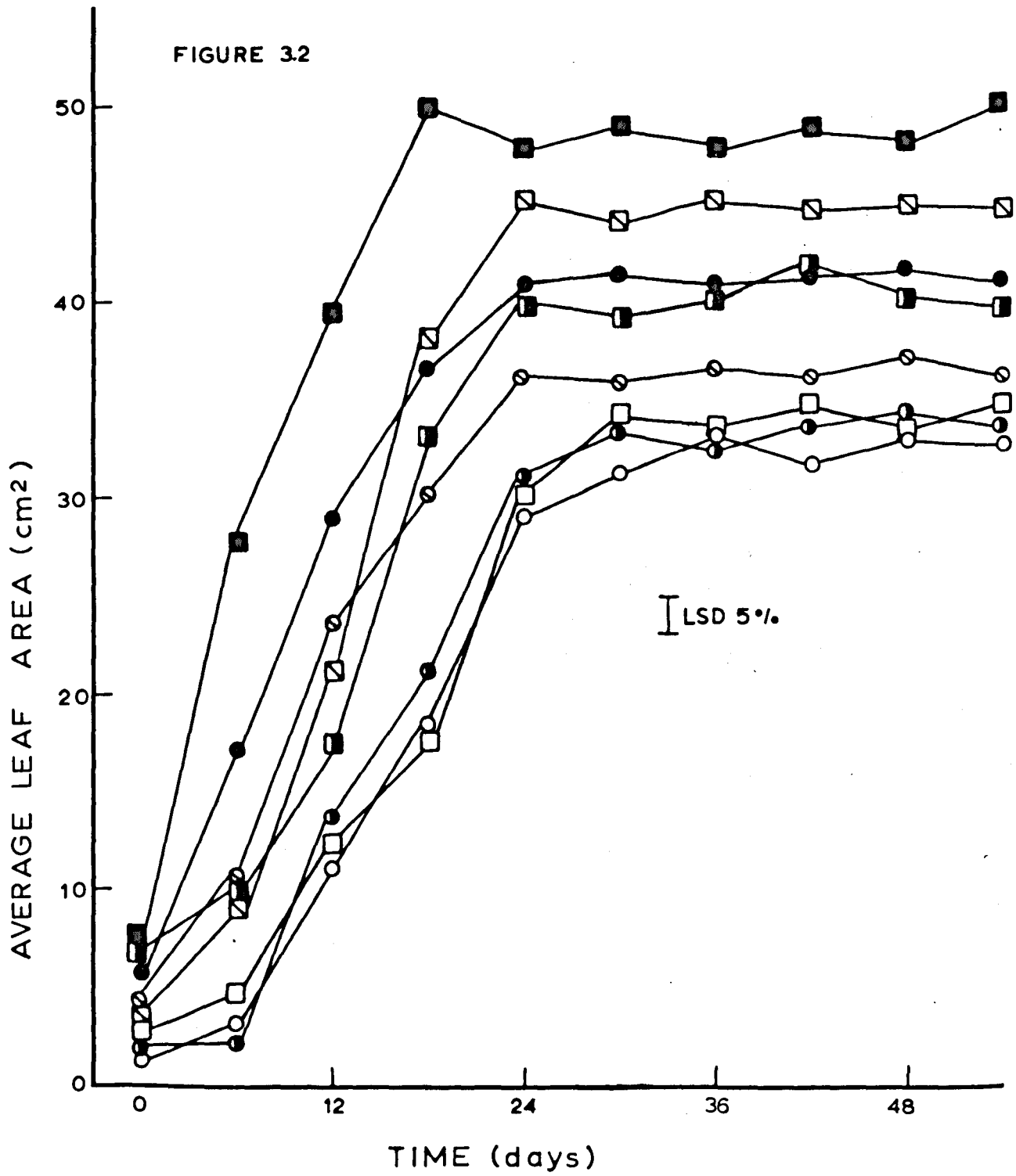


FIGURE 3.2 Changes during leaf expansion: Average leaf area
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

FIGURE 3.3

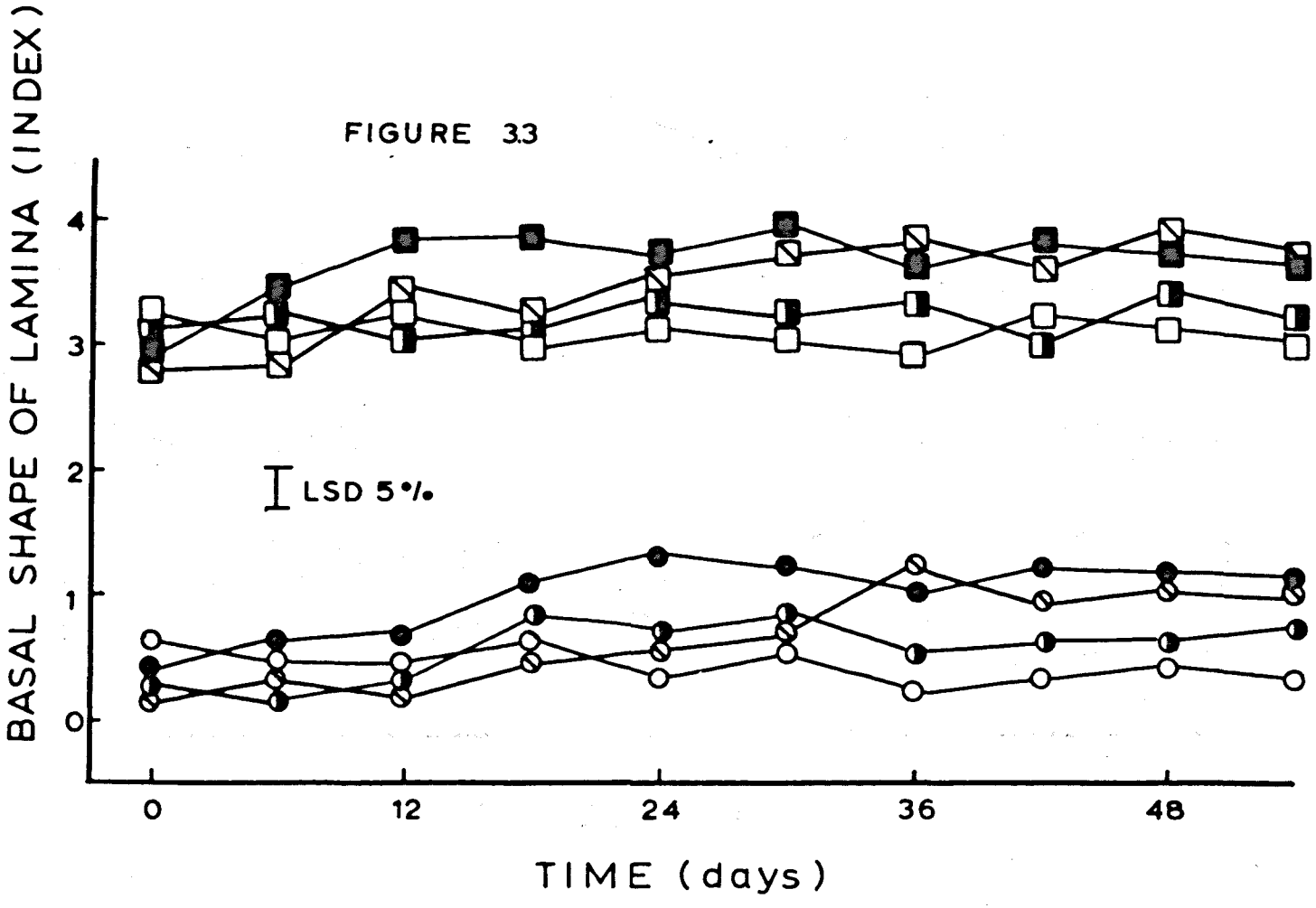


FIGURE 3.3 Changes during leaf expansion: Basal shape of the lamina
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

FIGURE 3.4

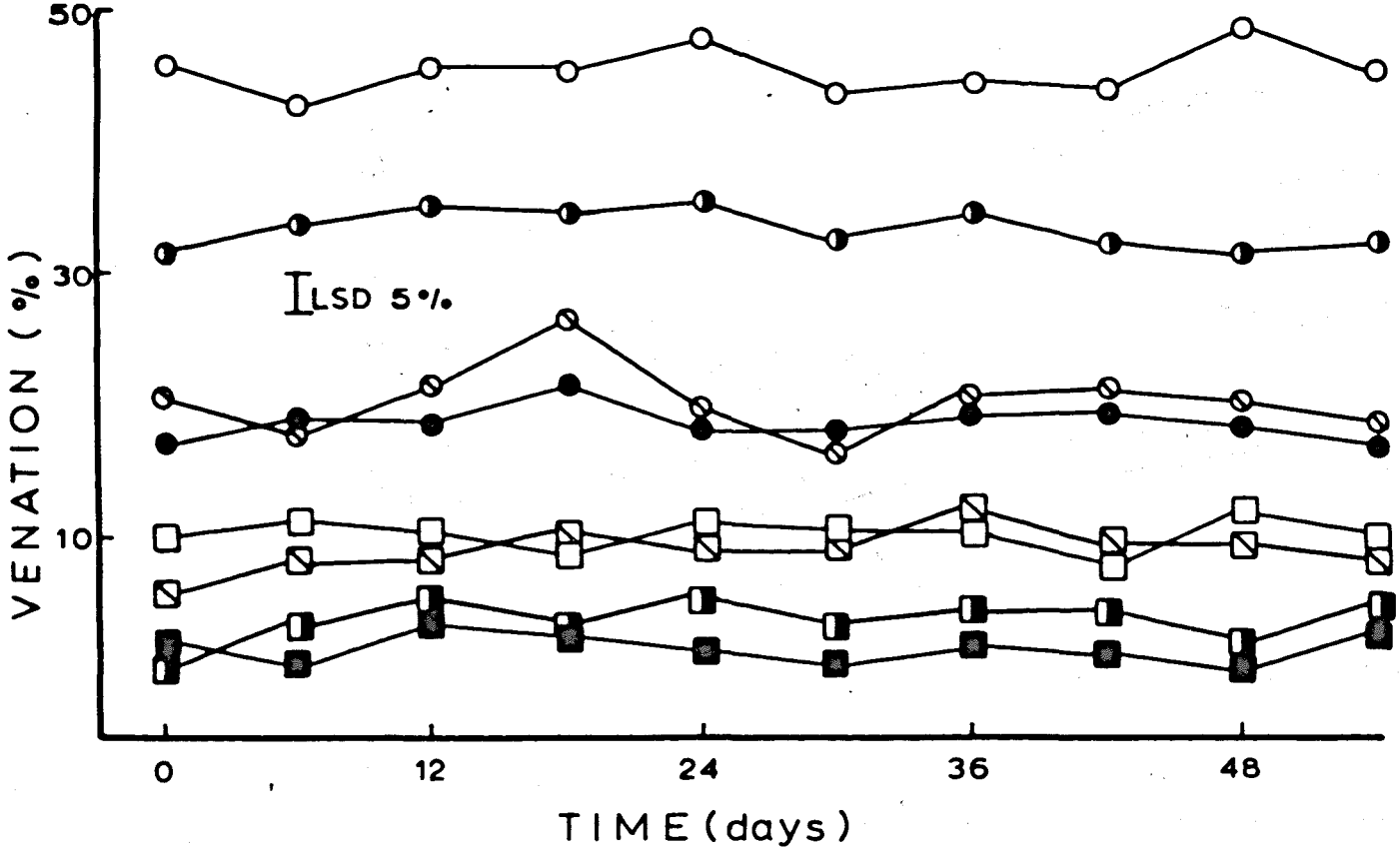


FIGURE 3.4 Changes during leaf expansion: Venation
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

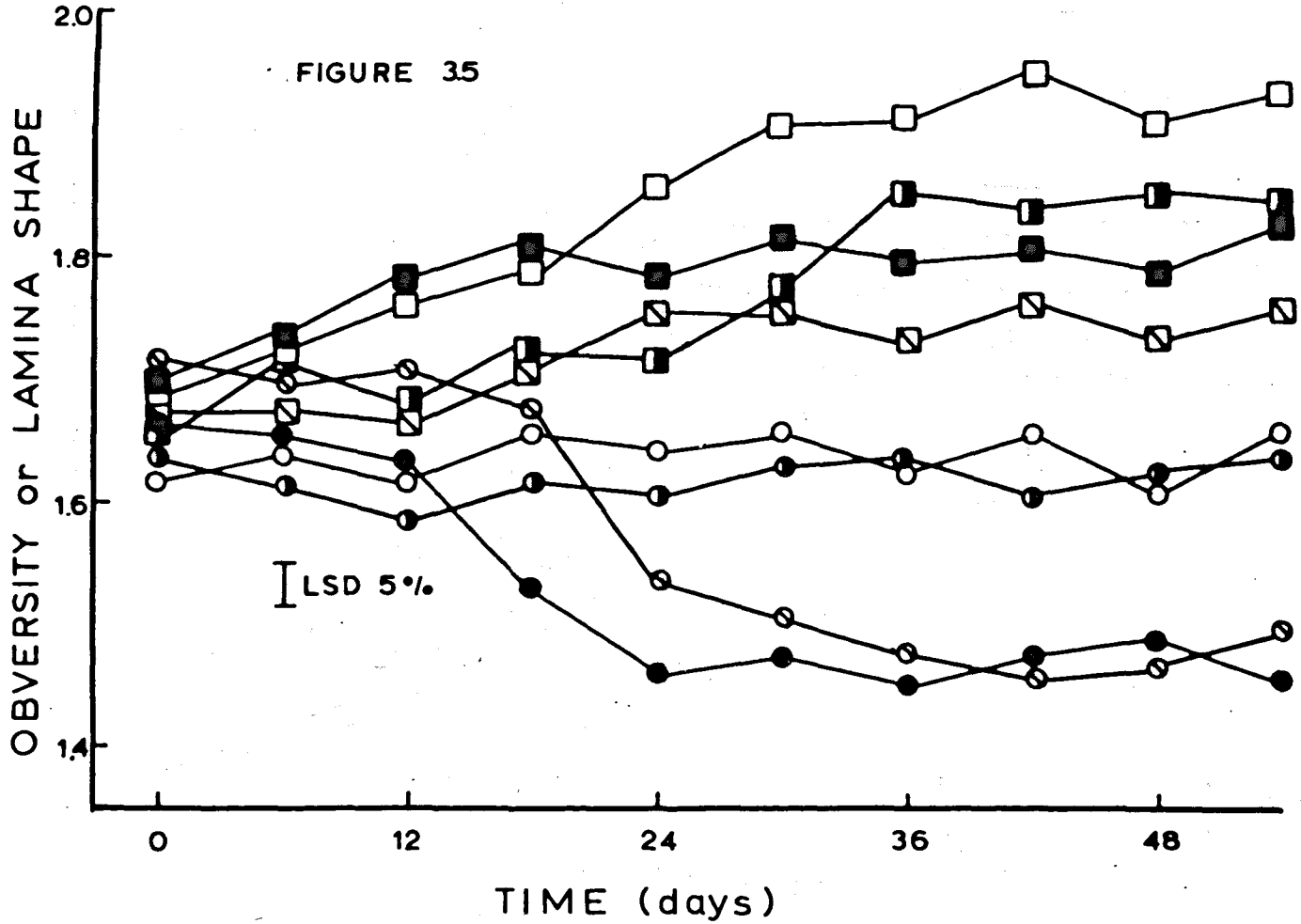


FIGURE 3.5 Changes during leaf expansion: Obversity
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

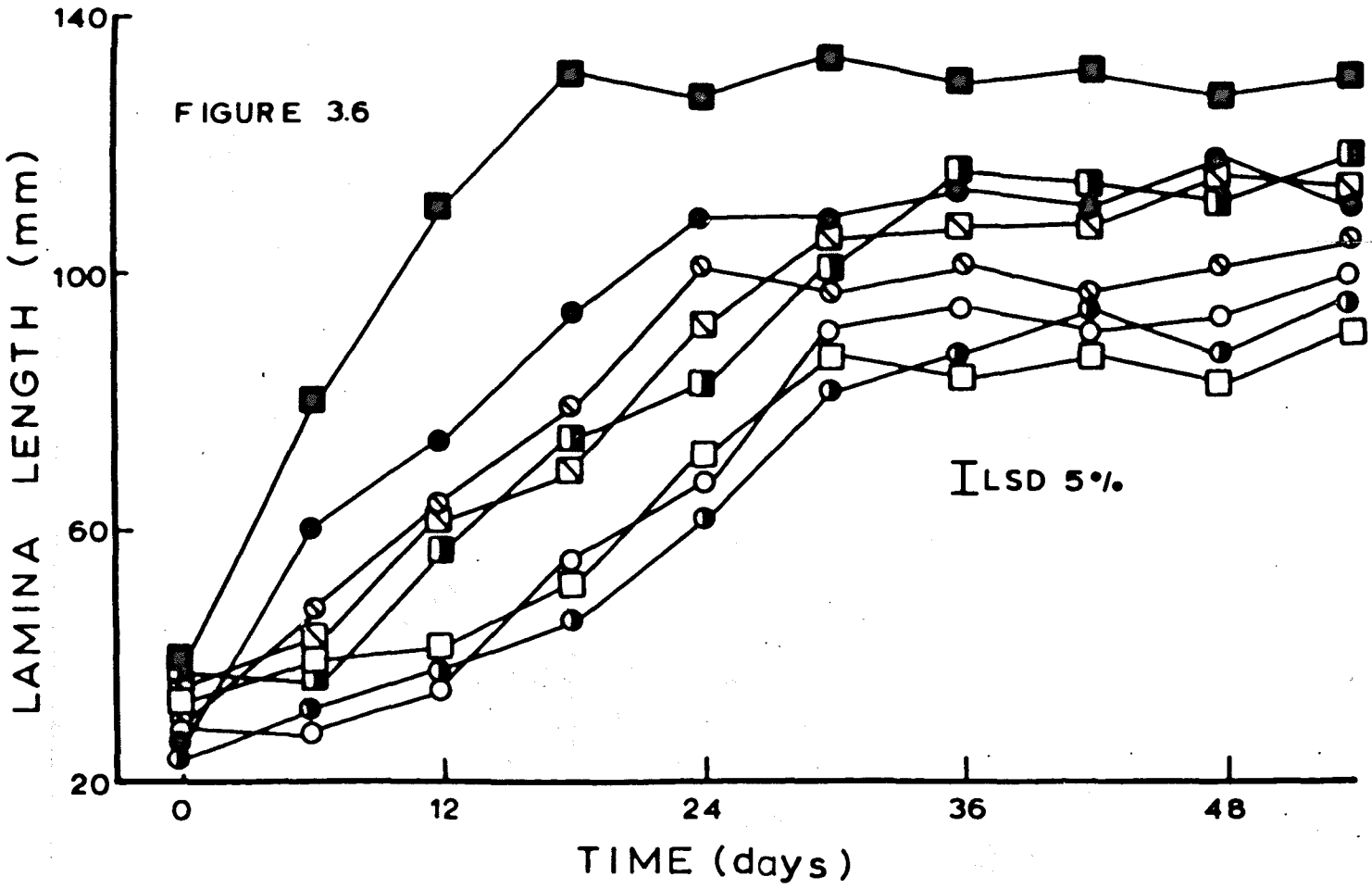


FIGURE 3.6 Changes during leaf expansion: Lamina length
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

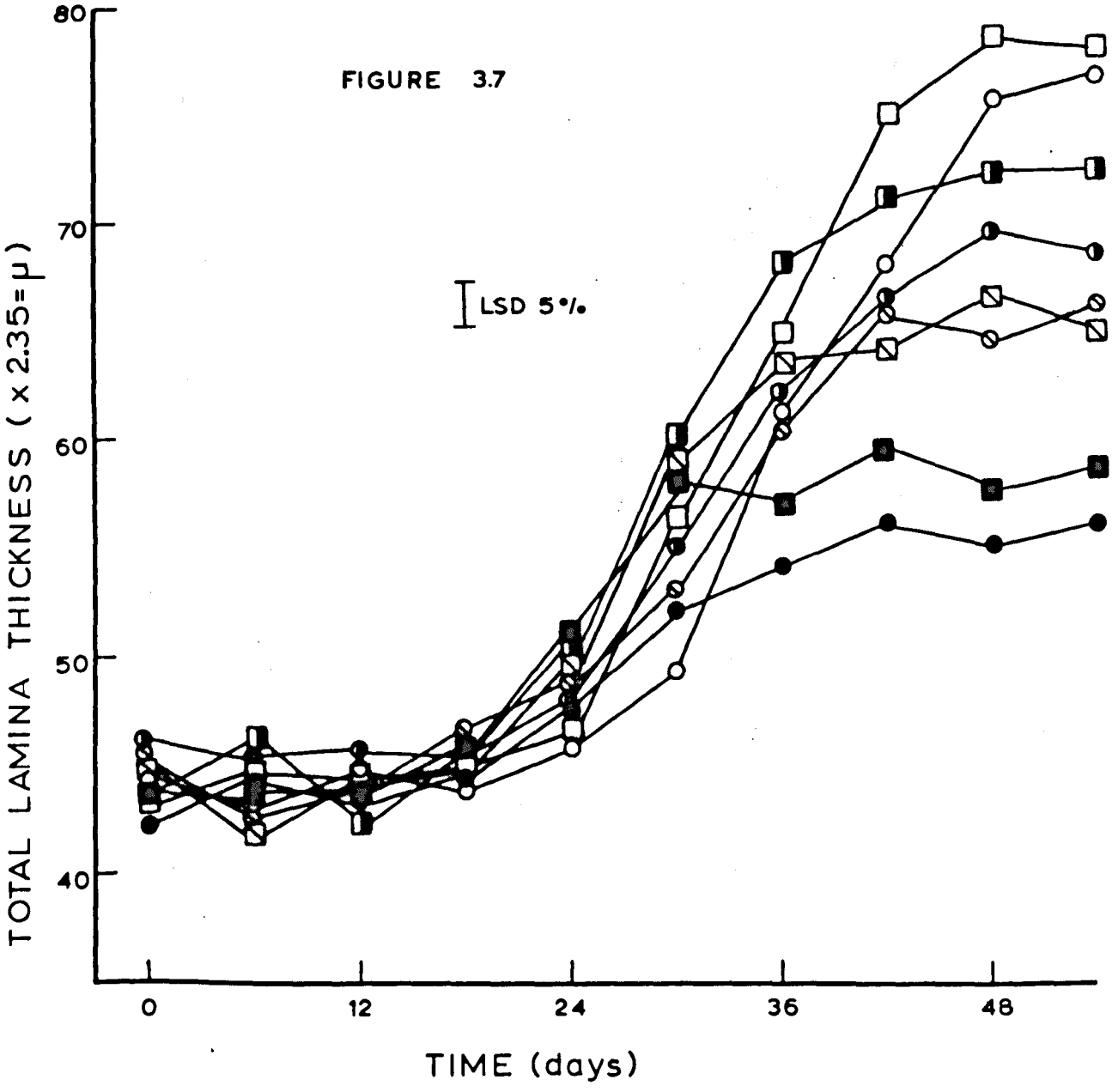


FIGURE 3.7 Changes during leaf expansion: Total lamina thickness
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

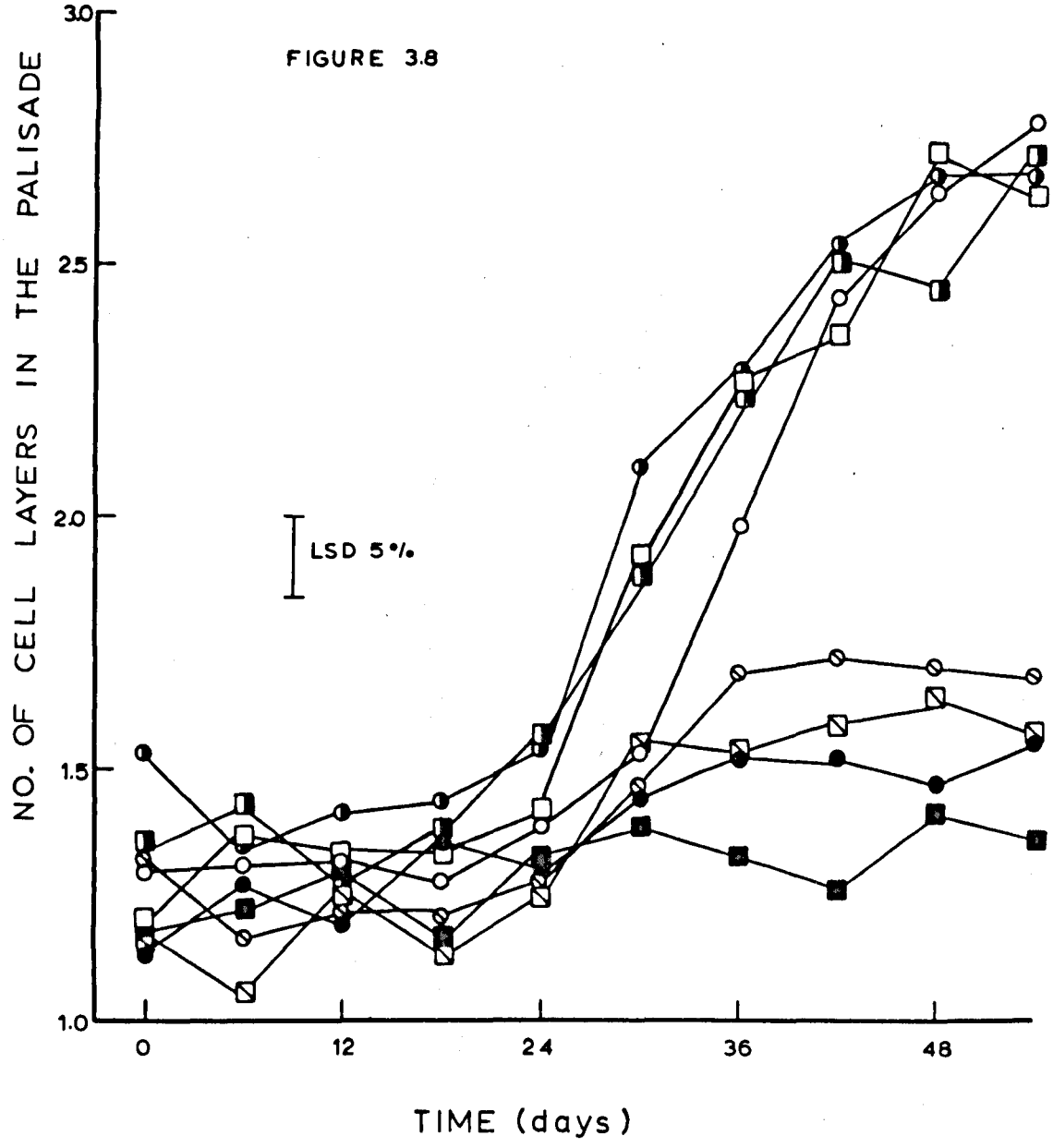


FIGURE 3.8 Changes during leaf expansion: Number of cell layers in the palisade
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◨ PSC; ■ PShC)

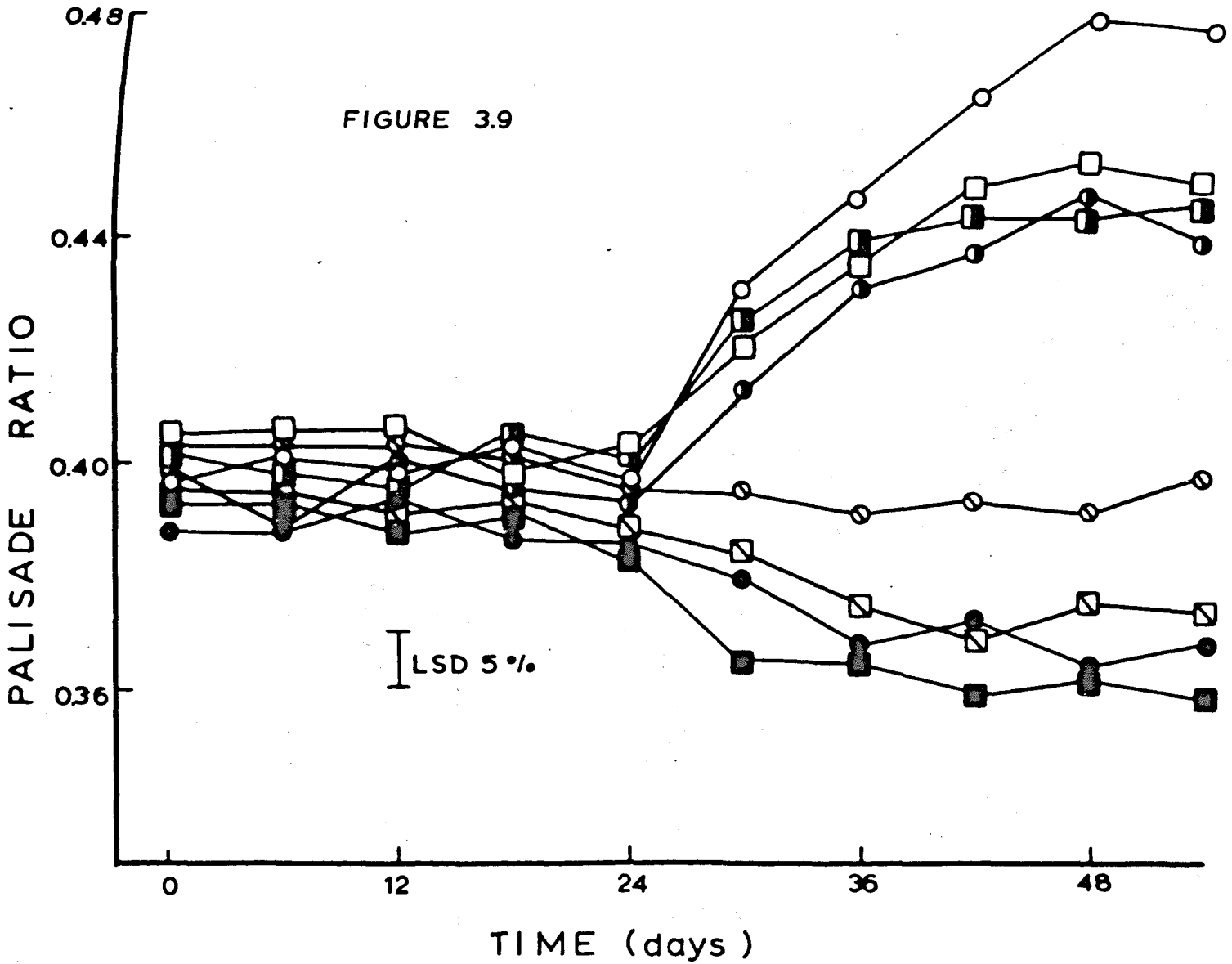


FIGURE 3.9 Changes during leaf expansion: Palisade ratio
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

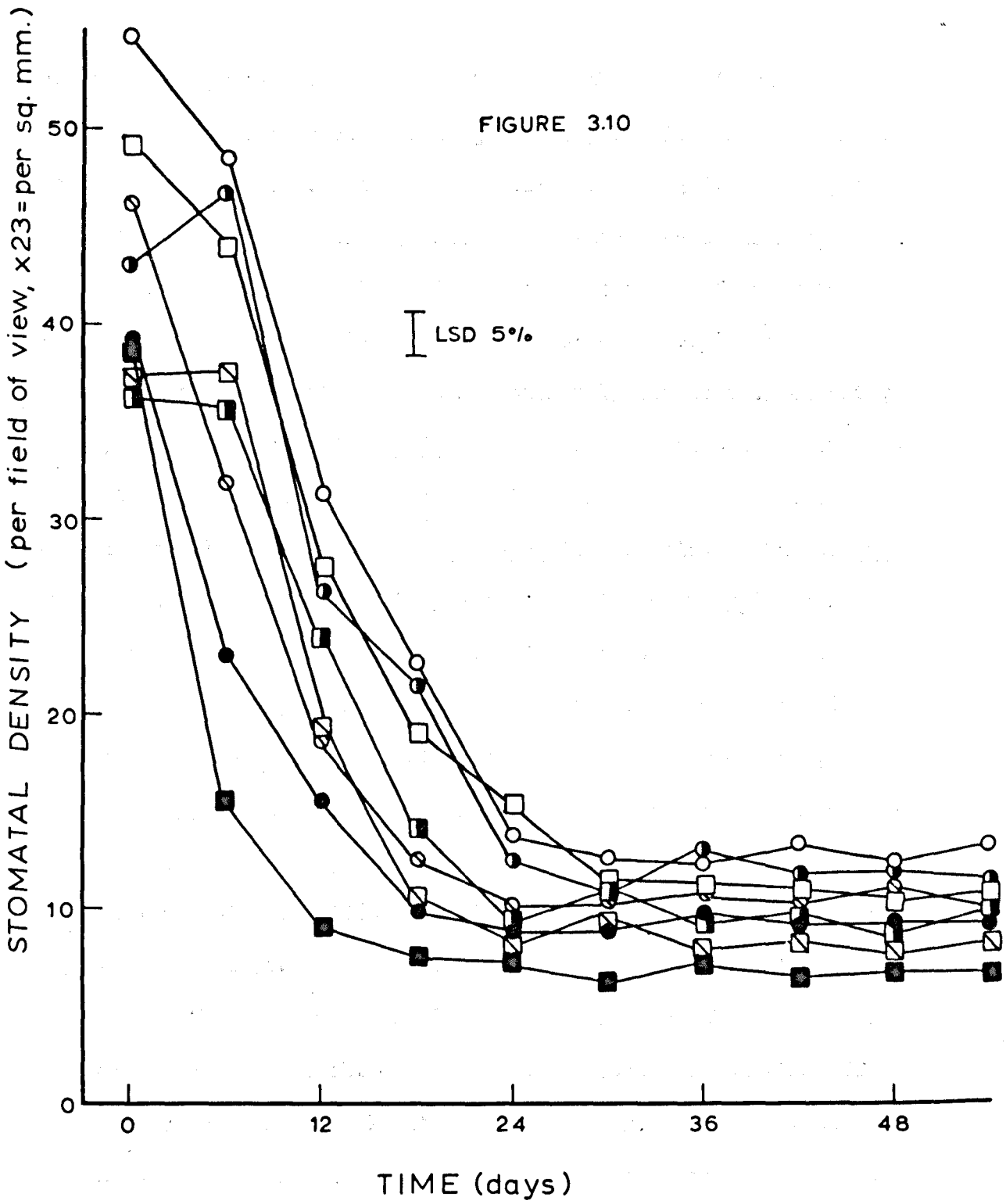


FIGURE 3.10 Changes during leaf expansion: Stomatal density
LSD 5% = least significant difference of means at the 5% level
See Appendix 2 for standard deviations
(○ RSO; ⊘ RShO; ◑ RSC; ● RShC
□ PSO; ▨ PShO; ◧ PSC; ■ PShC)

before a steady state is reached. Consequently shade leaves during their expansion phase must be experiencing changing conditions over a much greater period of time. It is obvious from Figure 3.2, however, that as far as leaf area is concerned, which represents expansion in two dimensions, in shade conditions, leaf area reaches its final value much earlier than the comparable sun leaves. For example, the open Q. robur sun leaf reaches a level state at approximately the seventh sampling period, whilst the closed Q. petraea shade leaf reaches an equivalent state at the fourth sampling period. It is also noticeable from Figure 3.2 that the leaf expansion for Q. robur is much slower than Q. petraea for comparable sites. Jones (1959) notes that "the Q. petraea leaf appears to expand the more rapidly ... " and this would seem to be upheld. The majority of leaf expansion takes place during the first 18-24 days, which agrees well with other workers. For example, Büsgen and Münch (1931) recorded 14 days for major leaf expansion in several oak species; leaves of Betula and Populus expanded within 14 and 21 days (Kozlowski and Clausen, 1966); Mounts (1932) noted leaf expansion in Vitis taking 40 days. The longer period taken for light intensity to level off in the shade might well be due to the fact that the sun leaves (generally above) are still expanding and consequently cutting off successively more and more light, and it is only when leaf expansion in sun conditions ceases that the drop in light intensity in the shade levels off.

Differences are apparent, however, between sun and shade situations even at the first sample (see analysis of variance and least significant difference of means in Appendix 2). One possible reason for this is the slight delay in sampling time. Although it is possible to obtain buds at the same stage of swelling and, although those buds produce leaves at the same time, not all the leaves of each bud are released from the bud

at the same time. In consequence, the first sampling had to be delayed until a sufficient number of buds had expressed sufficient numbers of leaves for collection. This meant that many of the leaves were already expanding rapidly when first collected and had in consequence experienced the environmental differences that existed between the different canopy sites. As can be seen from Appendix 2, the largest mean leaf area from the first sample is 7.38 sq. cm. (Q. petraea shade, closed canopy) and the smallest is 1.21 sq. cm. (Q. robur sun, open canopy). Consequently it may be concluded that although there might be differences in leaf area in the unswollen or swollen bud stage, differences appear shortly after the leaves are expressed from the bud. The small differences noted in these early samples appear to reflect very closely the larger differences apparent at later stages of leaf expansion, and this may be due to the influence of the environment on the leaf from expression of the leaf from the bud until the first sampling time, or may be due to environmental influences on the overwintering bud and during the previous growing season.

Anderson (1955) noted that in both Viburnum prunifolium and Cornus florida, the larger leaf area of shade leaves was produced by both earlier and larger increases in the growth rate of the shade leaves. This earlier development Anderson argued might well be related to temperature which is both higher and less extreme in shade situations. It is obvious from the diagrams of Hughes (1959) that leaf expansion is faster under shade conditions, but the diagrams, unfortunately, do not record the levelling off phenomena, so that it is not possible to determine if the sun or shade leaves reached their maximum leaf area first.

BS, AD and HR all behave very similarly with regard to leaf expansion, and only BS has been graphed (Figure 3.3). In the first

sample, the species are very different, but within each species, there are no differences consistent with the view that environmental influences have been important in the bud, i.e. the order of the means for the first sample bear no relation to the order of the final means. The slight changes which take place in BS and AD characters over the period of the sampling, particularly with respect to BS are probably due to the expanding leaf altering the characters. Cousens (1962) records auricles characteristic of Q. robur in unopened but swollen buds and the auricle condition characteristic of Q. petraea in Q. petraea buds in the same state. Leaf hairiness remains constant in Q. robur, but increases slightly in Q. petraea. This is probably due to poor observation - it is thought that the apparently larger number of hairs in the older leaves is a reflection of the fact that hairs in the younger leaves had not themselves fully expanded and were in consequence difficult to assess, and the increase in hairiness simply reflects the easier assessment of the character in the later stages.

Characters LN and V behaved similarly through the sampling period. Early samples of both characters showed the same expression of these characters as the fully expanded leaves. (Figure 3.4 shows the change in venation over the sampling period.) Consequently, it must be assumed that as far as these two characters are concerned, the expression of the characters is predetermined by the environment during the previous year. Mounts (1932) showed lateral veins to be present in Catalapa leaves of 2-5 mm. length. Cousens (1962) recorded both lobe number and main veins to be well developed in leaves of swollen but unopened buds. The findings here prompted confirmation of the result of Cousens. Examination of swollen, but unopened, buds showed both lobe number and venation to be well determined. However, what has not been confirmed is the time at which such determinations are manifest.

Moore (1909) using buds of the Norway maple and other species was able to show that no new organisation of tissue takes place within the buds during the winter and following spring. He found that the growth of the following year's buds began in March of the previous year. After initiation of the bud scales, the leaf primordia form and quickly produce the mid-rib of the leaf primordia. The tissue on both sides of the mid-rib becomes meristematic and is responsible for marginal growth. It would appear that leaf margin characters, at least as far as lobe number is concerned, are determined at this point in time. Foster (1936) notes that the actual period of marginal growth is of very short duration, and probably occurs in early/mid summer (almost certainly variable and dependent on species) when sun/shade differences are possibly at a maximum. Wieckowska (1972) showed that in Fagus sylvatica, venation appeared in leaf primordia in late July. Since in the present investigation, venation was apparently determined by the environment of the bud rather than the environment of the expanding leaf, if as in Fagus sylvatica, venation was determined in July, this would be the period at which differences between sun and shade would be a maximum.

Both LDR and OB are characters likely to be influenced by leaf expansion; differential expansion rates between the lobes and main body of the lamina influencing LDR, and differential expansion rates between the tip and base of the lamina influencing OB. Figure 3.5 graphs the change of OB with time; LDR follows a similar pattern and has not been included. The initial values for the different canopy sites and species are very similar, ranging from 1.61 to 1.72 for OB and 2.43 to 2.89 for LDR, although they diverge later. This would suggest that both characters are not predetermined in the bud. Wieckowska (1970) thought that leaf shape in Ulmus laevis was not determined by environmental

factors operating at the time of leaf expansion, but were operating as the leaf primordia were being formed. The results presented here would appear contradictory to this view. It is very interesting to note that, under shade conditions, these two characters level off before those under sun conditions, and also, for comparable sites, Q. robur levels off after Q. petraea. These differences between sites and species were also true of leaf area, and it therefore seems highly likely that these characters are closely correlated with leaf area expansion. LL and PL follow the same patterns as OB and LDR with regard to the levelling off of the characters in the different canopies and species. However, the species are separated at the first sampling period for both characters (Figure 3.6 shows the change in LL with time). PTR varies little with time as far as the Q. petraea leaves are concerned, suggesting that the petiole and lamina are expanding at the same rate thus preserving the ratio between them. Q. robur varies much more, particularly in the early stages, and this might well reflect the difficulty in measuring accurately the short petioles of the expanding Q. robur leaves.

The thickness of the oak leaves under study remained constant for the first four samples, and only began to increase after this sample - see Figure 3.7. This is a very different situation from that observed in the morphological characters; indeed lamina thickness does not begin to increase until lamina area expansion is almost complete (at about the time of sample 5). TTh, ETh, PTh and MTh all respond similarly, i.e. the increase in total thickness being due to increases in all the three components of TTh and not in any individual component. As with the morphological characters, the shade leaves and Q. petraea leaves reach their maximum values before the sun leaves and Q. robur leaves. The number of cell layers in the palisade tissue (Figure 3.8) shows interesting changes - in the initial stages, there being between 1 and 1½ cell layers, but as time progresses the shade closed canopy and the

shade open canopy of both Q. robur and Q. petraea remain at this level, whilst the sun closed canopy and the sun open canopy of both species show large increases in the number of cell layers. This result differs from that found by Isanogle (1944). In Cornus florida rubra and Acer platanoides she showed that the number of cell layers in the embryonic leaves was constant regardless of tree position, but that the number of cell layers increased in both sun and shade conditions. Avery (1933) showed, however, that the cell layers are well established in the leaf lamina when the leaf primordia is less than 5 mm. long. He showed that cell division stops firstly in the epidermis, next in the spongy mesophyll and lastly in the palisade tissue.

When expressed as the ratio of TTh, i.e. MR, PR and ER, parallel differences may be seen, eg. Figure 3.9. For the first four samples, both MR and PR remain constant, but thereafter changes occur; PR increasing in sun conditions and decreasing in shade conditions, MR increasing in shade conditions and decreasing in sun conditions. ER fluctuates wildly, but shows a general decrease towards the latter part of the investigation.

SD initially is very high, but drops rapidly as the leaf expands (see Figure 3.10) which again suggests that the number of stomata is fixed and density varies as leaf area varies.

We can now consider if these characters and investigations allow a working description of the neutral leaf. The characters may be divided in the following manner:

1. Those characters which appear to be influenced by environmental factors during the time spent by the leaf in the bud. V and LN belong in this grouping.

2. Those characters which do not appear to be influenced by environmental factors during the bud stage. These may be divided into those

which clearly differentiate the species at the very start of the leaf expansion and continue to do so throughout the period of leaf expansion and in the mature leaf, i.e. characters HR, BS, AD and PTR. Characters PL and LL may also be included in this group, although the differences between the species initially are distinct but small, and in the case of LL there is some slight overlap in the mature leaf. These constitute group 2(a). Group 2(b) includes those characters not influenced by environmental factors during the bud stage, but which only differentiate the species in the mature leaves, not in the young unexpanded leaves. Such characters are OB and LDR. AR and LL might also be considered as belonging to this group, although both fail to differentiate completely unambiguously between the species at the mature leaf stage. Group 2(c) includes those characters which fail to differentiate between the species at any stage of development of the leaf, i.e. TTh, ETh, MTh, PTh, SD, ER, MR, PR and CL. Both AR and LL might conceivably be included in this group.

Only characters in group 1 are characters that are influenced in the bud stage to the extent that the sun/shade differences exist in the unexpanded leaf, although it must be remembered that all characters have been influenced to some degree by the environment - no leaf can ever be completely isolated from the environment. As argued previously, the neutral leaf in the purest sense is that leaf defined by the genetic systems of the plant without the influence of the environment. The immature, unexpanded leaf comes close to being this concept of the neutral leaf with two provisos:

1. Some characters (those of group 1) have been influenced greatly by the environment and therefore must be discounted.
2. All characters of group 2 have been influenced to some degree by the environment, although to apparently no great effect.

If group 1 characters are ignored, therefore, the immature, unexpanded leaf possibly comes very close to our concept of a neutral leaf, and in the neutral leaf, there are differences between the species. Characters in group 2(a) differentiate the species in the young leaf stage, and these possibly reflect the important characters in differentiating the species. Consequently, when assessing variation within and between oak populations, the group 2(a) characters are perhaps the important ones.

CHAPTER FOUR

VARIATION IN SEEDLING MORPHOLOGY AND ANATOMY

Introduction

The environmental differences between different canopy sites have prompted several authors, eg. Humphries and Wheeler (1963), Daubenmire (1974), to suggest that the sun and shade leaf differences observed between sites are not simple manifestations of light intensity differences but are due to a combination of factors particularly light, temperature, humidity (and water supply) and wind speed. Certainly, each factor has been implicated in changes of leaf structure, eg. Blackman and Rutter (1948) - light; Cain and Potzger (1933) - water supply; England (1960) - temperature; Whitehead (1962) - wind speed. Hanson (1917) in a very detailed study of the physical conditions in sun and shade showed that in sun conditions, light intensity was higher, but so too were temperature, by as much as 2.8°C; wind speed, being twice as fast on average in exposed sun conditions; and humidity, varying between 1% and 16% higher in sun conditions. Hanson (1917) also pointed out some of the relationships he believed existed - humidity differences caused mainly by wind speed and temperature differences caused mainly by differences of light intensity. Some of the physical conditions of sun and shade have also been investigated by Fritts (1961) and Shreve (1931).

In order to try and ascertain which factor was mainly responsible for creating the leaf differences observed in oak canopies, attempts were made to set up field experiments, eg. artificial shading of branches, but these met with little success mainly due to the inability to isolate and vary one environmental variable at a time. Since mature oak trees pose insurmountable problems when considered for greenhouse-

type experiments, resort had to be made to seedling material. Unfortunately, the morphology of seedling leaves of Q. robur and Q. petraea are reported as being exceptionally similar. Jones (1959) affords the following description, which should be compared with the description of mature leaves given in Table 2.2:

"Q. petraea: the primary leaves are very variable in shape, they have fewer and shallower lobes than the adult leaves and are often oval and sub-entire or sinuately lobed, the base is cuneate and the petiole short. They are glabrous above and have scattered appressed simple hairs below, especially on the veins: the characteristic stellate hairs are not produced before the second year; but the petioles remain short and the leaves do not fully lose their juvenile features for several years. The first-year stem is green and smooth with scattered appressed hairs.

Q. robur: the primary leaves closely resemble those of Q. petraea and it is not possible to distinguish between the seedlings in the first year."

Consequently, any experimental work with oak seedlings must take account of two things: the apparent inability of taxonomists to clearly distinguish between the species and the change in leaf morphology that is likely to take place as the plants produce new and therefore more mature foliage.

On balance, light intensity has probably been shown to be the more influential environmental parameter in regulating leaf structure - it also has the advantage of being the easier parameter to control under experimental conditions. Since the differences in both humidity and temperature between sun and shade are small (Hanson, 1917), it is not possible to design growth chamber experiments to mimic such differences due to the problems of variation of temperature and humidity in pre-set growth chambers, eg. temperature differences between sun and shade were a maximum of 2.8°C (Hanson, 1917) which is within the range of variation of many growth chambers. Wind speed, although possible to control and vary on a small scale, requires specially designed chambers to carry out large scale experiments. Light intensity is the environmental

parameter showing the biggest difference between sun and shade - this chapter reports the influence of light intensity on seedling morphology and anatomy.

Experimental Design

Since both greenhouse space and growth chamber accommodation were at a premium, it was necessary to design a single experiment to consider the morphology and anatomy of Q. robur and Q. petraea leaves under the following headings:

1. Basic differences between the species
2. Change of leaf characters through time as juvenile foliage gave way to more mature leaf forms
3. Effect of light intensity on seedling leaf characters
4. The influence of the environment on the developing bud as a factor in determining foliage characters

The experiment essentially consisted of raising seedlings under uniform conditions, transferring to different light intensities and then transferring the seedlings back to uniform conditions. By studying leaf morphology and anatomy after each treatment, it was hoped to provide information on the above points.

Method

During September and October 1968, acorns were collected from two forests, Uffmoor Wood and Wyre Forest. Acorns were removed from trees so that their maternal parentage could be ascertained. Storage of acorns under water is regarded as one method of overwintering acorns (Palmer, 1955), but Jones (1958) reported that although the acorns of Q. robur will tolerate submergence in water, those of Q. petraea will not. The acorns were stored therefore in plastic boxes filled with damp Sphagnum sp. moss at 5°C. Acorn size is known to influence the

size of the final seedling (Jarvis, 1963) and consequently the acorns were weighed immediately after collection.

The acorns were sown, one per $4\frac{1}{2}$" black polythene pot in a sterilised seed compost on 10th January 1969. Throughout the whole of this experiment, the pots were watered every third day. Of the 280 acorns sown, approximately 15% failed to germinate, and of these about one third were found to be infected with the fungus Sclerotinia pseudotuberosa Rehm. which converts the whole acorn to a black sclerotium (Hauch, 1923). The 280 acorns chosen for sowing had the following weights:

Q. robur	mean weight - 2.894 gms.
	range 1.994 - 3.876 gms.
Q. petraea	mean weight - 2.646 gms.
	range 2.247 - 3.364 gms.

Very small acorns and very large acorns were not used. The pots were placed in random positions in a small greenhouse. By 12th March, the majority of the acorns had germinated - the young seedlings were divided into five groups, each group containing 18 seedlings of each species. These 180 seedlings were randomly arranged within the same greenhouse. The greenhouse did not have temperature regulation, and supplementary lighting was not given, the conditions representing uniform conditions for all seedlings.

During October 1969, the seedlings were moved to the University of York, and placed in a 'walk-in' growth chamber.

The growth chamber contained two steel, movable frames on each side of an aisle, and above each frame were six mercury vapour lamps and one supplementary incandescent lamp. Each half of the chamber was sub-divided into two halves. It was possible to vary light intensity in the chamber in two ways: at different heights from the lamps in the ceiling, and by layers of muslin suspended between the lamps and the

floor. Experimentation showed that the following light intensities could be achieved:

1. At 160 cm. from the base of the growth chamber - no intervening muslin (= 100% light intensity)
2. At 140 cm. from the base of the growth chamber - no intervening muslin (= 75% light intensity)
3. At 140 cm. from the base of the growth chamber - with one layer of intervening muslin set 170 cm. above the floor (= 50% light intensity)
4. At 40 cm. from the base of the growth chamber - with one layer of intervening muslin set 120 cm. above the floor (= 25% light intensity)

Under each light intensity, one group of seedlings consisting of 18 Q. robur and 18 Q. petraea seedlings were arranged in random order on a steel tray. These trays were so arranged that the leaves of the seedlings came to lie at approximately 160 cm. (= 100% light), 140 cm. (= 75% light), 140 cm. under muslin (= 50% light) and 40 cm. (= 25% light) from the base of the growth chamber. Temperature of the air in the chamber was maintained at 15°C, relative humidity was set to 95% and a 16 hour light/8 hour dark regime imposed. Jones (1959) noted that provided young oak plants are kept at a sufficiently high temperature, growth continues and the plants do not lose their leaves. Moving the seedlings early enough in autumn ensured that the seedlings kept their leaves and did not lapse into dormancy. From each seedling three leaves were removed: these were scored for morphological leaf characters whilst still fresh, then each leaf was halved, one half being fixed and the other cleared as described in Chapter 2. These leaves, the first harvest, represented leaves produced under uniform greenhouse conditions.

The light intensity experiment was finally set up in November 1969 and was continued until July 1970, using the temperature, humidity and light intensities noted above. For a 24-hour period during February

1970, the temperature rose to 25°C due to malfunction of the chamber's refrigeration unit. Since all new leaves had fully expanded by this time, this temperature fluctuation was thought to be unimportant. Normal temperature fluctuations were never more than ± 2°C. Regular temperature and relative humidity readings were taken above the seedlings under the four different light intensities. Although the seedlings were set at different heights from the lights and under muslin, the constant exchange of air between the chamber and the exterior meant that although there were minor differences between the treatments, these never reached significant levels - temperature differences being approximately 1°C and relative humidity 2-5%, but these were inconsistent differences, i.e. one treatment higher on one occasion, another treatment on another.

The fifth group of 18 Q. robur and 18 Q. petraea seedlings were kept in the 'roof' greenhouse. The greenhouse had supplementary and erratic heating but this was sufficient to prevent the seedlings becoming dormant. At the start of the period in the greenhouse, three leaves were removed from each seedling and treated in similar fashion to the leaves from the growth chamber seedlings. This last group represented a control group, since they were as far as possible under the same environmental regime as experienced in the greenhouse at Birmingham.

In July 1970, three leaves were again removed from each of the 180 seedlings and measured, fixed and cleared as previously described. The seedlings from the growth chamber were removed and placed with those from the 'roof' greenhouse in a randomised arrangement in the 'roof' greenhouse. The conditions were kept as previously described. In March 1971, a further three leaves were removed from each seedling.

Throughout the course of this experiment, which lasted two years, the seedlings were not repotted, but the seedlings were 'fed' every

three months of the experiment with equal quantities of a general liquid fertiliser. Some root disturbance took place on moving the seedlings from Birmingham to York, as the seedlings had rooted into the sand bed of the greenhouse bench, but this did not check growth in any observable way. Gardiner (1968) informed me that the Forestry Commission sow acorns in 40 cm. drainpipes filled with soil so as to minimise root disturbance on transplanting. Jones (1959) noted that the tap root may be 10-20 cm. long before the plumule appears, and may be up to 40 cm. long at the end of the first year. The experience of the present author would suggest that the elaborate precautions taken by the Forestry Commission are unnecessary.

The experimental design described here is summarised in Table 4.1.

It was important that only leaves developed during the previous growth regime were collected and for this reason, at the end of each period, individual leaves were marked so as not to confuse them with those produced during the current growth period. During each growth period, in the greenhouses and growth chambers, the seedlings expanded buds and produced new leaves and it was these leaves that were sampled at the end of the growth period.

The light intensities were described above as 100%, 75% etc., but these were relative only to the highest light intensity in the growth chamber. Table 4.2 below records the actual light intensity measured using a dome solarimeter, compared with light intensity under greenhouse and natural conditions.

During the last growth period in the greenhouse, another group of of acorns was sown in sterilised seed compost in $4\frac{1}{2}$" diameter black polythene pots. These were acorns collected in Autumn 1970 from the same trees as sampled two years earlier.

	MARCH 1969 - OCTOBER 1969 (8 months)	NOVEMBER 1969 - JULY 1970 (9 months)	AUGUST 1970 - MARCH 1971 (8 months)
GROUP 1	GREENHOUSE	100%	GREENHOUSE
GROUP 2	GREENHOUSE	75%	GREENHOUSE
GROUP 3	GREENHOUSE	50%	GREENHOUSE
GROUP 4	GREENHOUSE	25%	GREENHOUSE
GROUP 5	GREENHOUSE	GREENHOUSE	GREENHOUSE

Each group consists of 18 Q. robur seedlings and 18 Q. petraea seedlings.

Three leaves were removed from each seedling after every period, i.e. in October 1969, July 1970 and March 1971

TABLE 4.1 EXPERIMENTAL DESIGN FOR INVESTIGATION OF SEEDLING LEAF MORPHOLOGY AND ANATOMY

	Light intensity (cals/cm^2/min)	Light intensity as a percentage of daylight	Light intensity as a percentage of greenhouse light
Daylight	0.4195	100.0	159.2
Greenhouse	0.2665	62.8	100.0
Growth Chamber 100%	0.0877	20.9	33.3
Growth Chamber 75%	0.0659	15.7	24.9
Growth Chamber 50%	0.0441	10.5	16.7
Growth Chamber 25%	0.0218	5.2	8.3
(Growth Chamber 10%	0.0088	2.1	3.3)

TABLE 4.2 COMPARATIVE LIGHT INTENSITIES FOR THE SEEDLING EXPERIMENT

Approximately 24 acorns of each species were sown under each light intensity - after germination, the numbers were reduced to 18 seedlings of each species under each light intensity. No comparable greenhouse control was carried out - the seedlings were maintained at 15°C, 95% relative humidity and a 16 hour day/8 hour night. A fifth light intensity (10%) was introduced into the chamber by constructing a tray on the base of the growth chamber directly underneath the tray holding the seedlings experiencing 100% light. In order to prevent water falling onto these seedlings from above during watering, a drip-tray was constructed under the 100% seedlings. Watering was carried out on every third day. Three leaves were harvested from each seedling as described earlier after nine months. This part of the experiment lasted from November 1970 to July 1971.

The characters measured on the leaves collected from the different stages of the growth chamber experiment were the same as those recorded for adult leaves in Chapters 2 and 3.

Results

The results are presented in tabular form in Appendix 3, which

lists the means and standard deviations for the twenty leaf characters, for the two species and three sampling times, and presents a three-way analysis of variance for the data in which the main effects are species, sampling periods and seedling groups. For each character, the results for the subsidiary growth chamber experiment are presented together with a two-way analysis of variance in which the main effects are species and light intensities. Least significant differences of means are presented with the three-way and two-way analyses of variance.

Discussion

a) Differences in seedling leaf morphology and anatomy

The morphological characters generally differentiated the species in the older foliage produced at the end of the experiment, and some characters differentiated the species in the foliage first produced by the seedlings. Such characters initially separating the species were AU, HR, V and AR. Figures 4.1 and 4.2 show the change of AU and V through the three sampling periods. HR and V remained more or less the same throughout the experiment, but AU and AR did not; these two characters showing more divergent expression between the species at the end of the experiment than at the beginning. Jones (1959) recorded that the seedling leaves of Q. petraea did not produce stellate hairs until the second year - the Q. petraea seedlings in this investigation produced a mean score of 3.78 (in a range of 0-4) and this score suggests that most leaves had a dense covering of stellate hairs.

Five other morphological characters, LN, PL, BS, OB and LL only differentiated the species clearly at the third sample, whilst the remaining two characters PTR and LDR failed completely to differentiate the species at any stage. Figures 4.3 and 4.4 show the changes in PL and LDR respectively for the three sampling periods. Since adult leaves

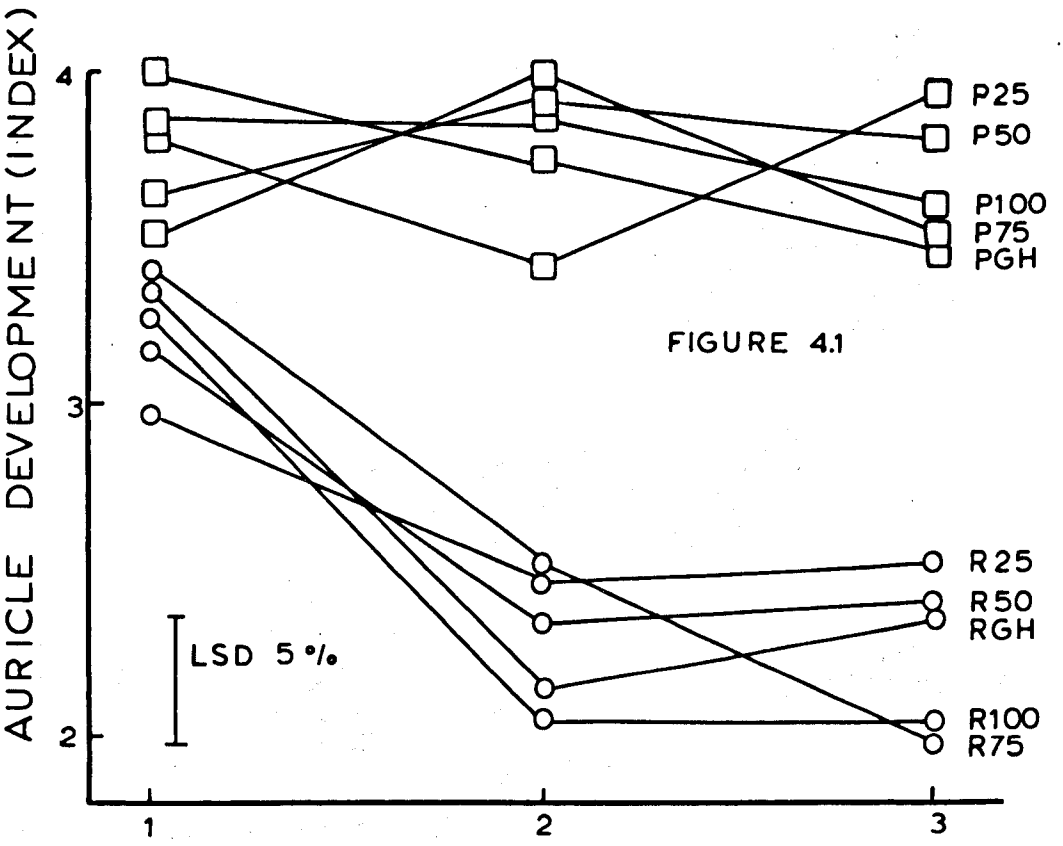


FIGURE 4.1

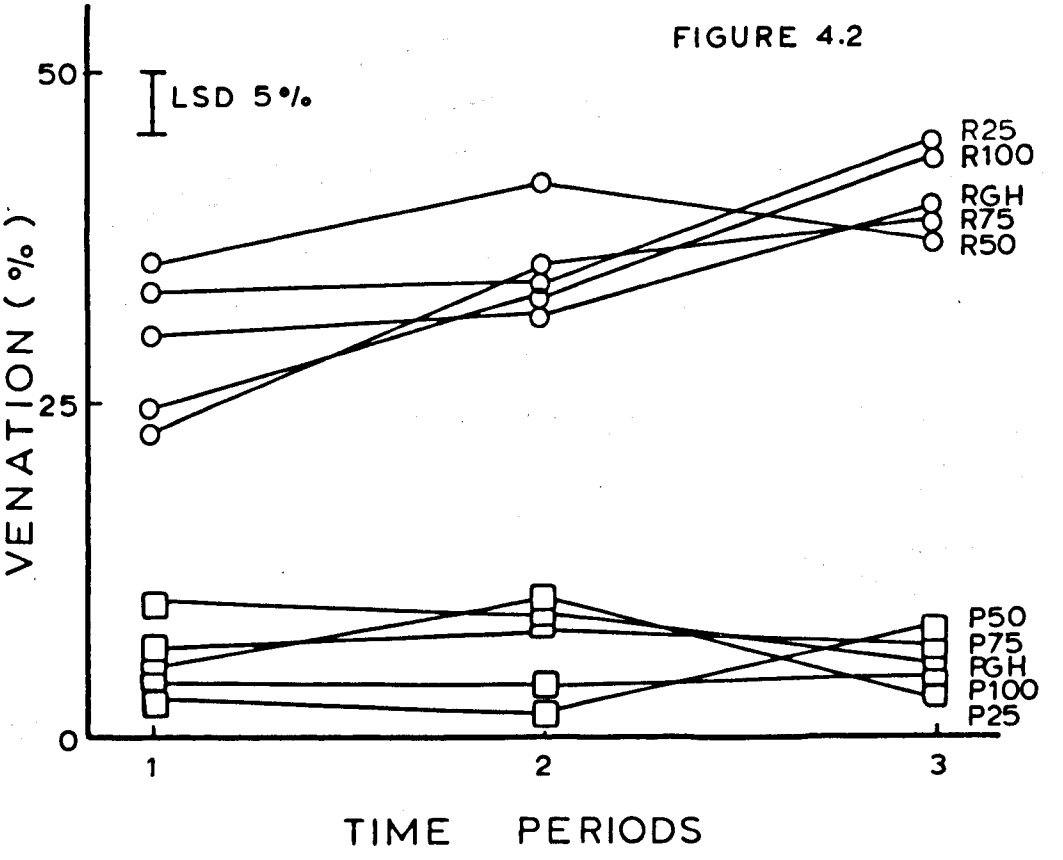
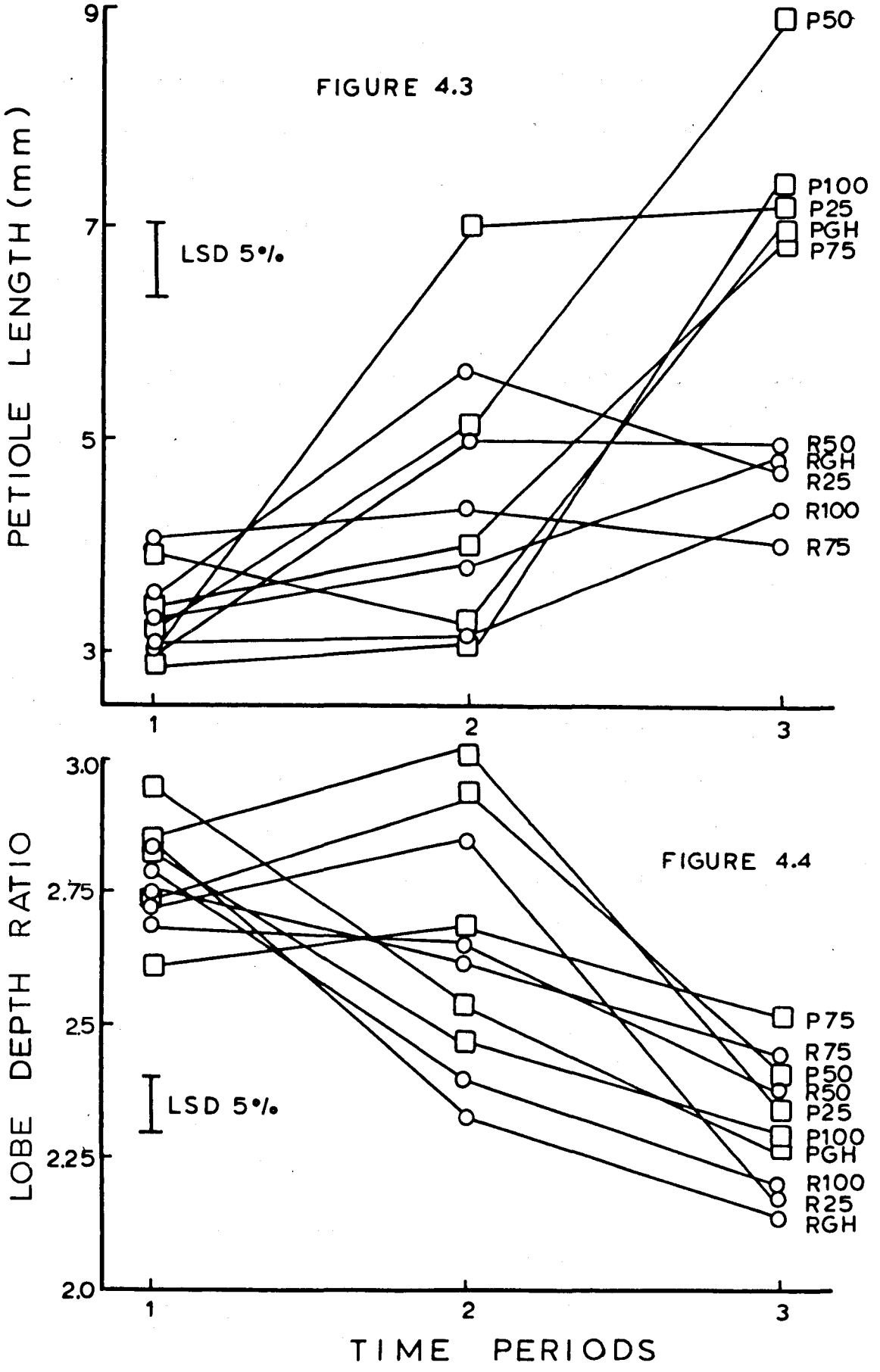


FIGURE 4.2

FIGURES 4.1 and 4.2 Change of seedling leaf characters with time: Auricle development and Venation respectively
1 = all seedlings under greenhouse conditions
2 = seedlings under different light intensities
3 = all seedlings under greenhouse conditions
LSD 5% = least significant difference of means at 5% level
See Appendix 3 for standard deviations
○ Q. robur; □ Q. petraea



FIGURES 4.3 and 4.4 Change of seedling leaf characters with time: Petiole length and Lobe Depth Ratio respectively
1 = all seedlings under greenhouse conditions
2 = seedlings under different light intensities
3 = all seedlings under greenhouse conditions
LSD 5% = least significant difference of means at 5% level
See Appendix 3 for standard deviations
○ Q. robur; □ Q. petraea

can be differentiated using PTR and LDR it must be concluded that after two years growth the seedlings had failed to produce mature foliage as far as these two characters are concerned. Table 4.3 lists the means for all characters for the two species for the first and third samples and compares these with adult leaves at the south lower sun aspect in open canopy.

Characters LL, PL, PTR, AU, BS and AR failed to reach adult form in the Q. robur seedling leaves at the third sample although characters LN, LDR, V, HR and OB were of the adult type. The final sample of Q. petraea seedling leaves was also different from adult leaves in characters LL, PL, PTR, LN, LDR and AR but not in characters V, HR, AU, BS and OB.

Anatomically, there appeared little difference between the seedling and adult leaves or between the first and third seedling sample, eg. Figure 4.5 shows the change in PTh through the three sampling periods. CL and SD proved exceptions. CL was lower in the first sample than the third, and both samples were significantly lower than the adult leaves. Since PTh is remarkably similar in samples of seedling and adult leaves (see Table 4.3), individual cell size differences must be quite large to account for the CL and PTh values (see Table 4.4).

	Average length of palisade cell (PTh/CL)	
	Q. robur	Q. petraea
Sample 1	17.78 ± 1.62	17.39 ± 1.58
Sample 3	14.25 ± 1.47	14.08 ± 1.46
Adult	12.98 ± 1.53	13.61 ± 1.43

TABLE 4.4 AVERAGE LENGTH OF PALISADE CELLS IN SEEDLING AND ADULT LEAVES (× 2.35 = μ).

These differences are much greater than observed between the different canopy sites noted in Chapter 2, and suggests a major difference between adult and seedling leaves. Initially, SD was high, but decreased

	Sample 1		Sample 3		Adult (SLS open)	
	Q. petraea	Q. robur	Q. petraea	Q. robur	Q. petraea	Q. robur
LL	29.64	28.62	75.33	53.62	98.62	95.21
PL	3.38	3.42	7.50	4.57	15.61	2.78
PTR	9.81	9.25	11.07	12.58	7.32	35.26
LN	4.46	4.58	5.11	4.87	5.71	4.95
LDR	2.79	2.76	2.38	2.28	2.78	2.12
V	5.85	29.43	6.10	40.83	8.01	48.61
HR	3.79	1.08	3.78	1.16	3.76	0.91
AU	3.76	3.17	3.67	2.27	3.36	0.32
BS	3.71	3.74	3.81	2.74	3.34	0.34
OB	1.93	1.85	1.91	1.71	1.96	1.63
AR	8.99	7.71	23.42	13.95	34.23	30.72
TTh	67.26	70.48	69.33	69.36	78.92	77.42
PTh	31.13	33.24	31.97	32.30	35.67	36.85
MTh	21.72	23.81	22.36	23.25	28.96	25.47
ETh	14.48	13.41	14.99	13.81	14.28	15.09
PR	0.463	0.478	0.449	0.466	0.452	0.476
MR	0.322	0.318	0.328	0.335	0.367	0.329
ER	0.215	0.198	0.218	0.199	0.181	0.195
CL	1.790	1.869	2.269	2.266	2.620	2.840
SD	13.25	13.74	11.35	10.98	12.97	12.62

TABLE 4.3 MEAN CHARACTER VALUES FOR SEEDLING AND ADULT LEAVES OF Q. robur and Q. petraea

(See Appendix 3 for units of measurement)

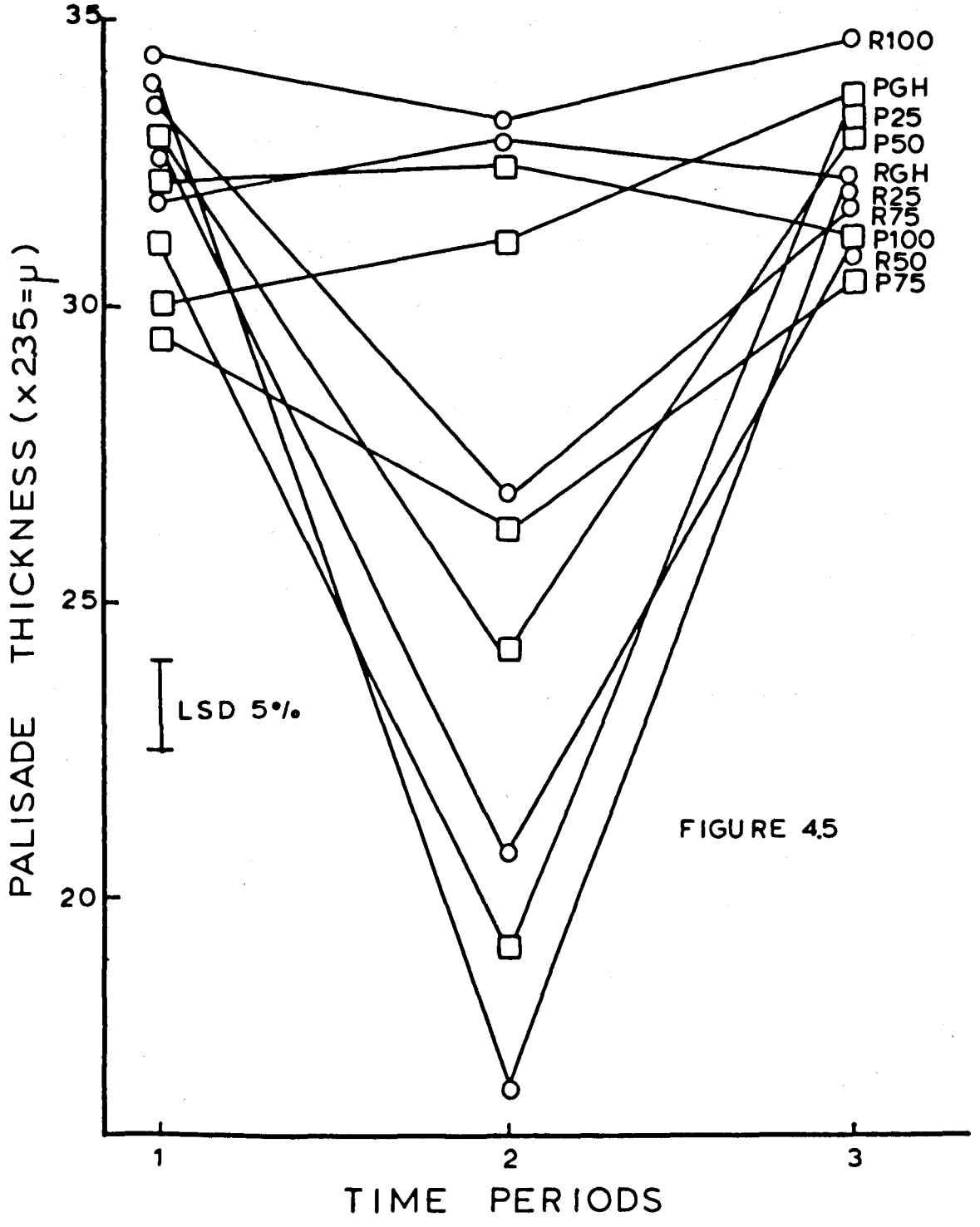


FIGURE 4.5

FIGURE 4.5 Change of seedling leaf characters with time: Palisade thickness
1 = all seedlings under greenhouse conditions
2 = seedlings under different light intensities
3 = all seedlings under greenhouse conditions
LSD 5% = least significant difference of means at 5% level
See Appendix 3 for standard deviations
○ Q. robur; □ Q. petraea

significantly by the third sample and this is due probably to a general increase in leaf area that occurred at the same time. However, if this argument is extended to its logical conclusion, adult leaves which are much larger than even the third seedling sample (see Table 4.3) should have an even lower SD. Unfortunately they do not, the SD of adult leaves falling midway between that of the first and third seedling sample. It would appear, therefore, that the relationship established between SD and AR for adult leaves in Chapter 2 is different from that between SD and AR for seedling leaves.

Seedling leaves are some 10% thinner than adult leaves and this appears to be due to thinner palisade and spongy mesophyll layers but not to any large differences in epidermal thickness. Major differences between the species with respect of their anatomical characters are not particularly obvious, a general conclusion broadly in agreement with the findings of the investigation of anatomical characters discussed in Chapter 2.

b) Effect of light intensity on seedling leaves

It is possible here to consider the results of the two light intensity experiments, the first in which four light intensities were used and the second in which five light intensities were used, but the results for the second growth chamber experiment were similar to those for the first, and therefore these will be discussed together. The growth chamber light intensities represented 2-20% daylight, the range of light intensities normally encountered by seedlings in nature.

Most characters responded to changes in light intensity, the exceptions being V, HR, BS and AU, which remained relatively unchanged at the different light intensities (see Figures 4.6 and 4.7). Other characters showed a response to changing light intensity - the leaves under lower light intensities being thinner, with thinner palisade

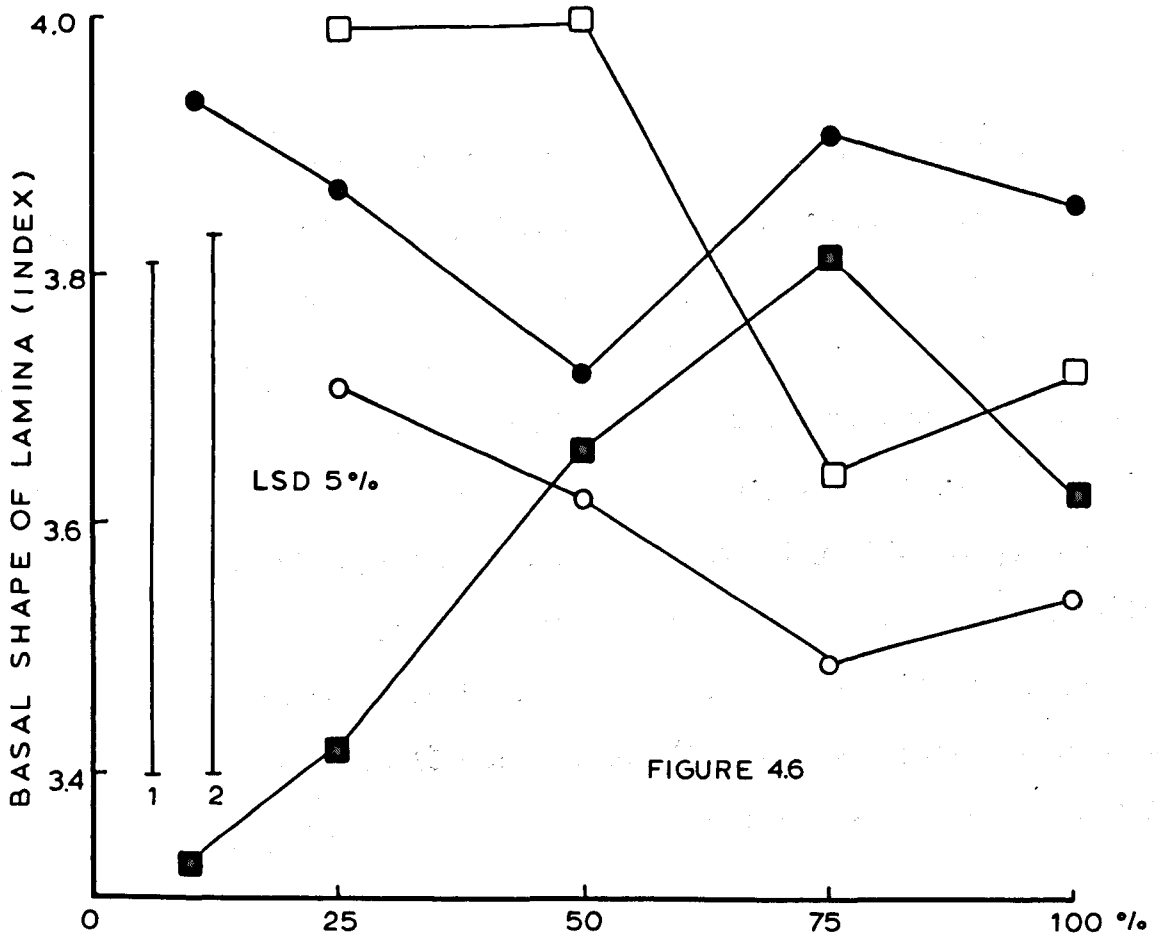


FIGURE 4.6

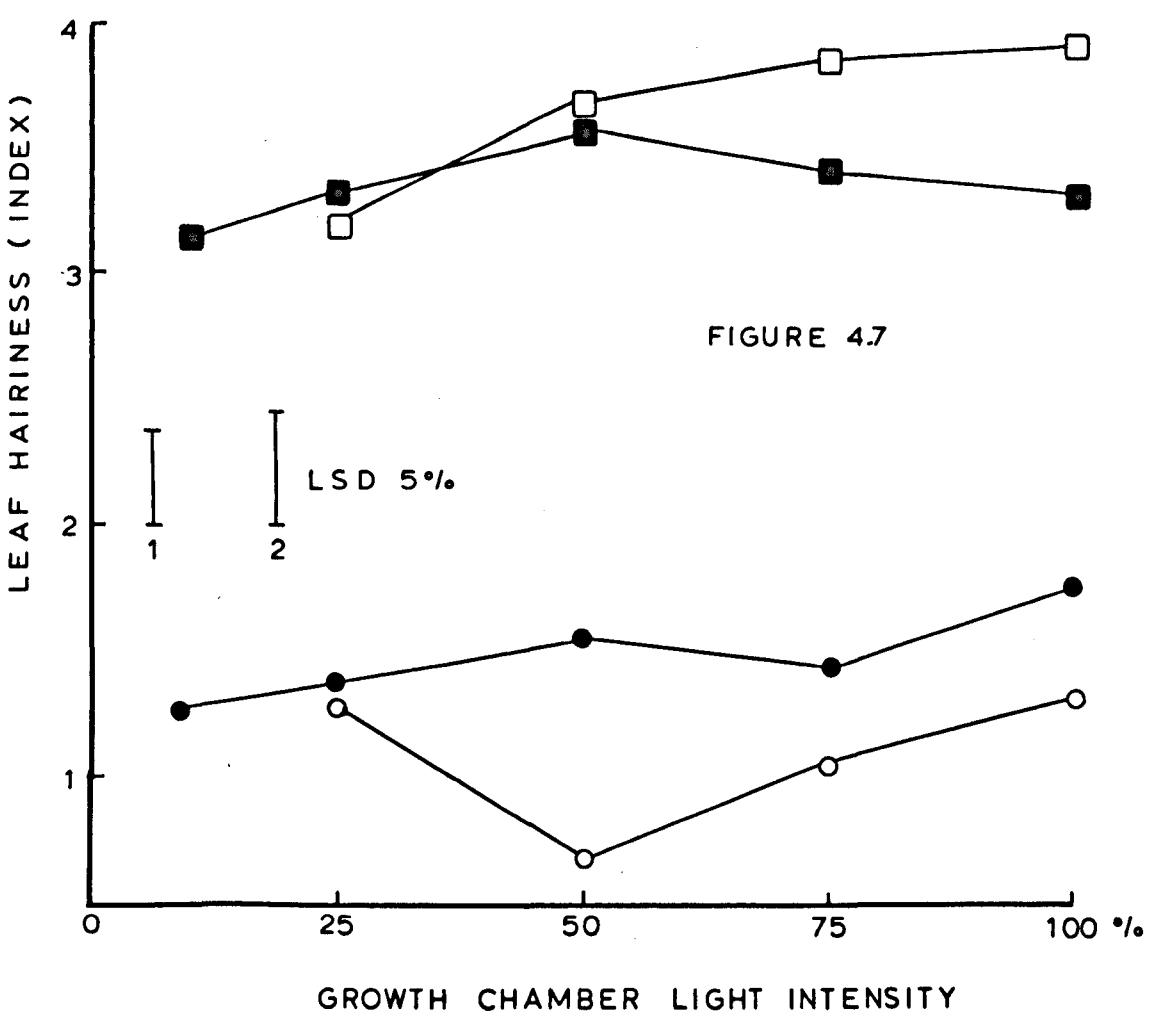


FIGURE 4.7

GROWTH CHAMBER LIGHT INTENSITY

FIGURES 4.6 and 4.7 Change of seedling leaf characters with light intensity; Basal Shape of Lamina and Leaf Hairiness respectively

○ Q. robur 18 months old; □ Q. petraea 18 months old
● Q. robur 9 months old; ■ Q. petraea 9 months old

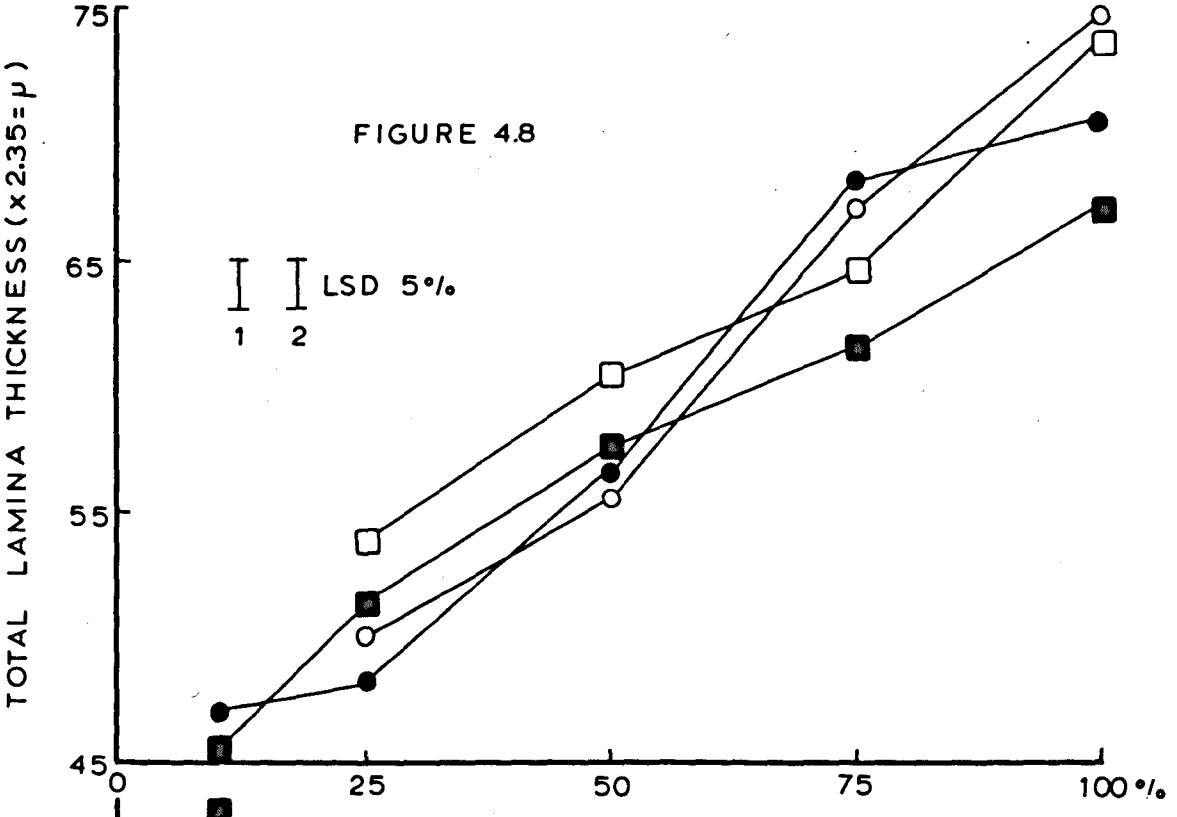
LSD 5% = least significant differences at 5% level for 1, the 18 months old seedlings and 2, the 9 months old seedlings

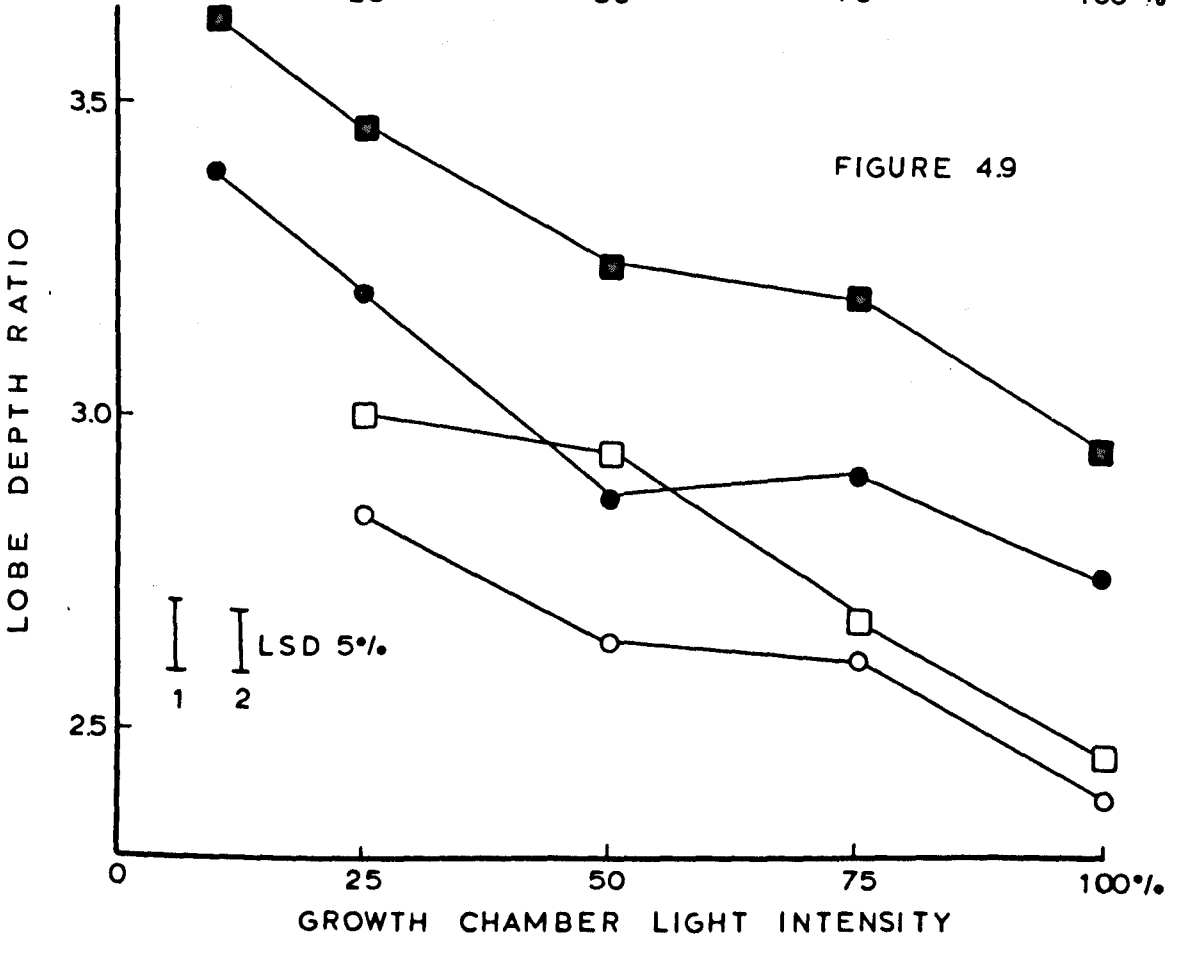
See Appendix 3 for standard deviations

tissue, thinner epidermis, thinner spongy mesophyll, larger MR, smaller PR, with a lower CL and SD, shallower, fewer lobes and a smaller PTR (Figures 4.8 and 4.9 show the change of TTh and LDR with light intensity respectively). These differences parallel closely the differences observed between the sun and shade leaves of mature trees described in Chapter 2. Since the conditions in the growth chamber were constant between treatments apart from the light intensity difference, the results presented here would provide very good support for the theory that light intensity is the major environmental factor in determining canopy leaf differences. Nescjarovič and Smirnova (1969) have reported that seedlings of Q. robur from eastern, southern and south-western areas of Russia had a more xeromorphic leaf structure than those from north and north eastern areas, suggesting that the differences between leaves under different light intensities reportedhere have wider geographical application.

Two characters, AR and LL, did not show a simple decline or increase with light intensity (Figure 4.10 shows the change of AR with light intensity). Leaf area and lamina length were both small at the high light intensities, increased with decreasing light intensity, but decreased again at the very low light intensities. Such a gradient is apparent within mature forest canopies. The leaves in the deepest parts of the canopy (not collected during the sampling for Chapter 2) are very small, and poorly developed, very like those from the lower light intensities of this experiment. Cowart (1936) found that apple leaves had their largest area at the median part of the branch but that the leaves in the densest part of the canopy were very small. Reed and Hirano (1931) also described the largest leaves of citrus trees being one third to one quarter of the distance from the base to the shoot apex.

Other characters produced somewhat anomalous results. Two





FIGURES 4.8 and 4.9 Change of seedling leaf characters with light intensity; Total Lamina Thickness and Lobe Depth Ratio respectively

○ Q. robur 18 months old; □ Q. petraea 18 months old
● Q. robur 9 months old; ■ Q. petraea 9 months old

LSD 5% = least significant differences at 5% level for 1, the 18 months old seedlings and 2, the 9 months old seedlings

See Appendix 3 for standard deviations

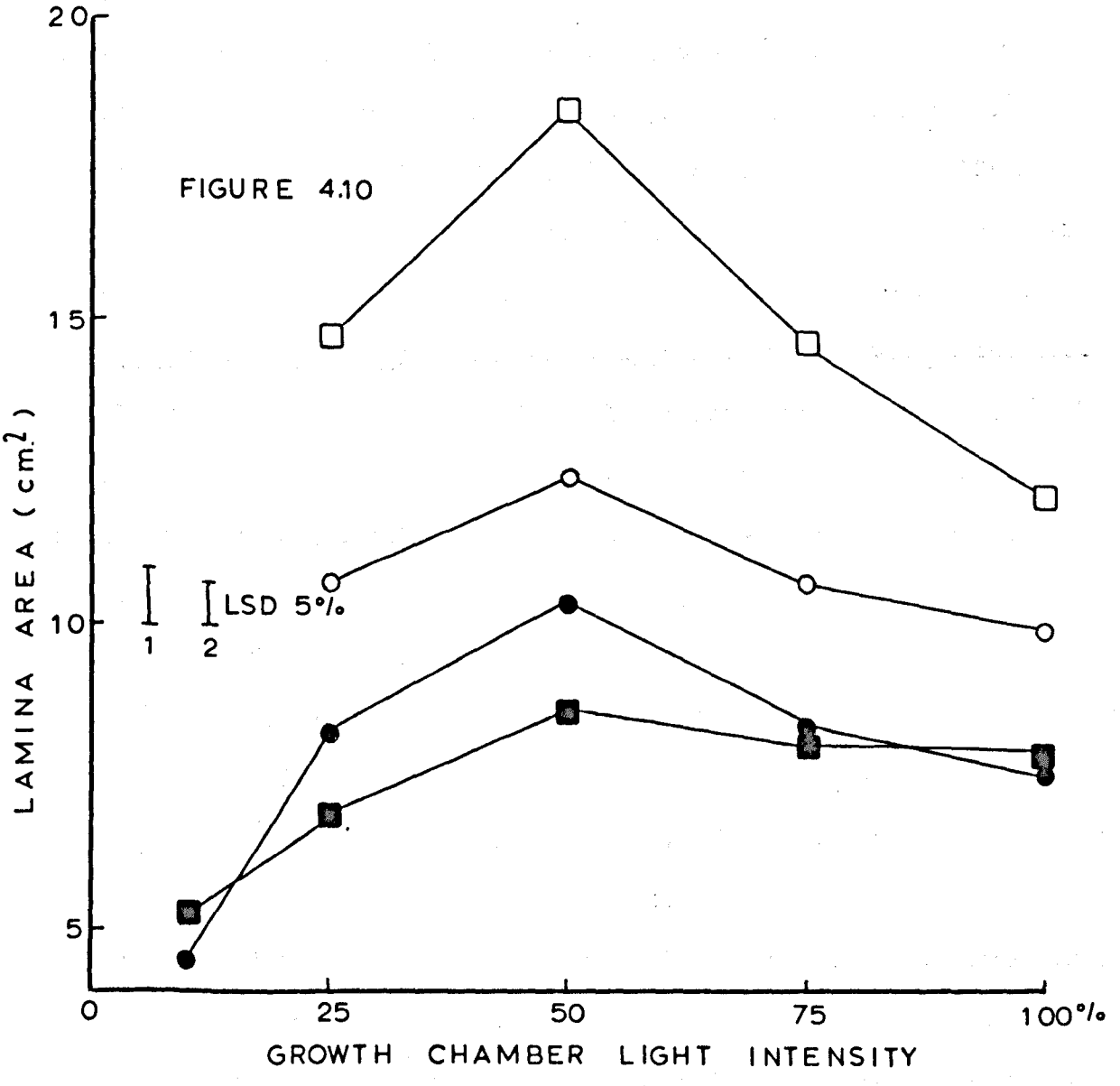


FIGURE 4.10 Change of seedling leaf characters with light intensity; Leaf Area
○ Q. robur 18 months old; □ Q. petraea 18 months old
● Q. robur 9 months old; ■ Q. petraea 9 months old
LSD 5% = least significant differences at 5% level for 1, the 18 months old seedlings and 2, the 9 months old seedlings
See Appendix 3 for standard deviations

characters, PL and OB, showed increase, and one character ER showed decrease with decreasing light intensity, but this response was only observed in the experiment using the older seedlings (i.e. the one using four light intensities), the three characters showing different behaviour in the second experiment. PL increased with decrease of light intensity until the 10% light intensity where PL began to fall (in both Q. robur and Q. petraea). This would appear to be related to the area and lamina length differences at low light intensities noted above. OB varied much more in the experiment with younger seedlings with little apparent pattern and this might possibly be due to the changes of leaf area, and the etiolation effect of the very low light intensities affecting leaf shape. In the experiment with older seedlings, ER generally declined with decrease of light intensity, but the same pattern was not observed in the younger seedlings where Q. robur increased and Q. petraea decreased with light intensity. No particular reason, other than the different ages between the seedlings of the two experiments was considered to be responsible for the behaviour of these characters, although modifications of both gross morphology and anatomy can be brought about by changes of light quality (Miller et al, 1968), and the apparently abnormal response of these characters might be due to the quality of the growth chamber light.

c) Residual Effect

The possible influence of the previous growing conditions on leaf form can be deduced from the results of these experiments by careful inspection of the data. If the previous conditions do have a major effect on leaf form then some evidence should show in the following year's leaves, eg. leaf thickness under uniform growth conditions at the third sample should still show differences, and differences of the same type, as the leaves when grown under the different light intensities.

Two characters, V and LN, were closely examined since these had been shown in the previous chapter to be characters predetermined at the bud stage and therefore likely to be influenced by previous, not current environmental parameters. There appeared to be no correlation between the third sample of leaves and the light intensities of the second sample. Initially, a surprising result, there is a possible explanation.. Lobe depth in the seedling leaves can be very shallow, particularly in the early stages of the seedling's growth, and since lobe depth is the only criterion apart from the presence of a vein that the taxonomist has for determining whether a lobe is present or not, the lobe depth is important in recognising the number of lobes. As Jones (1959) points out, the leaves of oak seedlings may be oval or only sinuately lobed. Lobe depth is also affected by environmental factors to some degree, and this might well mask the presence of lobes. Venation is obviously correlated with anddependent upon lobe number, since the number of veins are expressed as a ratio of the number of lobes and can therefore be influenced by difficulty in detection of lobes. One other possible explanation might be that other environmental factors other than light intensity operative in the bud stage are responsible for predetermining LN and V in canopy leaves.

Of the other characters, only CL showed the response expected of a 'predetermined' character, i.e. the response of the character in the third sample was correlated with the response of the character in the second sample. During leaf expansion, (see Figure 3.8) CL increased with time but only in those leaves in sun or open conditions, not in the shade or closed conditions. The seedling leaves were all experiencing low light intensities in the growth chamber even at the 100% light intensity (= 21% daylight), and this is reflected in the CL values in the growth chamber compared to the first and third greenhouse samples

(see Table 4.5), i.e. the growth chamber seedlings have a lower CL than even the first sample.

	Sample 1	Sample 2 *	Sample 3
Q. robur	1.869 ± 0.371	1.665 ± 0.313	2.266 ± 0.516
Q. petraea	1.790 ± 0.388	1.663 ± 0.392	2.269 ± 0.475

* including all seedlings except the greenhouse controls

TABLE 4.5 MEAN NUMBER OF CELL LAYERS IN THE PALISADE TISSUE OF SEEDLING LEAVES

If there were a true residual effect, then the changes between the different light intensities from Sample 2 to Sample 3 could be expected to be the same. This is not shown in the data (see Table 4.6). The seedlings at the lower growth chamber light intensities show very much greater changes between Sample 2 and Sample 3 than the ones at higher growth chamber light intensities (see Table 4.6).

	Q. robur	Q. petraea
100%	0.337	0.290
75%	0.364	0.534
50%	0.548	0.783
25%	0.890	0.675
Control	0.480	0.301

TABLE 4.6 CHANGE IN THE NUMBER OF CELL LAYERS OF THE PALISADE TISSUE OF SEEDLING OAK LEAVES BETWEEN THE SECOND AND THIRD SAMPLE

Since the differences of CL at the third sample between treatments are only just significant (see Appendix 3) and because of the arguments presented above, it cannot be concluded that CL is a character showing a true 'residual' response. As noted in Chapter 3, Avery (1933) showed that the cell layers are well established when the leaf primordia is very small, but Isanogle (1944) reported changes of the number of cell layers as leaves expanded. The results presented here would support the findings of Isanogle, i.e. the number of cell layers is not predetermined.

General Conclusion

It is possible, using careful measurements to distinguish the seedlings of Q. robur and Q. petraea although both the light intensity of the habitat of the seedling and the seedling's age must be taken into account. This is somewhat unfortunate, since it creates difficulties in recognising hybrid seedlings. The light intensity difficulty may be overcome, but the age problem is more difficult to solve. Grazing frequently reduces plants to ground level, but these are able to regenerate from buds in the axils of the cotyledons (Jones, 1959). Consequently, a small plant need not be a young plant, and little is known of the leaf form of such regenerated seedlings. If they continued to produce leaves of seedling type, they would present no problem; if, however, they produce more mature type leaves, they would present important problems in the detection of hybrid seedlings.

The results presented and discussed in this chapter would lead to the general conclusion that light intensity is a major if not the major influence in determining morphological and anatomical differences between sun and shade leaves of oak. Such a conclusion has implications in sampling oak populations for the purposes of population description and analysis.

SECTION THREE

THE POPULATION

CHAPTER FIVE

THE OAK POPULATION: A TAXONOMIC INVESTIGATION

Introduction

The lack of any single diagnostic character capable of differentiating Q. robur from Q. petraea would indicate that if hybrids are to be successfully detected within populations, a multivariate approach is required. The analysis of pictorialised scatter diagrams noted in Chapter 1 is in one sense a multivariate method because it enables the taxonomist to view the whole range of variation exhibited by a population for all characters measured, at one and the same time. Such diagrams (in practice) rarely permit a multivariate view since no matter how well-trained the observer is, he cannot assimilate all the possible relationships between characters and individuals on such a diagram. PSD's also represent 'information loss', i.e. in reducing the raw data, measurements, etc. to a form convenient for encoding onto the metroglyp, results inevitably in individuals with different measurements for a character being given the same index score with a resultant loss of information about these individuals. The arbitrariness of the choice of axes for the construction of the PSD would also appear to be contrary to the movement of objectiveness in modern taxonomy. Refinements to the PSD in special circumstances, eg. the 'Introgression Path' of Cousens (1965) although of some possible practical value represent at present only a theoretical assessment of a complex situation, which requires further evaluation before final acceptance. Love and Nadeau (1961) have also argued against the methods which rely on the research worker having to grasp difficult, variable situations, and suggest the use of the Hutchinson polygraph in which the radiating spokes of a circle represent characters, and variation is expressed by marking on each spoke the corresponding character

expression and joining these to form a polygonal figure. The method has been used successfully on Xanthium for recognising hybrids and for investigating introgression in Empetrum (Love and Nadeau, 1961).

The analysis of population material must inevitably be multivariate if the population structure is to be understood, but few oak taxonomists have availed themselves of the wide range of multivariate analyses at present in use in the fields of numerical taxonomy, genecology, ecology, etc. Such analyses have been successfully employed in the study of a wide range of complex situations from soils classification (Bidwell and Hole, 1964), autecology (Van Andel and Nelissen, 1973), and problems of fossil fish classification (Hemmings and Rostron, 1972) through to more purely taxonomic studies, eg. Homopteran taxonomy by Boratyński and Davies (1971). Indeed the use of such analyses has been advocated for many years (Fisher developed his discriminant analysis as early as 1936), and particularly recently, emphasis has been placed on the usefulness of multivariate methods in the study of variation in taxonomic research. Jeffers (1967a) has recently reviewed the techniques available for the study of variation in what are essentially taxonomic problems, and Pettet (1965) has used one multivariate technique, Factor Analysis to study variation in the genus Viola.

Of the investigations at present reported into introgression between Q. robur and Q. petraea, only Wigston (1971) has applied multivariate statistics in the form of discriminant function analysis. Few investigations of introgression have resorted to the use of multivariate analysis - Ledig et al.(1969) being one of the few exceptions. The methods of classification, using cluster analysis and of ordination using principal component analysis represent a variety of different lines of investigation which have proved successful in previous studies into problematical taxonomic situations, eg. Jeffers and Richens (1970).

This chapter reports an investigation into the variation observed within populations of British oaks, using the techniques of cluster analysis, principal component analysis and discriminant function analysis.

Methods of Numerical Taxonomy

Methods of Description:

The fundamental unit of numerical taxonomy is the operational taxonomic unit or OTU which represents the lowest ranking taxon employed in a given study. They may be, therefore, individuals, populations, subspecies, species, genera, etc. In the present study, the OTU represents individuals or populations. In order to carry out analyses on OTU's, each OTU must be scored for a list of characters or attributes to produce an OTU x character matrix. (The two terms characters and attributes will be used synonymously in this thesis, although some taxonomists would distinguish between them. Sneath and Sokal (1973) provide a full discussion on differences between characters and attributes, and some of pitfalls in using the terms.)

Taxonomists are faced, therefore, with a choice as to which characters are to be used. The view taken in this work was to use as many characters as possible with the proviso that they did not infringe the legality of taxonomic characters as documented by Sneath and Sokal (1973) and that they should be quickly scored, with little possibility of error. The characters chosen were generally those used previously and described in Chapter 2. Details of these characters are discussed later in this chapter.

Character weighting, i.e. the emphasis on one character rather than another has caused great concern among numerical taxonomists. A posteriori weighting may be justified, but Sneath and Sokal (1973) would argue that a priori weighting is objectionable on the grounds that is presupposes

knowledge either about the classification of the organisms or about presumed significance of their characters. Sneath and Sokal (1973) fail to point out, however, that characters are naturally weighted. To choose a character in preference to another weights that character, and although numerical taxonomists would argue for the inclusion of all possible characters in a given study, not all characters can be included for many reasons. For example, a character that is time consuming to measure, or expensive to measure, would be ignored. Even those characters finally chosen for study will inevitably be weighted - measurements, by nature of their variability, would be weighted in any analysis of variability unless steps are taken to standardise the data. This is not to advocate a priori weighting of characters, but to point out that no scheme of choosing or assessing characters will be without weighting - weighting is inherent in both the choice and assessment of characters, and to believe it is not is misunderstanding the whole process of character sampling and character judgement.

A posteriori weighting can be developed as a consequence of multivariate analysis, since these frequently determine which characters are useful in separating groups, and how useful in relation to other characters. In numerical form, these represent possible weights, although the use of weighting is so disreputable in numerical taxonomy that few studies have applied a posteriori character weighting from one analysis to another. The view taken in this thesis is that numerical a priori weighting is unjustified, but realising that weighting has probably already been introduced into the analysis by character choice, by the method of scoring etc. Although a posteriori weighting is not attempted, the importance of characters in differentiating populations is discussed, and from the principal component analysis, numerical weights (the eigenvectors) are derived, but their value is not assessed.

Methods of analysis

The three techniques to be used, Discriminant Function Analysis (DFA), Cluster Analysis (CA) and Principal Component Analysis (PCA) differ in both their initial view of the data, and in the results of the analysis. They all, however, use the initial N OTU x m variable matrix. It is possible to consider the placement of each OTU in an m-dimensional hyper-space where each dimension m represents a single variable. If m were equal to 2, then the space would be contained in a simple bivariate scatter diagram. It is the analysis of this multi-dimensional hyper-space that is the important factor in multivariate analysis.

Discriminant Function Analysis:

The technique of DFA is to test the validity of groupings previously defined or to test the validity of assigning new samples to pre-existing groupings. The analysis considers a population assessed for m variables to form a cluster of points in an m-dimensional space. Other populations also described by the same m variables can also be considered in the same m-dimensional space. The analysis determines an (m-1)-dimensional plane that effectively separates the two clusters and this plane is the discriminant function. Individuals within each cluster become placed in one or other of the groups depending on which side of the (m-1)-dimensional plane they fall. In reality, the discriminant function is chosen as a linear function (designated as Z) of a series of characters originally used to describe the OTU's that weight the characters so that as many OTU's from one group have high values for Z, and as many OTU's from the other group have lower values for Z.

The basic steps of the analysis are:

1. Calculation of the variances and covariances between characters to produce an m x m matrix. These are used to produce a pooled within-groups variance-covariance matrix, W.

2. Calculation of the discriminant function Z by multiplying the inverted matrix W^{-1} by a vector a:

$$Z_{JK} = W^{-1}a_{JK}$$

where

Z_{JK} = the discriminant function between J and K, a vector quantity

W^{-1} = inverted matrix W

a_{JK} = $(\bar{X}_{1J} - \bar{X}_{1K}), (\bar{X}_{2J} - \bar{X}_{2K}) \ldots\ldots (\bar{X}_{mJ} - \bar{X}_{mK})$

Where $\bar{X}_{1J}$ = character centroid for taxa J, character 1, etc.

3. The vector Z consists of a series of 1 to m weights for characters 1 to m, which may be multiplied through the observed character values of an individual b, to produce a discriminant score:

$$DS_b = Z_1X_{1b} + Z_2X_{2b} + \ldots\ldots Z_mX_mb$$

4. Reference scores for taxa may be obtained from the centroids for the taxa:

i.e. $DS_J = \bar{X}_JZ$

$DS_K = \bar{X}_KZ$

A score for the midpoint between the taxa may also be calculated as:

$$DS_{MP} = \tfrac{1}{2}(\bar{X}_J + \bar{X}_K)\,Z$$

5. The value of DS_{MP} defines a plane midway between the centroids, and consequently discriminant scores falling to one side would be allocated to taxon J, those to the other side to taxon K. The individual discriminant score DS_b can thus be adjudged in its relationship to taxa J and K.

6. The analysis also permits the membership of groupings to be tested in statistical procedures.

It is not proposed here to explore the mathematical procedures of DFA, excellent accounts already exist, eg. Cooley and Lohnes (1971) who provide worked examples and a computer program.

DFA represents the only multivariate procedure used by oak taxonomists - Wigston (1971) and Ledig et al. (1969). Ledig et al. (1969) were able to show using DFA that the analysis yielded few individuals exactly intermediate between the two parental types, Q. prinus and Q. alba, but yielded a bimodal peak, the two modes being one either side of the bi-parental mean suggesting that backcrosses occur in both

directions. A larger proportion of types between the bi-parental mean and the Q. primus mean did, however, suggest there was more gene flow to Q. primus. Wigston (1971) has used the DFA in combination with PSD whilst studying oak populations in south-west England. This enabled him to conclude that the populations of both species exhibited the same range of variation, and that some hybridisation was evident. DFA has been used on a variety of material, eg. Jolicoeur (1959) examined geographic variation in the wolf using skull measurements and DFA, and Rostron (1972) has used the related and more recently developed study of canonical variates to investigate taxonomic delimitation of gazelles using skull measurements, but the analysis is not as widely used in taxonomic research as the following analyses.

Cluster Analysis (CA):

DFA requires a taxonomy for analysis, CA on the other hand creates the taxonomy de novo. The basis of CA is that the OTU's are grouped together on the evidence of their similarity, but different measures of similarity and different methods of grouping results in the number of clustering techniques available being almost infinite. Sneath and Sokal (1973) discuss the 'taxonomy' of different clustering methods, and these can be summarised thus:

1. Agglomerative v. Divisive methods

Agglomerative techniques seek to group OTU's together in successively larger groupings, starting with the case where each OTU is a separate group, and ending with all OTU's in the same group. Divisive methods assume all OTU's belong to the same initial group, and seek to subdivide this group until either each subgroup contains only one individual or until some stopping criterion is applied. The latter technique is represented in ecology by Association Analysis (Williams and Lambert, 1959, 1960) and will be used in the following chapter.

2. Hierarchic v. Non-hierarchic classifications

As normally used, a hierarchical system is one where the groups of a taxonomy are always subgroups of higher groups, but Sneath and Sokal (1973) have recently argued that in the strictest definition of a hierarchical system not all members of a subgroup must belong to the same higher grouping. Non-hierarchic classifications are those in which subsidiary taxa do not become members of larger, more inclusive taxa.

3. Overlapping v. Non-overlapping classifications

In overlapping classifications, taxa at any one rank are not mutually exclusive, i.e. OTU's may be members of more than one taxon. This is illegal in non-overlapping systems.

A series of agglomerative, hierarchical, non-overlapping techniques were evaluated at the start of this work, and from these the most useful were chosen. The basis of all these clustering methods is as follows: The analysis considers the OTU's to consist of N groups, or clusters with one OTU per cluster. At each of the N-1 fusion steps, those two clusters which are most 'similar' are combined. Similarity is determined by means of a variable parametric transformation of the similarity coefficients between OTU's. This transformation is expressed as:

Let two clusters P and Q be fused, then the similarity between a cluster R and the new cluster (P+Q), i.e. S(R,P+Q) can be obtained from:

$$S(R,P+Q) = AP * S(R,P) + AQ * S(R,Q) + B * S(P,Q) + G * |(S(R,P) - S(R,Q))|$$

where AP, AQ, B and G are given different values in different analyses

Seven initial clustering methods were used, with the following values:

1. Ward's Error Sums of Squares Method:

$$AP = (NR + NP)/(NR + NP + NQ)$$
$$AQ = (NR + NQ)/(NR + NP + NQ)$$
$$B = - NR/(NR + NP + NQ)$$
$$G = 0$$

2. McQuitty's similarity analysis:

   $AP = AQ = 0.5 \qquad B = G = 0$

3. Gowers median method:

   $AP = AQ = 0.5 \qquad B = -0.25 \qquad G = 0$

4. Single Linkage (Nearest Neighbour)

   $AP = AQ = 0.5 \qquad B = 0$
   $G = -0.5$ (if dissimilarity coefficient used) or $G = 0.5$ (if similarity coefficient used)

5. Complete Linkage (Furthest Neighbour)

   $AP = AQ = 0.5 \qquad B = 0$
   $G = 0.5$ (if dissimilarity coefficient used) or $G = -0.5$ (if similarity coefficient used)

6. Average Linkage:

   $AP = NP/(NP + NQ)$, $AQ = NQ/(NP + NQ)$, $B = G = 0$

7. Centroid method

   $AP = NP/(NP + NQ)$, $AQ = NQ/(NP + NQ)$, $B = -AP * AQ$, $G = 0$

   where NP, NQ and NR = cluster sizes, and B and G are parameters or constants whose value is determined by the analysis.

This permits the evaluation of a similarity coefficient to be computed from previously evaluated similarities without the necessity to return to the original similarity matrix.

At each fusion cycle therefore the two most similar clusters are joined, and so on until all OTU's belong to the same cluster. The most useful clustering method was found to be Ward's Error Sums of Squares method used with Squared Euclidean Distance as the similarity coefficient.

This transformation is defined as the sum of the distances from each individual to the centroid of its parent cluster, and fusion occurs between those two clusters P and Q whose fusion yields the least increase in the error sum. The other methods are fully discussed in Sneath and Sokal (1973). Williams (1971) reviews the present state of cluster analysis particularly emphasising some of the difficulties.

Of seemingly wide application, cluster analysis has not been used often in hybrid studies, its use tending to be restricted to description rather than the problem solving investigations usual in hybrid studies. Examples of its purely classificatory use can be found in Sneath and

Sokal (1973). CA has been used fairly extensively in plant taxonomy, eg. Hsiao (1973) has studied the clustering of OTU's of the genus Platanus using both morphological and leaf and fruit phenolic characters.

Principal Component Analysis (PCA):

Williamson (1972) recognises the objective of PCA to be the partitioning of the variance within rows of a data matrix into new variables, the Principal Components. These new variables are extracted from the data such that the first accounts for the largest variance that can be found, and the second orthogonal to the first and accounting for the largest amount of the remaining variance and so on. The following sequence of calculations and examinations characterise the extraction of Principal Components:

1. Construction of the basic data matrix consisting of N individuals and m variables.
2. Transformation of the basic data if required. Standardisation of taxonomic data is normally obligatory since characters are a mixture of lengths, weights, indices, etc.
3. Calculation of an m x m matrix which can either be a covariance matrix (if each character standardised to zero mean) or a correlation matrix (if each character standardised to zero mean and unit variance).
4. Calculation of the eigenvalues (or latent roots) and the eigenvectors (or vectors) of the covariance or correlation matrix.
5. Examination of the eigenvalues, and their interpretation. Each eigenvalue is normally calculated with a value of the percentage of variance for which the eigenvalue accounts. Examination of these percentages permit the researcher to determine how much variation is accounted for by say 2, 3 or 4 eigenvalues. Frequently, the number of eigenvalues accounting for a large part of the original variation is much smaller than the original number of variables.
6. Examination and interpretation of the eigenvectors. The eigenvectors represent the weighting given to each of the basic variables. These can be used to determine which variables are important in contributing to the extracted eigenvalues. These can be expressed in several standardised forms - so that the sum of the squares of their elements equals unity (Williamson, 1972), or so that the maximum element is unity (Jeffers, 1967b) or as here so that the sum of the vectors squared equals the corresponding latent root.
7. Calculation of transformed values by multiplying the transformed data matrix and vector matrix to produce the Principal Components matrix in which each individual is located along each new component axis. These axes may be plotted against each other in pairs, and the distribution of individuals examined. The number of axes

examined will depend on how much variance they account for, if the first two axes extract say 90% of the variance, these represent good summaries of the data.

The Principal Components represent, therefore, an attempt to summarise in as few dimensions as possible the relationships which existed between individuals in the original multidimensional space, with the minimum amount of distortion of these relationships.

The mathematics of PCA are detailed in Cooley and Lohnes (1971) who also provide a worked example and computer program.

PCA has many applications. It has been used for example in relating tree growth to environmental parameters (White, 1972), in the analysis of the geographical distribution of zooplankton (Colebrook, 1964), and in more taxonomically oriented studies of Jeffers (1964, 1967b). It has been used also by Gardiner (1972) in the study of variation in birch populations, a similar use to the one reported here.

As a method of analysis, PCA is frequently used in conjunction with cluster analysis, i.e. the same data is both clustered and analysed by PCA, so that in trying to evaluate the PCA ordination, the classification produced by clustering can be super-imposed, and correlations between the analyses sought. This is now accepted practice in ecological research, and some examples exist in taxonomy, eg. Lubke and Phipps (1973), Sims (1966).

Sampling Populations

The choice of populations to be sampled should be random but two factors would militate against this. Firstly the 'known' populations that would normally be included in a population sample either because they represented an extreme type, or had previously been sampled for other purposes or indeed a variety of reasons. Secondly, the problems of sampling a population of populations, when the distribution of the

populations is unknown. It is possible to generate random geographic co-ordinates for a given area, but there is little likelihood of finding an oak population at the generated co-ordinate, and finding the closest oak to a given point might well take some time. Consequently, a rather liberal view was taken of the random sample.

It was argued that in the first instance, Botanical Recorders of the Botanical Society of the British Isles would know of oak woodlands in their recording area that would be possible sites; they might also know the history and management of woodlands that were available. A circular was sent to these Recorders, and from their replies, a list of possible populations was compiled. Before this, however, a decision had to be made on geographically limiting the sample. Birmingham was the original base for the investigation, and it seemed sensible to concentrate on The Midlands, Wales and East Anglia, since little previous work had been done in this area and it provided a complete range of soil types, altitude and areas of stability and disturbance. The area was delimited therefore by the 52° 30' N line of latitude in the north and the 51° 30' N line of latitude in the south. This was extended south in south Wales around Barry and Cardiff, since to divide off a small part of Wales as does the line of latitude seemed rather arbitrary. The total area was, therefore, 200 kilometres from north to south and almost 500 kilometres from east to west. The list of possible populations was restricted to populations found in the defined limits. These populations were supplemented by others in the following manner: whilst visiting these 'known' populations, likely-looking sites derived from the Ordnance Survey 1" to 1 mile maps (which distinguish coniferous and deciduous forest) were also visited and, if oak, were sampled. In this way, it was found possible to produce a good coverage of populations over the geographical area without the time wastage inevitable in a purely random

collection.

A list of the 135 populations sampled with their code letters, grid reference and name is given in Appendix 4.

Definition of a Population

A perennial problem in sampling in genecology is the problem of defining a population, and more importantly the bounds of a population. Davis and Heywood (1963) give the commonsense definition as "any group of individuals considered together at any one time because of features they have in common" but this is not applicable to sampling problems. The definition given by population geneticists as "a reproductive community of sexual and cross-fertilising individuals which share in a common gene pool" is more useful, but still difficult to interpret in the field. Quercus is a wind pollinated genus, and pollen flow can occur over a wide geographical area. For example, Semerikov and Glotov (1971) have investigated the degree of genetic isolation shown by populations of Q. petraea and determined that trees on opposite sides of a glade full of shrubs and 500 m wide belong to the same population, the distance and obstacles not providing a barrier to pollen transport. Under less sheltered conditions, therefore, the pollen may well spread much further. In large forests of oak spreading over many square miles, it might be argued that trees at either side are reproductively isolated and therefore in different populations. The view taken here would be that such trees would be considered as members of different sub-populations.

A working definition of an oak population is therefore proposed as a group of trees spatially isolated from other groups of oak trees by at least 1 kilometre. Samples from large groups of trees, spreading over large geographical areas are considered as sub-populations of the main population as too are groups of trees closer than 1 kilometre apart.

Certainly, the possibilities of gene flow between sub-populations and populations may still be quite high particularly under conditions favourable to long distance pollen transport.

The sampling of individual populations

The investigation of variation of leaf morphology and anatomy in oak canopies (Chapter 2) although useful in determining the range of variation expected of a particular genotype did not prove particularly useful in deciding where to sample a particular tree, since each site seemed as variable as every other. There did appear to be a greater difference between the species at the sunnier parts of the canopy, and since Cousens (1963) had sampled from the south aspect, this position was chosen for sampling purposes. Each tree was sampled, therefore, at a height of 6 m on the southern aspect.

Finney and Palca (1949) have reported edge effects in sampling forests, and attempted to eliminate such effects using the Laurie sampling scheme. In work of the type presented here, complex techniques were to be avoided due to the large number of populations to be sampled. A small pilot scheme was completed, therefore, in which the taxonomic status of a population was assessed from trees within the centre of a forest and from trees at the edge. A hybrid index (see later) was calculated for each tree, and a frequency histogram produced for the two samples of the population (Figure 5.1). The edge sample was found to be significantly different from the centre sample, and in the Q. robur population chosen for this study, the edge sample was found to be more towards the extreme Q. robur, a possible effect of the extreme environmental conditions at the edge of a forest. For this reason, samples were carried out wherever possible inside the forest, avoiding the edge. Normally, 50 trees were sampled in the population, but on occasions, due

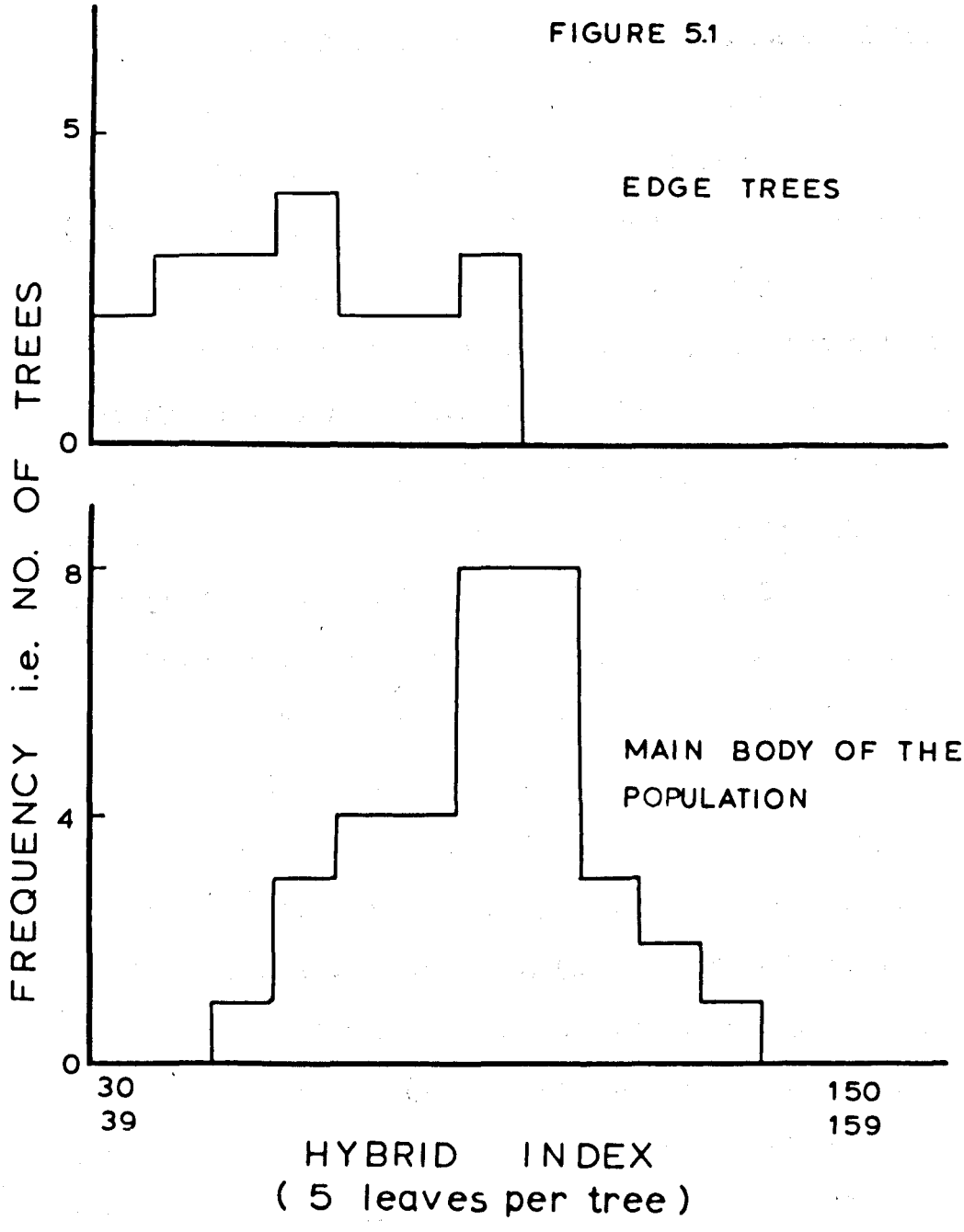


FIGURE 5.1 The 'edge' effect in sampling oak populations. The histograms record the Hybrid Index for two samples of the same population, using only edge trees and trees in the centre of the population

to lack of time or the small size of the population, this was reduced accordingly, usually to 25 trees. A small branch was removed from each tree from the correct height and aspect, transported back to a laboratory, where five leaves were later removed from each branch in the mid-shoot region, pressed in an herbarium press and oven-dried. After this, the leaves from each tree were stored in envelopes in herbarium boxes until they could be scored.

The number of trees and leaves sampled in a population is critical in characterising the population. The number of leaves used varies greatly from 2 (Cousens, 1963), 10 (Carlisle and Brown, 1965) to 16 (Benson et al. 1967), but in few cases is the number ever justified. Experience during the intensive canopy sampling had persuaded the present author that the samples used in that study were possibly too large, and that five leaves would have been sufficient to characterise a canopy site, although the mean value of the larger sample would probably have had a higher degree of confidence attached to it. Five leaves were subsequently used for the population sample. The number of trees is also important, since too small a sample might result in missing a small percentage of hybrids, whilst too large a sample might result in too heavy a work load. The tree sample size is regulated to some extent by the population size itself - it is thought that the sparse distribution of oaks in Scotland meant that populations were small and hence the small samples of Cousens (1963) (a range of 6-31 trees with a mean sample size of 13 trees), but his samples of Yugoslavia (Cousens, 1965) were of the same size (a range of 10-54 trees with a mean of 23 trees). The sample sizes of Roudsea Wood made by Carlisle and Brown (1965) were 118, 54 and 48 trees, but for the purposes of detailed analysis, these were reduced to 20. Wigston (1971) used leaf litter samples, a useful technique, since it meant that samples could be collected through the

year, but the results presented here in Section 2 and the possible influence of lammas shoot production might pose problems in a definitive assessment of the taxonomic status of such oak populations. Furthermore, Wigston(1971), because of his sampling method, had to treat each leaf as an OTU, it being impossible to examine the range of variation observed within an individual.

Taxonomic characters

The characters used for determining the taxonomic status of the oak woods varied from those previously described in Chapter 2 when investigating variation of leaf structure in canopies in two important respects:

1. The addition of new, and the deletion of characters
2. The use of linear measurements as well as ratios

The second is an important point, since in most taxonomic studies, quantitative characteristics such as petiole length, lobe depth, etc. are corrected for leaf size by expressing them as a ratio. However, there are no a priori reasons for supposing that the uncorrected characters might give just as good a taxonomic separation as the corrected characters. In the present study, therefore, single measurements have been used as well as the ratios derived from them, since not only may they be useful in separating the species, but by only using ratios, 'information' is lost. Jeffers and Richens (1970) have used similar arguments against the use of ratios in the study of elm populations, but they also disregarded ratios for the actual analysis of their results. Sokal and Hunter (1955) too have used individual measurements, but they introduced ratios into their computations as in the present investigation. The following characters were scored for each leaf for each population:

Leaf regularity

The lobe characteristics of Q. robur have been described by

Jones (1959) as very variable, the leaf possessing an irregular outline, whilst Q. petraea is described as having a much more regular outline. Such differences are difficult to quantify, and therefore the following qualitative scale was used:

A perfectly regular leaf scored 4, and one unit was deducted for each of the irregularities listed below:

1. Presence of sub-lobes, i.e. subsidiary lobes developed on the sides of the main lobes
2. Lobe depths of corresponding sides of the leaf markedly different
3. A different number of lobes on one side of the leaf from the other
4. The leaf outline of the left and right side of the lamina varied markedly

A leaf showing all the above traits scored zero, a leaf showing two of the traits scored 2, and so on.

Basal shape of the lamina

As described in Chapter 2.

Auricle development

As described in Chapter 2.

Leaf hairiness

In Chapter 2, leaf hairiness was considered a single character, but for the purposes of the population work, it was considered to be two characters - Simple hairiness and Stellate hairiness. These were scored on an abundance scale, from 4 being the most abundant to 0 being a glabrous leaf.

Number of lobe pairs

As described in Chapter 2.

Sinus number

The number of veins to the sinuses was not considered a separate character in Chapter 2, but here it was used as an individual character.

Venation

As described in Chapter 2.

Petiole ratio

As described in Chapter 2.

Obversity

As described in Chapter 2.

Lobe depth ratio

As described in Chapter 2.

Individual lengths comprising the last three ratios noted above were also used as characters, these being: petiole length, lamina + petiole length, lamina length, length from the basal part of the lamina to the widest part of the lamina, width of the lamina at the widest part of the lamina, and the depth of the sinus for the lobe at or just below the widest part of the lamina. Of the seventeen characters, five were ordered multistate qualitative characters, the rest quantitative characters.

The Hybrid Index

It became necessary during the analysis of the populations to determine a hybrid index score for each character, so that individuals could be compared on a common basis. The qualitative characters, by the very nature of their multistate condition, and their original definitions, were in Hybrid Index form; all that remained as far as these were concerned was to confirm that the extreme states were representative of the two pure species. The quantitative characters had to be organised into an ordered multistate form, such that the extreme forms were also representative of the two pure species.

It was important, therefore, that reference material of the two species be obtained for comparative purposes and scored for the seventeen characters. This reference material was obtained from a variety of sources, namely herbaria, fresh material from specimen trees in arboreta

and botanical gardens and from trees sampled in the wild. Published accounts of the range of variation of individual characters were also utilised, as well as the results reported in Chapter 2. For each of the quantitative characters measured frequency histograms were constructed, in order to determine the separation of the species. The frequency histograms for each species were well separated for each character, and usually in the form of a normal distribution. These were divided into the following character states:

State	Description
0	A range encompassing the extreme 66% of the normal distribution of the Q. robur histogram.
4	A range encompassing the extreme 66% of the normal distribution of the Q. petraea histogram.
1,2,3	The range between the end of state 0 and state 4 was divided into three equal states, the one closest to Q. robur being 1, the one closest to Q. petraea being 3, and the intermediate state 2.

It should be noted that states 1 and 3 encompass part of the distribution expected of 'pure' species.

The qualitative characters were also checked to determine if they corresponded to the 'pure' species types - minor modifications were made at this time to these qualitative characters. The conversion of the quantitative characters to the Hybrid Index score is given in Table 5.1. The conversion of quantitative characters to a hybrid index is normally to be avoided, since it results in the loss of 'information' which might otherwise be important. The addition of characters, which the Hybrid Index permits, to give an overall value for an OTU and therefore allow OTU comparison can also be brought about by character standardisation, a technique that will be explored later in this chapter.

Hybrid Index

Characters	0	1	2	3	4
Lobe number	3	4	5	6	7
Sinus number	5	4	3	2	1
Venation	> 54.00	53.99 -42.00	41.99 -30.00	29.99 -18.00	< 17.99
Petiole length	< 3	4- 7	8- 11	12- 15	> 16
Lamina + petiole length	< 77	78-97	98-117	118-137	>138
Petiole ratio	< 3.00	3.01 - 6.00	6.01 - 9.00	9.01 - 12.00	>12.01
Length of lamina to widest part	< 48	49-56	57- 64	65- 72	> 73
Lamina length	< 74	75-90	91-106	107-122	>122
Obversity	< 1.505	1.506- 1.645	1.646- 1.785	1.786- 1.925	> 1.926
Lamina width	>39	35-38	31- 34	27- 30	< 26
Sinus depth	> 22	18-21	14- 17	10- 13	< 9
Lobe depth ratio	< 1.880	1.881- 2.120	2.121- 2.360	2.361- 2.600	>2.601

TABLE 5.1 CONVERSION OF QUANTITATIVE LEAF CHARACTERS TO A HYBRID INDEX SCORE

(All measurements in millimetres)

Reproductive characters

Six reproductive characters have been shown to be useful in characterising the oak species: acorn shape, acorn colour, acorn stripe, peduncle length, peduncle diameter and peduncle pubescence, but these cannot be scored on the same material. For example, acorn stripe cannot be scored on the mature acorn, but acorn colour can. Populations were mainly sampled late in the season, during late September and early October, when many acorns were already mature. Acorns collected in the green state matured naturally before they could be scored for acorn stripe, and also storage of the acorns for a long period before scoring meant that much of the dark colour of the acorns was lost, leaving acorns of both species a light fawn. Peduncle pubescence was also difficult to score since friction between the peduncle and adjacent branches may have removed the hairs. Cousens (1963) has noted a similar phenomenon. Q. robur, with a much longer peduncle, is more likely to be influenced by such treatment, and therefore it too proved a poor character.

Acorn material and peduncles collected during the population sampling were scored for three characters: acorn shape (as a ratio of length : width), peduncle length (from point of attachment to first acorn) and peduncle diameter (measured at the centre of the peduncle). Correlation between these characters and the seventeen leaf characters is presented in Table 5.2, using both raw data and the leaf data converted to a hybrid index. For this latter set of correlations, the reproductive characters too were converted to a hybrid index value based on herbarium material, and published accounts of the species. Peduncle length and peduncle diameter showed high correlations with a large proportion of the leaf characters (Peduncle length: 14 significant correlations with raw data, and 15 significant correlations with hybrid

	Raw Data			Hybrid Index Data		
	Acorn Shape	Peduncle Length	Peduncle Diameter	Acorn Shape	Peduncle Length	Peduncle Diameter
Leaf regularity	-0.044	0.305	-0.222	0.101	0.311	0.248
Basal shape	-0.305	0.531	-0.339	0.249	0.530	0.405
Auricle development	-0.279	0.489	-0.402	0.203	0.466	0.432
Simple hairs	-0.096	0.466	-0.359	0.079	0.500	0.384
Stellate hairs	-0.155	0.517	-0.427	0.141	0.538	0.433
Lobe number	0.052	0.310	-0.190	0.051	0.332	0.271
Sinus number	0.038	-0.347	0.339	0.010	0.515	0.415
Venation	0.000	-0.387	0.345	-0.029	0.541	0.443
Petiole length	-0.165	0.570	-0.405	0.168	0.625	0.472
Lamina + petiole	-0.163	0.358	-0.129	0.160	0.356	0.189
Petiole ratio	-0.142	0.520	-0.400	0.132	0.601	0.465
Lamina to widest part	-0.093	0.029	0.071	0.097	0.039	-0.061
Lamina length	-0.148	0.271	-0.045	0.134	0.264	0.107
Obversity	-0.081	0.368	-0.187	0.060	0.344	0.234
Lobe depth	0.075	0.036	0.146	0.021	-0.042	0.044
Lamina width	-0.152	0.211	-0.007	-0.111	-0.230	-0.147
Lobe depth ratio	-0.030	0.083	-0.159	0.153	0.212	0.265
Acorn shape	1.000	-0.126	0.044	1.000	0.156	0.077
Peduncle length	-0.126	1.000	-0.626	0.156	1.000	0.648
Peduncle diameter	0.044	-0.626	1.000	0.077	0.648	1.000

Significance levels: 5% - 0.159 1% - 0.208

TABLE 5.2 THE CORRELATION BETWEEN LEAF AND REPRODUCTIVE CHARACTERS

index data; Peduncle diameter: 12 significant correlations with raw data, and 13 significant correlations with hybrid index data). Acorn shape was less related to leaf characters showing only four significant correlations with both raw and hybrid index data. Since few populations were represented by large numbers of trees with reproductive material, and since the peduncle characters showed such high correlation with the leaf characters, the large scale analysis of populations was completed on leaf characters alone. The poor correlation between acorn shape and leaf characters was possibly due to collection of acorns in varying states of maturity mentioned earlier when specific shape differences had not been produced. It was possible to analyse 15 populations which were represented by leaf and sufficient reproductive material, and there was exceptional agreement between these results and those using only leaf characters.

The scoring of populations

Since several characters were of a qualitative nature, and since some of the quantitative characters relied on judgement in certain instances, eg. whether a vein was ajudged to go to a sinus or not, a system of rescoring and checking was introduced in order to verify that the 'judgement of the taxonomist' was not altering with time. The following procedure was, therefore, adopted:

After every 20 leaves scored, the first leaf was rescored, after every ten trees, the first tree was rescored, and after every eight populations, the first population was rescored. The degree of error acceptable during this process was as follows:

A rescored leaf - two characters, were allowed to deviate by one unit on the hybrid index score, i.e. a leaf scoring for example 40 (in a range of 0-68) could under rescoring be from 38-42, but no character could deviate more than one unit.

A rescored tree - the range of a tree could fall between 0-340. The acceptable error was 5 hybrid index units no more than two of which could be attributable to one leaf, i.e. a tree scoring for example 200 could under rescoring range from 195-205.

A rescored population - all trees had to lie within 5 hybrid index units of their original score.

If minor errors were found in scoring, these were easily rectified, but if major errors arose, this entailed completely rescoring up to eight populations. Such a situation did arise after a gap of several months between population assessment.

The analysis of the population data

A matrix of individuals x characters was prepared for each population on computer cards, one card per individual. This matrix was incomplete in the sense that characters derived as ratios of other characters were not incorporated on the card, but were calculated during data input to the various programs. The populations were first put through an initial data sorting program DATAC which performed the following elementary preliminaries to analysis proper:

a) Calculation of ratios, and printing of raw data matrix.

b) Calculation of character means, standard deviations, maximum and minimum values for the population.

c) Standardising the raw data such that the mean of each character was zero, and the variance unity. The standardised character values for each tree were summed and also output at this stage.

d) Conversion of the raw data to hybrid index values, and outputting this new matrix. The total hybrid index for each tree, and hybrid index character means, standard deviations, maximum and minimum values for the population were also output.

e) Standardising the hybrid index data such that the mean of each character was zero, and the variance unity. The standardised hybrid index character values for each tree were summed and also output at this stage.

f) A hybrid number for the population was calculated and output.

The hybrid number is a single value originally used by Gay (1960)

for comparing hybrid populations. It is derived from the hybrid index totals for each tree as:

$$HNO = \frac{\sum_{i=1}^{i=N} (HI_i \text{ or if } HI_i > 170,\ 340 - HI_i)}{N}$$

where HNO = hybrid number
N = population size
HI_i = hybrid index of the ith tree

It can be compared to the mean population hybrid index; if they are equal then no tree in the population exceeds the mid-point value between the extremes, if the hybrid number is greater than the hybrid index, some tree or trees have scored over the mid-point score, and may be of hybrid origin.

An initial analysis was to consider the frequency histograms of hybrid index scores and test these for normality - it being argued that if all characters for a population were normally distributed, and the population composed of 'pure' trees, the frequency histogram should not deviate from normality. The histograms were tested for normality using χ^2, and for skewness and kurtosis using parameters g_1 and g_2 (Sokal and Rohlf, 1969). The results for this analysis although useful in some instances were not good overall and the analyses were not used in the population investigation. The basic problem was the inability of the analyses to deal with the outlying individual. For example, a perfectly 'pure' population with an approximately normal distribution would be analysed incorrectly if a member of the other species were present. Such 'mixed' populations were frequent, leading to cases where the analysis could not be used. An alternative to the hybrid index frequency histogram was that of using standardised characters. The central problem to all hybrid index type researches is the inability to ensure that each character discriminates the species correctly, and that all intermediate values are correctly assigned. For example, if one or a small group of

characters consistently overestimated the intermediacy of a population due to poor establishment of the initial indices for that character or characters, then populations might be scored with a tail or skew, a situation reminiscent of the hybrid index 'tails' noted by Anderson (1949) as being indicative of introgression. In this case it would be due to poor establishment of indices, not hybridisation. This problem, which can be difficult to detect in large samples can be overcome by standardising each character to the same mean and variance, and then calculating individual totals and from this a frequency histogram. Such standardisation can be on raw data or hybrid index data, but it has the disadvantage that once calculated, it becomes impossible to compare different populations as they would have been standardised to the same mean and variance. Examples of standardised histograms are given, together with their corresponding hybrid index frequency histograms in Figure 5.2.

The reference population:

Discriminant function analysis requires a pre-existing taxonomy for its operation so that the analysis may test the validity of the taxonomy or fit new OTU's into the taxonomy. It was necessary, therefore, to create a taxonomy for the analysis. In reality, it became necessary to supply the analysis with two taxonomic groupings, the reference group and the test population and to determine if after the analysis any of the test group had been classified with the reference group. Several alternatives were available for the composition of such a group:

1. A 'population' where each individual was represented by characters scored from an herbarium sheet. Included here could also have been material from specimen trees.
2. A spurious 'population' artificially created from published accounts of the species diagnostic characters and their ranges.
3. The results for an actual population.

Neither of the first two alternatives was tenable, the first would have consisted of only 'pure' types, and populations never occur in this form

FIGURE 5.2

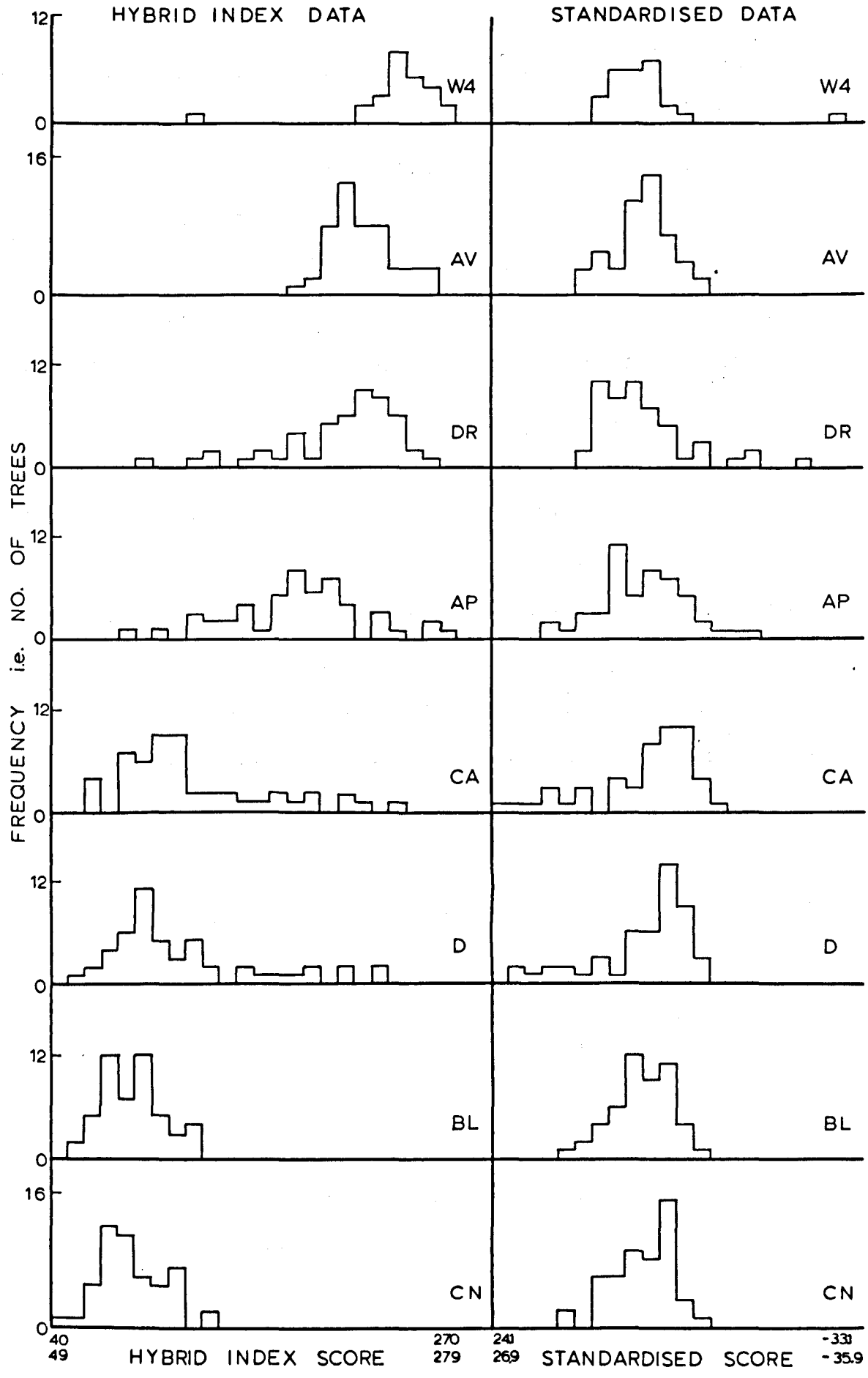


FIGURE 5.2 Hybrid Index frequency histograms for eight populations, together with histograms of the same populations in which each character for each population has been standardised to zero mean and unit variance, and totals calculated for each tree.
BL and CN = pure Q. robur populations
D and CA = introgressed Q. robur populations
AP and DR = introgressed Q. petraea populations
AV and W_4 = pure Q. petraea populations

except occasionally as a result of planting, the second would have been difficult to set up and almost certainly subject to bias. The third alternative was chosen, and the populations sorted for suitable candidates. It was finally decided to use two populations, one representing a 'pure' Q. robur, A_2 and one a 'pure' Q. petraea population, W_5. Both populations had been sampled on two consecutive years, and both had been used for subsidiary studies, so that the composition of the woods were intimately known. The population sample from population W_5, the Q. petraea population, had three Q. robur trees, although the population itself showed no signs of introgression or hybridisation. In using these populations for analysis therefore, only 22 trees were used for W_5, the three Q. robur trees having been removed, and 25 trees were used for A_2. It was anticipated that these populations might prove unusual and have to be discarded; in the event, they were retained for the complete analysis of the populations. Evidence is presented later in this thesis (Chapter 7, Table 7.3) that on pollen viability grounds these populations may also be considered to be of 'pure' status..

Having established these reference populations out of necessity, it was decided to use the same populations in the Cluster and Principal Component Analysis, as reference points. For example, a Cluster Analysis on a single population will produce clusters of OTU's, but these are only comparable with other populations if reference OTU's are included. Similarly with PCA, ordinations cannot sensibly be compared if they contain no common 'information' - the common information in this case being the reference populations.

The characterisation of populations

The basis of the present study was to gain an insight into the structure of the populations and to do this each population was analysed

in combination with W_5 and A_2, the proposition being that populations of similar composition to either W_5 or A_2 will cluster with them using CA, or fail to be discriminated from them with DFA or will ordinate with them using PCA. Populations diverging from W_5 or A_2 will similarly depart from them under these analyses. Spurious test data, in which artificially designed populations were analysed, worked well; the analyses distinguished 'odd' inidividuals and 'odd' populations consistently and as expected.

Each population was combined with W_5 and A_2 and analysed as follows:

1. DFA - the test population and two reference populations were set up as different taxonomic groups. The analysis was used to check the validity of these groups to produce a listing of the discriminant scores for each OTU, i.e. tree, and to produce a hit/miss table for group membership. Some associated tests of significance were also output by this analysis. The program used was contained in the ICL Statistical Package XDS3.

2. CA - the use made of CA was to combine W_5, A_2 and the test population, and to treat each tree as a separate OTU. Membership of clusters was determined at the end of the analysis, together with the character means and standard deviations of each cluster. The method used for this analysis was Ward's Error Sums of Squares method using squared Euclidean distance as the coefficient. A unified approach, using the same method for all populations, seemed sensible. This and other clustering analyses are available in CLUSTAN 1A, a suite of FORTRAN IV programs from the University of St. Andrews.

3. PCA - as with CA, the individual trees of W_5, A_2 and the test population were treated as separate OTU's. As previously argued, since taxonomic data was being employed only the results of the correlation matrix were considered, i.e. standardisation of both means and variances of the variables. The characters differentiating the populations were

investigated using the vector scores, and the proportion of variance accounted for by the Components was examined. Following Jeffers (1964) all Components which had an eigenvalue with an absolute value of less than 1 was ignored. This reasoning is based on the fact that if all the basic variables had been completely uncorrelated, all the eigenvalues would be close to 1 and consequently any Component with an eigenvalue of less than 1 is a Component accounting for a smaller percentage of the variability than would be represented by each of the basic variables separately. The distribution of the three populations was examined from plots of the individuals in Component space. The production of Component scatter diagrams is time consuming and consequently a plotting procedure was developed for the PCA program that produced scatter diagrams on the computer line printer. Divall (1973) has recently advocated this as an excellent time saving method. An ALGOL procedure called ACTUALPLOT that plots scatter diagrams is given in Appendix 5. Generally only the first three Components were examined and for the majority of the work the first two Components proved sufficient.

In order to understand fully the examination and characterisation of the populations, an illustrative example will be used in which four populations are considered, DI,AAA, AO and AL. Populations AL and AO are thought to represent an introgressed Q. robur population and an introgressed Q. petraea population respectively. During the course of the examination of the hybrid index frequency histograms, several populations occurred in which the majority of the trees were morphologically intermediate between the two species; DI is such a population. Another type of population was also observed during PCA. These populations, although having hybrid index frequency distributions typical of a 'pure' population, showed complete separation from the 'pure' type in the PCA. Populations of both Q. robur and Q. petraea could be observed of this type; AAA is an example

of a Q. petraea type population. Figure 5.3 shows the hybrid index frequency histograms for these four populations, A_2 and W_5. The analyses discussed below have all been based on the hybrid index data matrix. Although all three analyses have been computed on both the raw data matrix and the hybrid index data matrix, the latter is more convenient to use for discussion purposes, since it allows mean values of the hybrid index to be calculated for each tree so that comparisons may be more easily made. (It should be noted perhaps at this stage that the use of terms 'introgression' and 'pure' are dangerous, since there is little evidence to support their use. They are used here for convenience - the evidence presented at the latter part of this chapter and in Chapter 7 lend support to the proposal that there are indeed 'pure' and 'introgressed' populations, and that these can be recognised by the analyses presented here. The use of such terms now pre-empts those conclusions.)

Populations A_2 and W_5

Initially, these populations were analysed together without test populations, in order to determine if the populations separated well, with no intermediate trees. The resultant cluster analysis for these two populations is given in Figure 5.4. As can be seen, at the ten cluster level, the two populations are each separated into five clusters. There is some evidence that three Q. robur trees of population A_2 are an extreme group (Cluster 9, mean HI = 73.9) and that six Q. petraea trees of population W_5 are an extreme group (Cluster 5, mean HI = 268.3). The two groups of five clusters do not join until the last fusion cycle. In PCA, the population scatters are well separated on the first Component which accounts for 56.11% of the total variance (see Figure 5.5). The distribution of the individuals along the first Component is given in Table 5.3, and again the populations are seen to be well separated. The characters responsible for this separation would appear to be all except

FIGURE 5.3

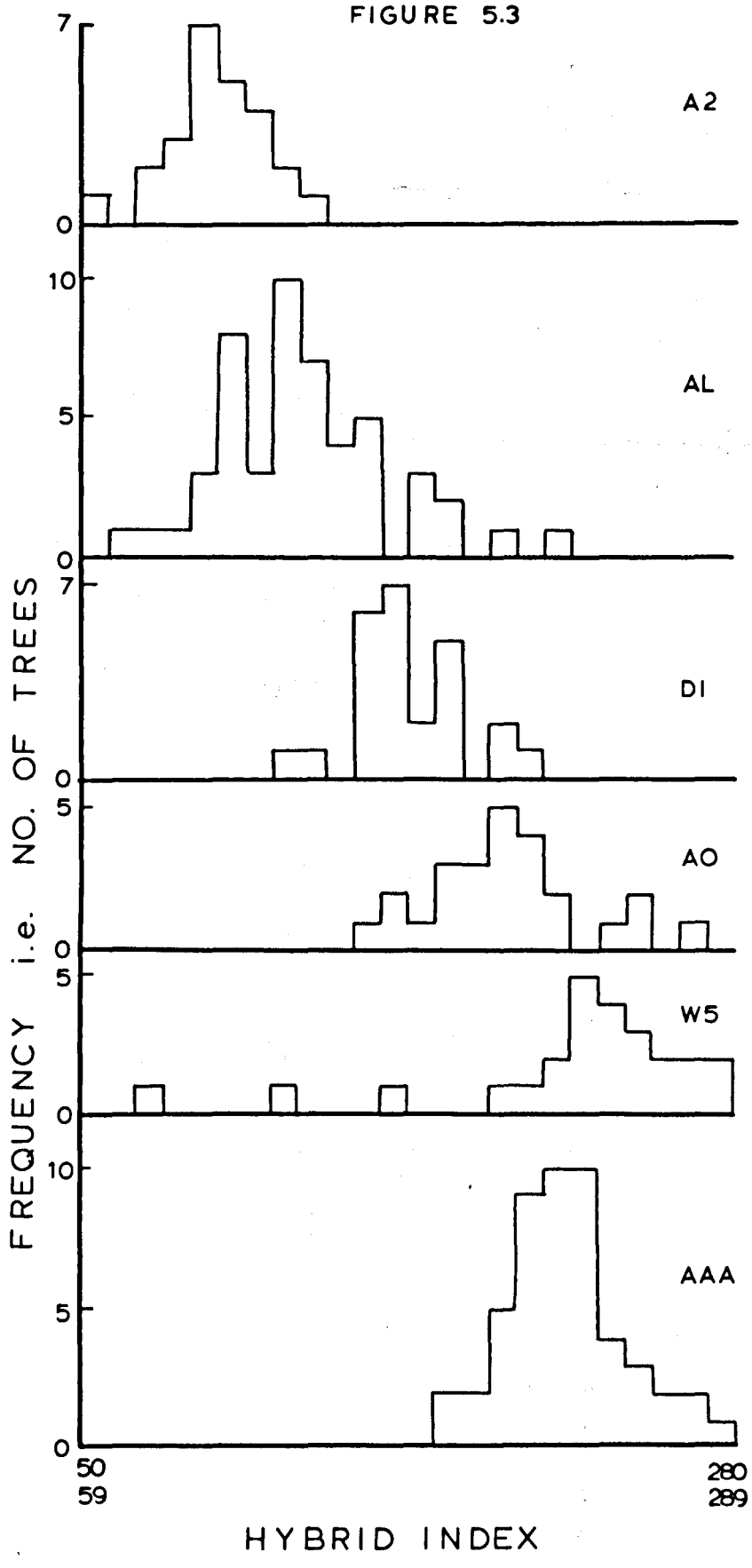


FIGURE 5.3 Hybrid Index Frequency Histograms for the reference populations A_2 and W_5 and the example populations used in the text. (Each tree represented by 5 leaves, 17 characters per leaf.)

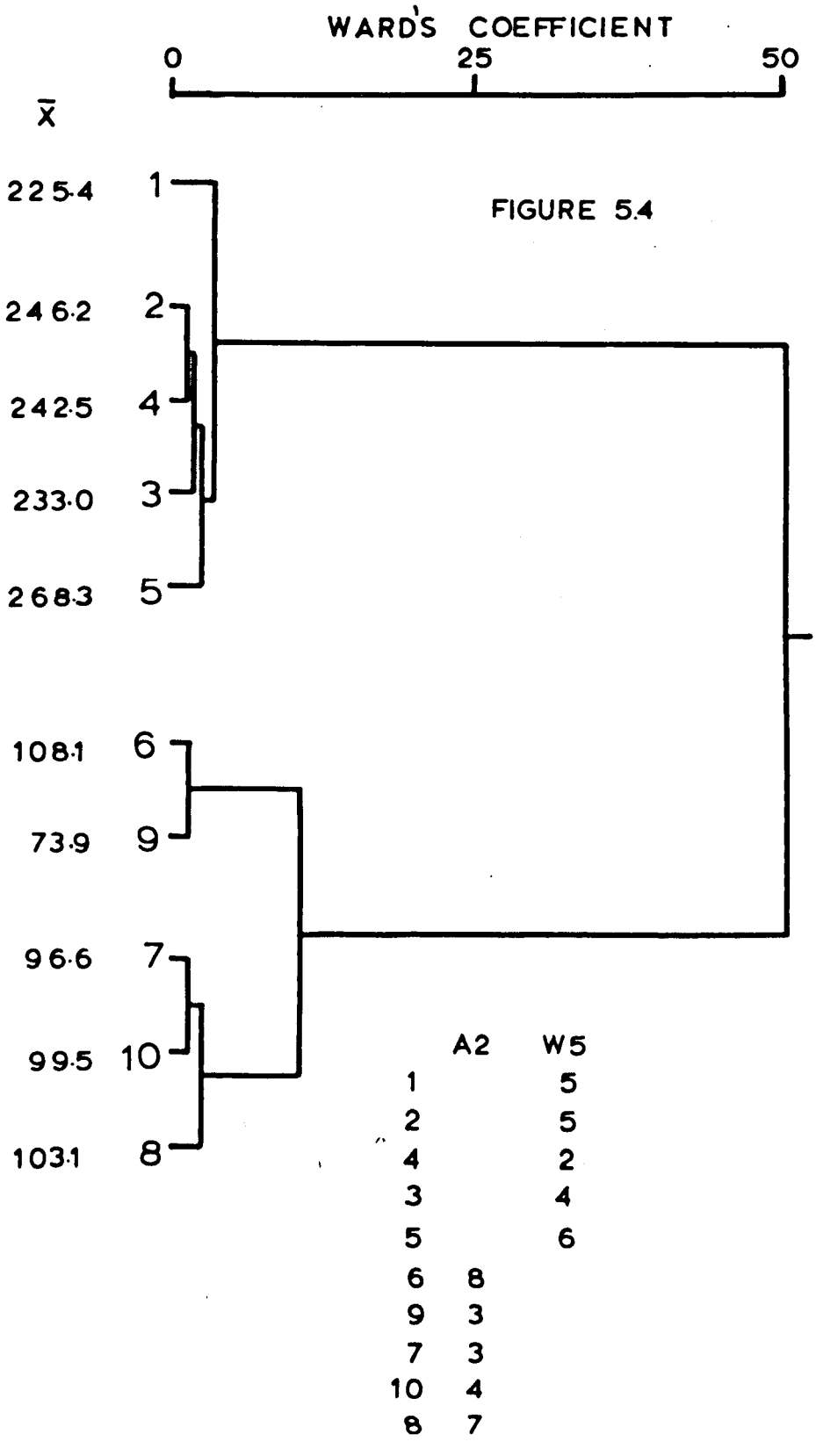


FIGURE 5.4

FIGURE 5.4 Ward's Error Sums of Squares Cluster Analysis on the reference Populations A_2 and W_5. The table at the bottom of the figure gives cluster membership; $\bar{x}$ refers to the Hybrid Index mean of each cluster.

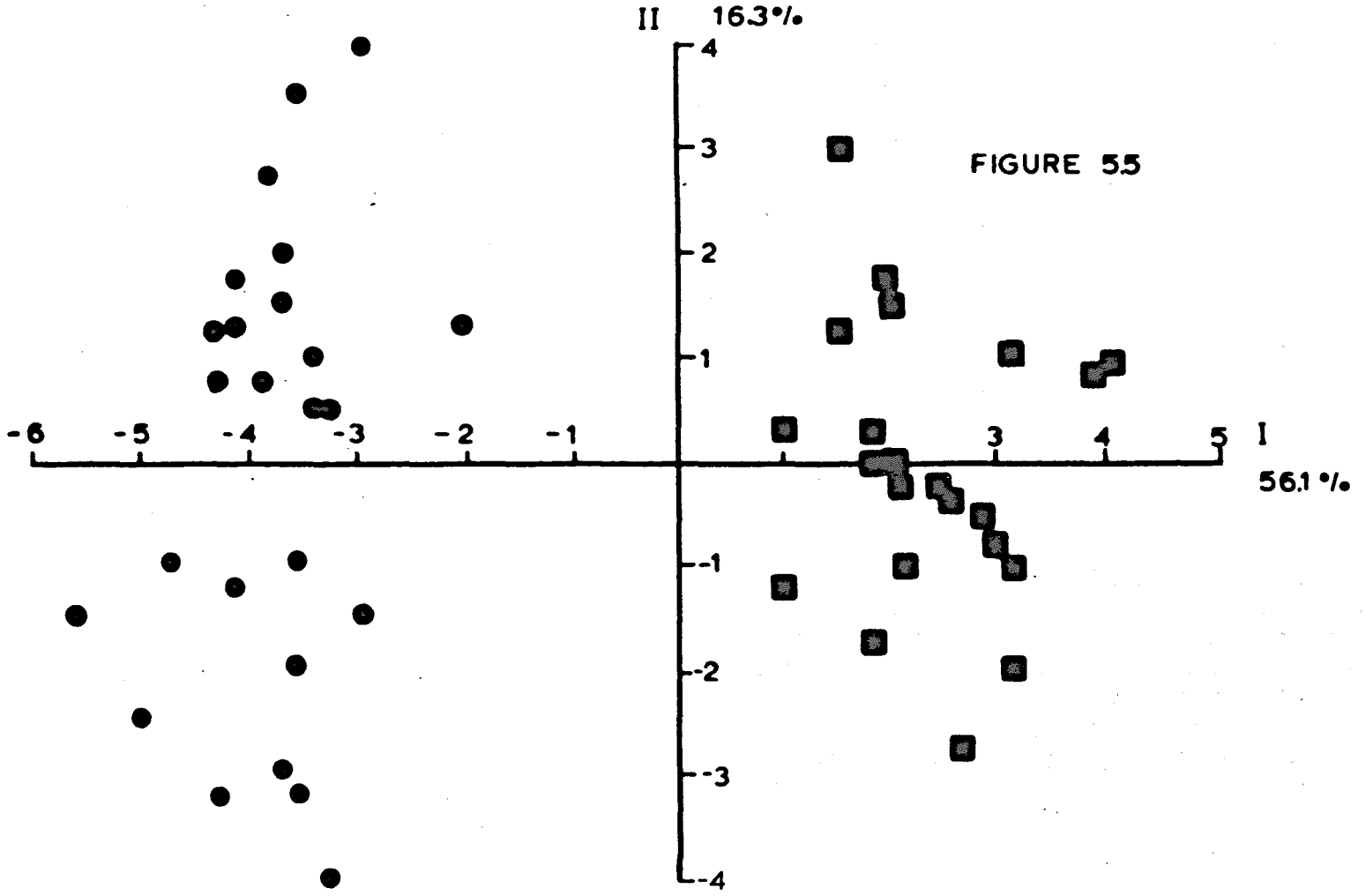


FIGURE 5.5 PCA of individual trees of the reference populations A_2 (pure Q. robur ●) and W_5 (pure Q. petraea ■)
The first two Components of the correlation matrix are shown, together with the amount of variance accounted for by each Component.

Component 1 Zones	A_2	W_5	A_2	W_5	AL	A_2	W_5	AO	A_2	W_5	DI	A_2	W_5	AAA
1	3			8		3			4				9	
2	21			12		21			20				11	12
3	1			2	2	1			1		9		2	36
4					4			9			13			2
5		4	1		18		4	10		4	3	4		
6		15	21		24		13	5		13		12		
7		3	3		2		5	1		5		9		

Component 2 Zones	A_2	AAA	W_5
1	1		2
2	1		4
3	3		9
4	6		5
5	3	4	1
6	4	10	1
7	4	19	
8	3	10	
9		7	

Note: for the sake of clarity, the difference between the maximum and minimum scores on the first Component has been divided into seven zones to produce the above distributions. The actual values can be seen on the corresponding PCA scatters (Figures 5.5, 5.8, 5.11, 5.14 and 5.16). Component 2, for population AAA has been treated similarly, but divided into nine zones.

TABLE 5.3 DISTRIBUTION OF INDIVIDUALS ALONG THE FIRST COMPONENT OF PRINCIPAL COMPONENT ANALYSES OF THE POPULATIONS SHOWN

lamina length to the greatest width and lamina width, the latter showing a negative correlation while the rest are positive (Table 5.4). DFA (Figure 5.6) also clearly separated the groups, with no overlap, the mean discriminant scores being -0.624 for A_2 and -2.622 for W_5. In this analysis, since there were only two initial groups, the single discriminant function accounted for all the variance in the data.

Populations AL and AO

From the hybrid index frequency histograms (Figure 5.3) both populations appeared to show evidence of a skewed distribution with several trees in the intermediate zone between the species, AO being a possible introgressed Q. petraea population, AL being a possible introgressed Q. robur population. Using CA (Figure 5.7) many of the individuals of AL clustered with A_2 in groups 1,2,3 and 5, but a number of them formed almost exclusive groups - clusters 4,6,9 and 10, and three of these clusters 6,9 and 10 with individuals of mean HI of 142.7, 190.8 and 126.5 grouped with each other before joining with a mixed AL/A_2 group. These three groups, containing 20 AL trees, could be thought of as being of possible hybrid origin. None of the AL individuals fused with the two W_5 clusters, 7 and 8. When subjected to PCA (Figure 5.8 and Table 5.3) AL produced a 'typical' reaction for this type of population. The largest group of the population remained with the bulk of the A_2 population, but in the intermediate zone between the species, there appeared another large group, well separated from population W_5, but also slightly shifted to one side of the A_2 population. Further beyond this group was another small group that tailed off into population W_5. In this case the first two Components accounted for 64.58% of the total variance. Again, all characters except lamina width and lamina length to the widest part were important in separating the groups (Table 5.4). DFA did not always produce results compatible with the

Eigenvalue	Percentage Variance accounted for by the first three Components				
	W_5	AL	AO	AAA	DI
1	56.11	45.29	49.02	42.90	46.70
2	16.33	19.29	17.84	27.39	19.61
3	7.71	9.42	8.91	7.41	8.32
TOTAL	80.15	74.00	75.77	78.70	74.63
	Eigenvectors - standardised so that the sum of the elements squared equals the eigenvalue				
Leaf regularity	0.80	-0.60	0.73	-0.74	0.70
Basal shape	0.88	-0.80	0.78	-0.88	0.76
Auricle development	0.86	-0.78	0.75	-0.85	0.78
Simple hairs	0.83	-0.73	0.80	-0.78	0.74
Stellate hairs	0.89	-0.83	0.79	-0.77	0.82
Lobe number	0.49	-0.42	0.52	-0.55	0.46
Sinus number	0.93	-0.83	0.87	-0.85	0.86
Venation	0.92	-0.82	0.89	-0.92	0.86
Petiole length	0.94	-0.92	0.94	-0.70	0.91
Lamina + petiole	0.77	-0.69	0.74	-0.31	0.71
Petiole ratio	0.93	-0.90	0.91	-0.81	0.86
Lamina to widest part	0.25	-0.22	0.22	0.09	0.21
Lamina length	0.63	-0.53	0.60	-0.18	0.56
Obversity	0.41	-0.38	0.41	-0.44	0.40
Lobe depth	0.52	-0.35	0.39	-0.57	0.39
Lamina width	-0.34	0.32	-0.36	-0.01	-0.35
Lobe depth ratio	0.76	-0.69	0.68	-0.64	0.70

TABLE 5.4 EIGENVALUES AND VECTORS FOR THE PCA OF REFERENCE AND SOME TEST POPULATIONS

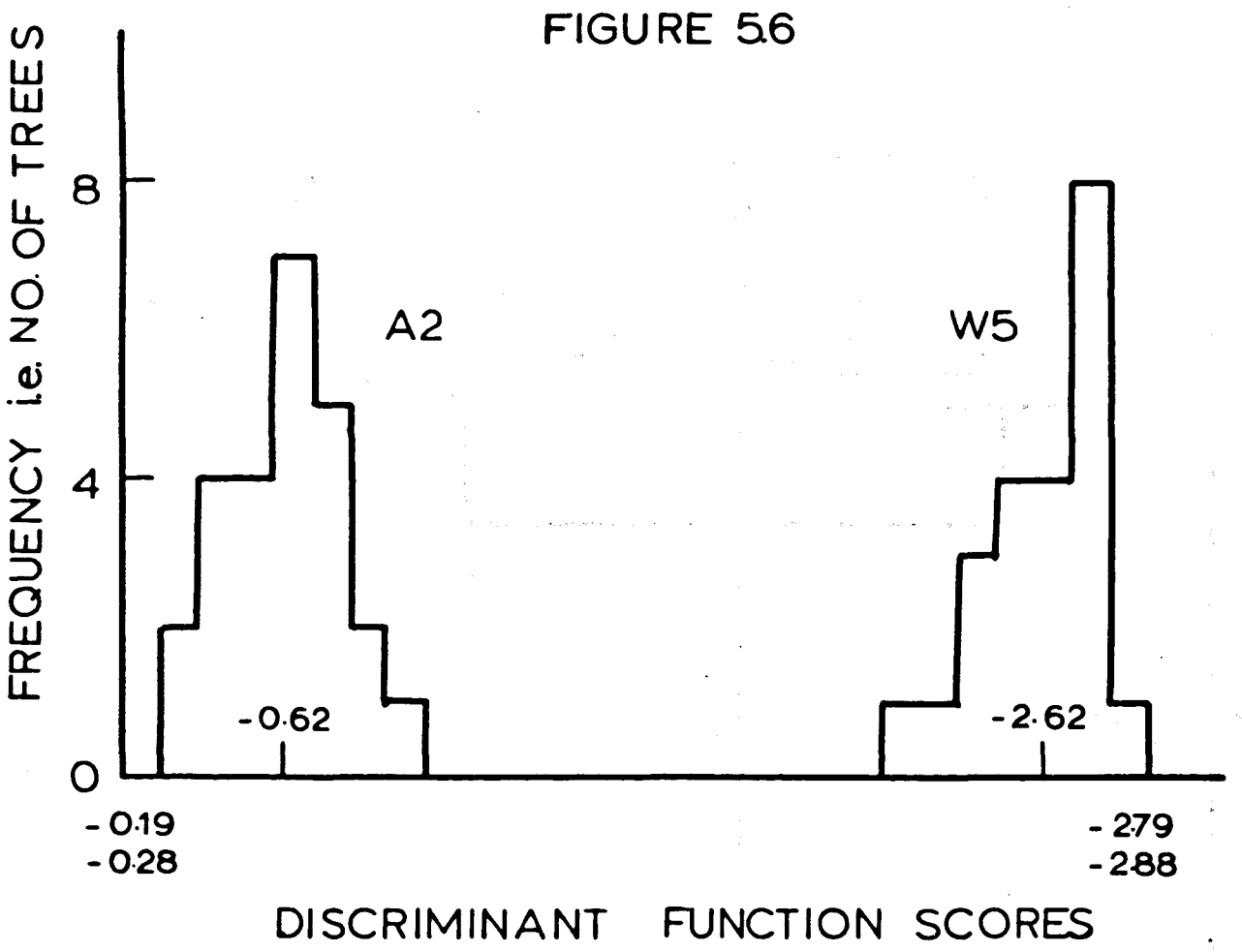


FIGURE 5.6 Frequency of Discriminant Function Scores of the two reference Populations A_2 and W_5. (The figures for each population refer to the population mean Discriminant Function Score.)

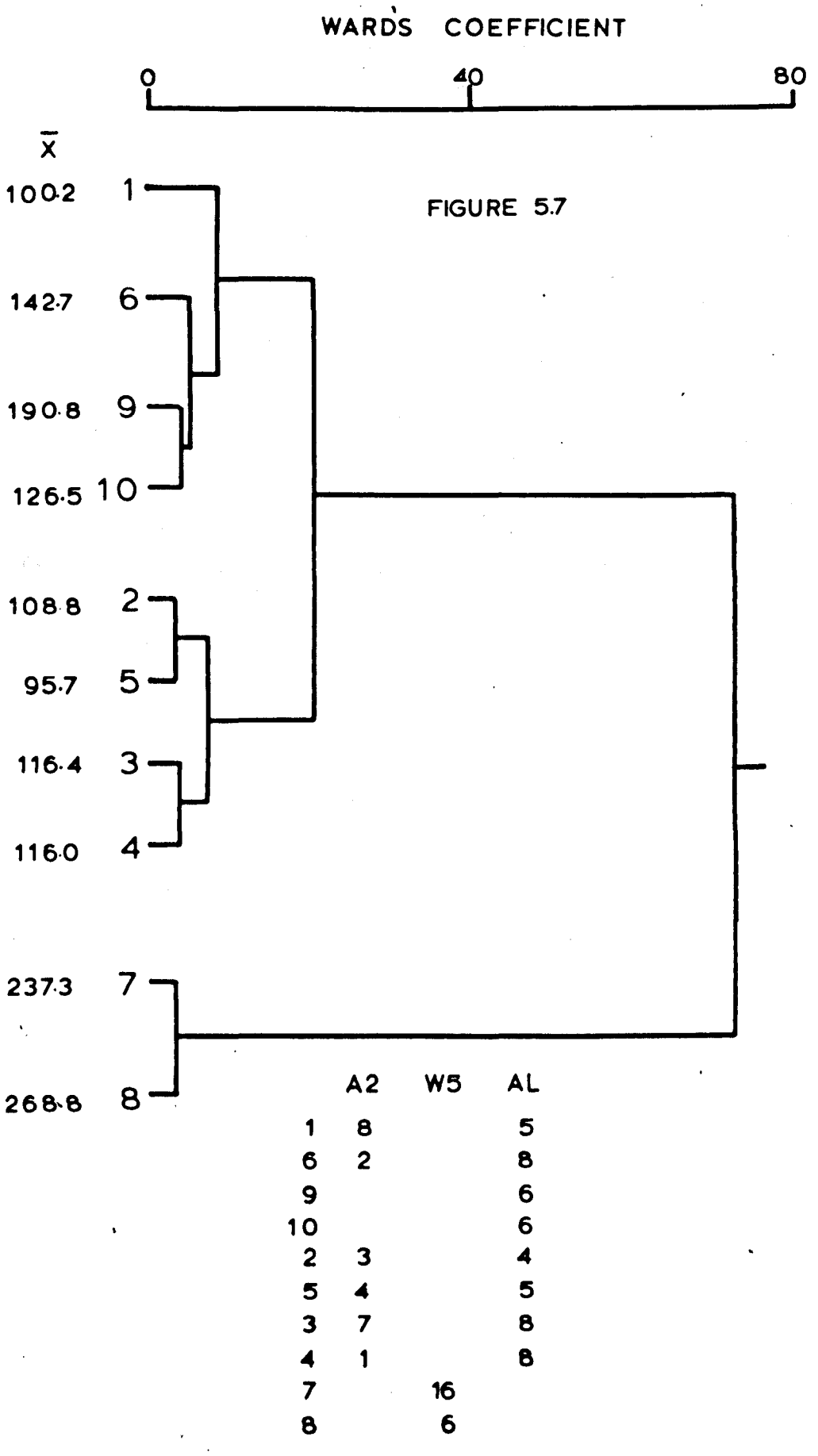


FIGURE 5.7

FIGURE 5.7 Ward's Error Sums of Squares Cluster Analysis on the reference Populations A_2 and W_5, and the test Population AL, a suspected introgressed Q. robur woodland. The table at the bottom of the figure gives cluster membership; $\bar{x}$ refers to the Hybrid Index mean of each cluster.

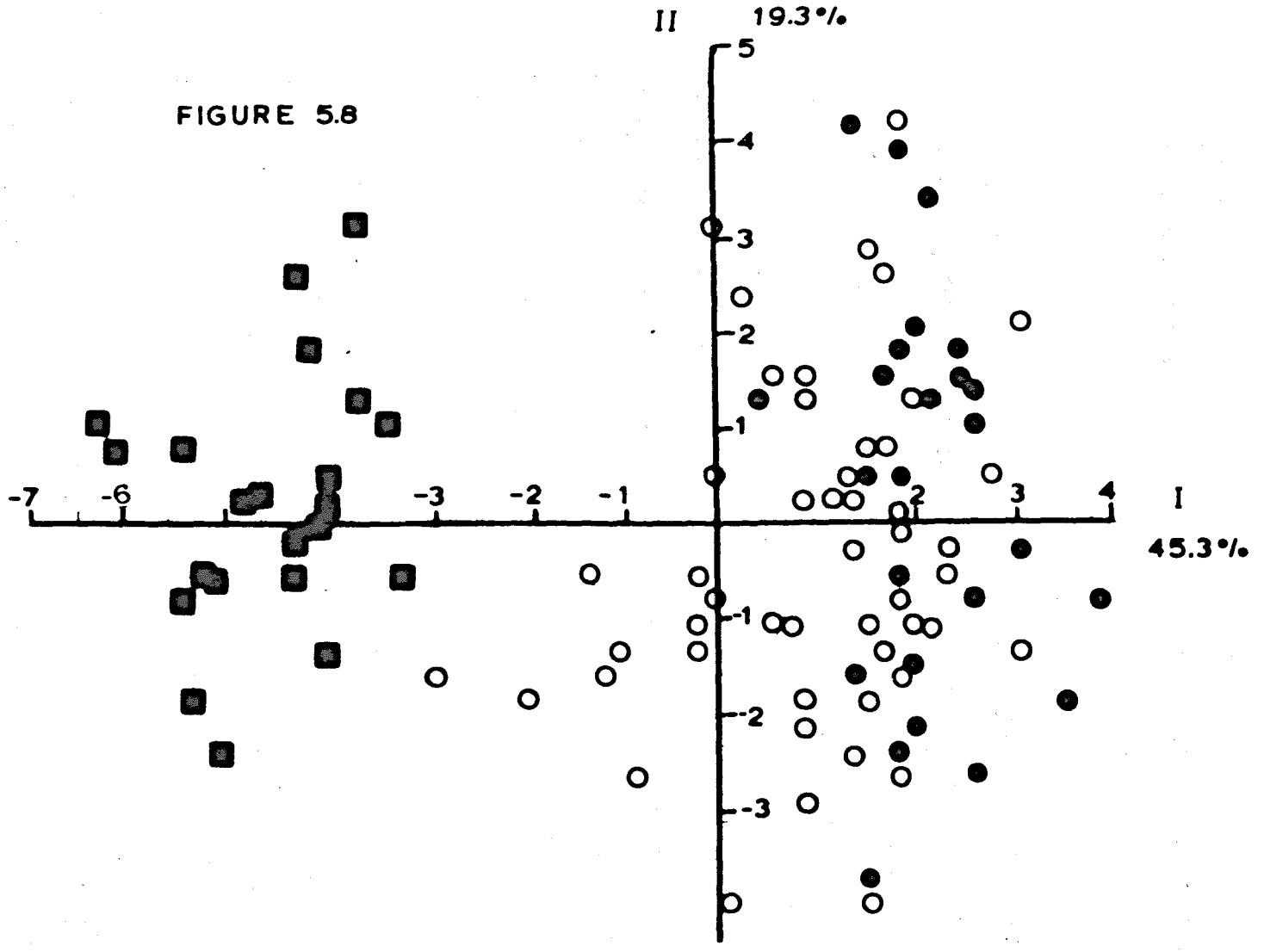


FIGURE 5.8 PCA of individual trees of the reference populations A_2 (pure Q. robur ●), W_5 (pure Q. petraea ■) and the test population AL (a suspected introgressed Q. robur population ○)
The first two Components of the correlation matrix are shown, together with the amount of variance accounted for by each Component.

results of other analyses, and the DFA of AL illustrates such a discrepancy (Figure 5.9). It might be expected that DFA would produce discriminant scores for the majority of population AL within the range of A_2, but to also produce some scores some distance away towards population W_5. In reality, it did not and the mean discriminant score for AL was indeed lower than A_2. No reason was forthcoming to explain situations such as this where results from DFA failed to show the same pattern as with PCA and CA. The number of occasions on which discrepancies arose was small - 5 populations in 135 or approximately 4%.

Population AO showed very much the same type of pattern as population AL, except that this population was a possible introgressed Q. robur population. In CA (Figure 5.10) AO produced three clusters (8,9 and 10) which contained only one alien tree from W_5, and a similar PCA result was obtained as that for AL, the first two axes accounting for 66.86% of the variance and the same two characters being unimportant in separation (Figure 5.11 and Tables 5.3 and 5.4). DFA (Figure 5.12) although discriminating between W_5 and AO, mean discriminant scores being 1.35 and 1.17 respectively and 0.38 for A_2, W_5 and AO showed some expected degree of overlap.

Population DI

During the course of the analysis of the populations, two rather distinct types of population were found, which showed interesting patterns of variation. DI is an example of the first, AAA is an example of the second. Population DI consisted of trees morphologically intermediate between both species with few trees that could be recognised as belonging to the pure species type. CA confirmed this view (Figure 5.13) the population grouping into three exclusive clusters 8, 9 and 10 with only three trees of the population clustering with W_5. Clusters 8, 9 and 10 finally fused with the A_2 clusters, 1, 2 and 3 before joining in the last

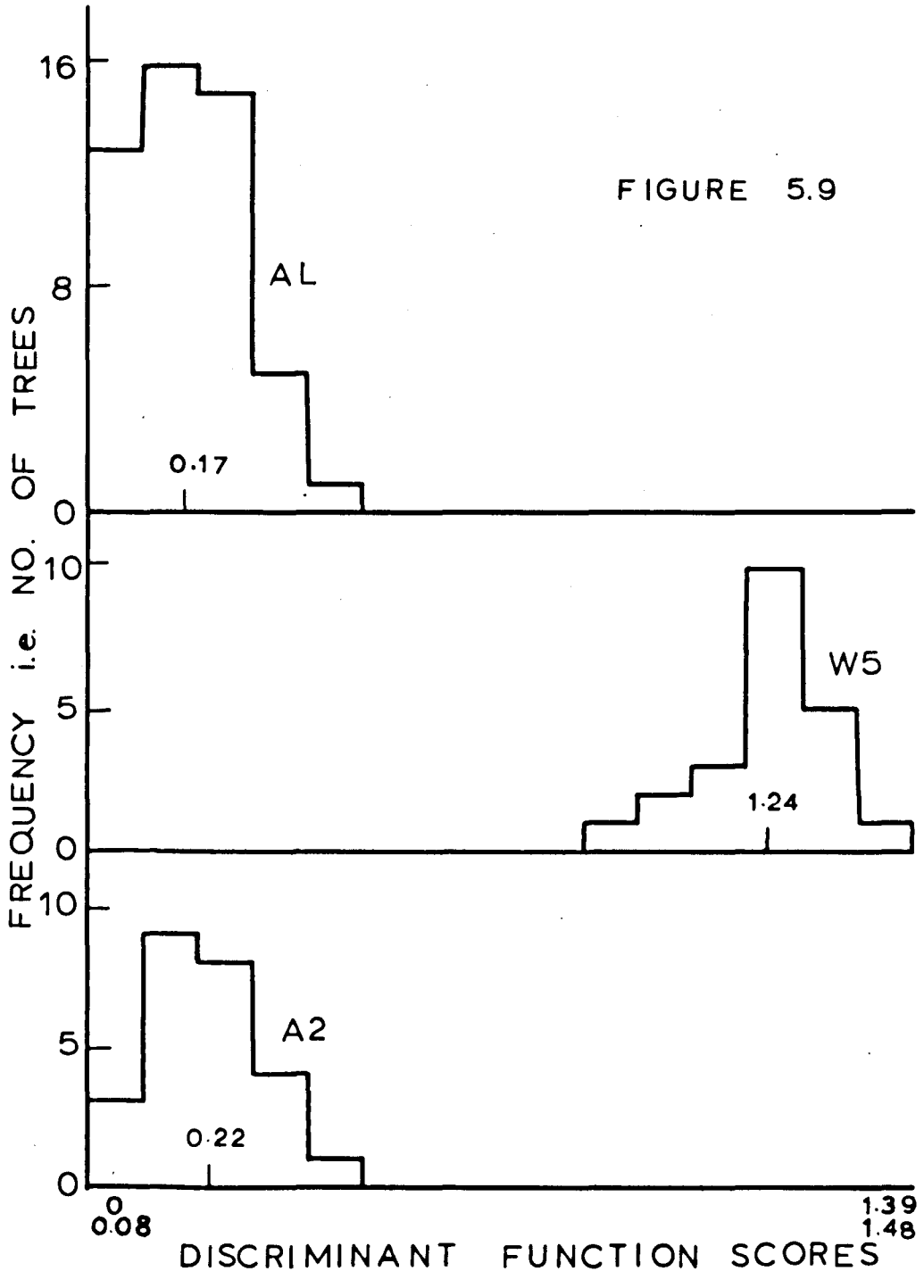


FIGURE 5.9 Frequency of Discriminant Function Scores of the two reference Populations A_2 and W_5 and the test Population AL, a suspected introgressed *Q. robur* woodland. (The figures for each population refer to the population mean Discriminant Function Score.)

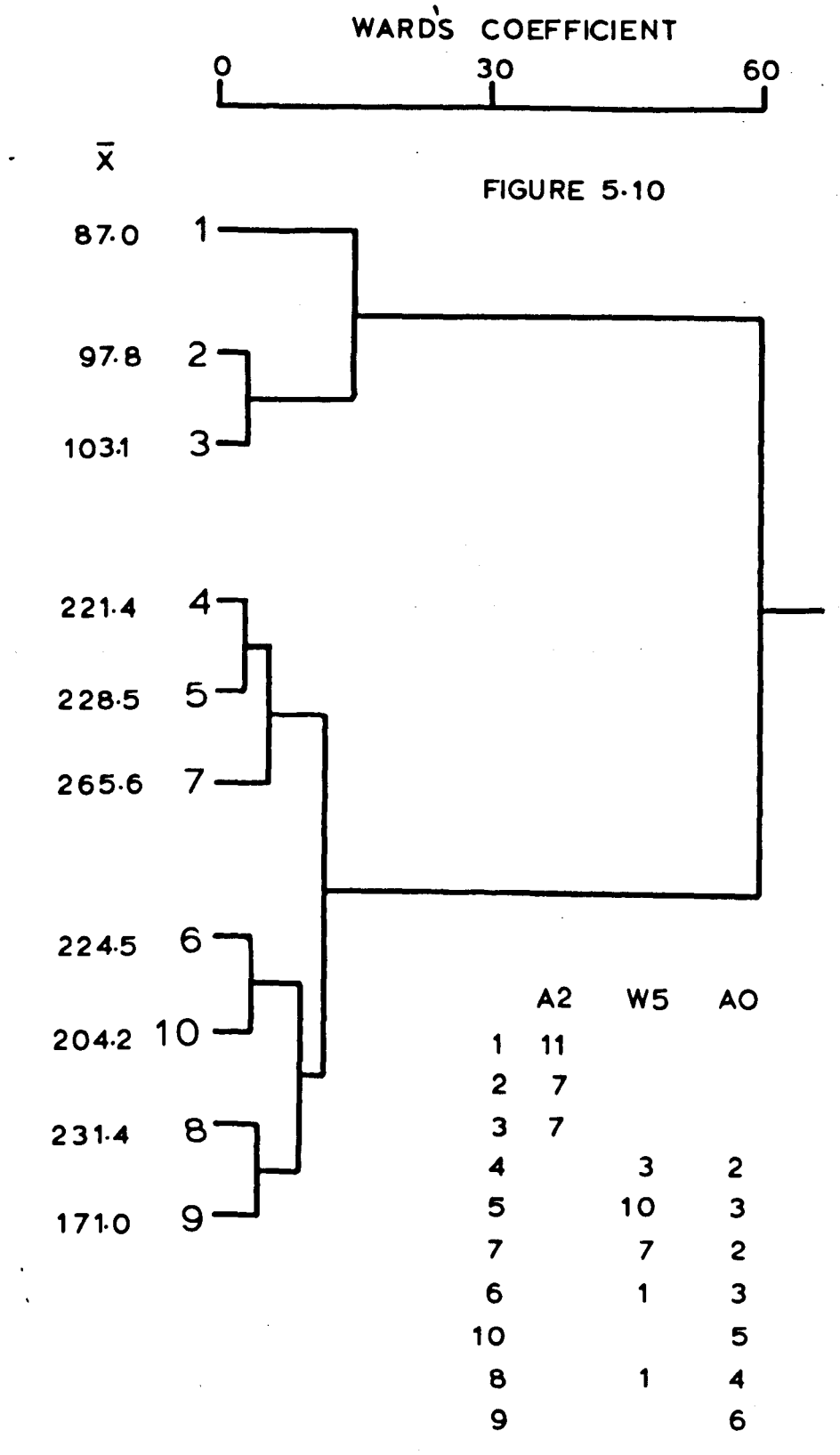


FIGURE 5.10

FIGURE 5.10 Ward's Error Sums of Squares Cluster Analysis on the reference Populations A_2 and W_5, and the test Population AO, a suspected introgressed Q. petraea woodland. The table at the bottom of the figure gives cluster membership; $\bar{x}$ refers to the Hybrid Index mean of each cluster.

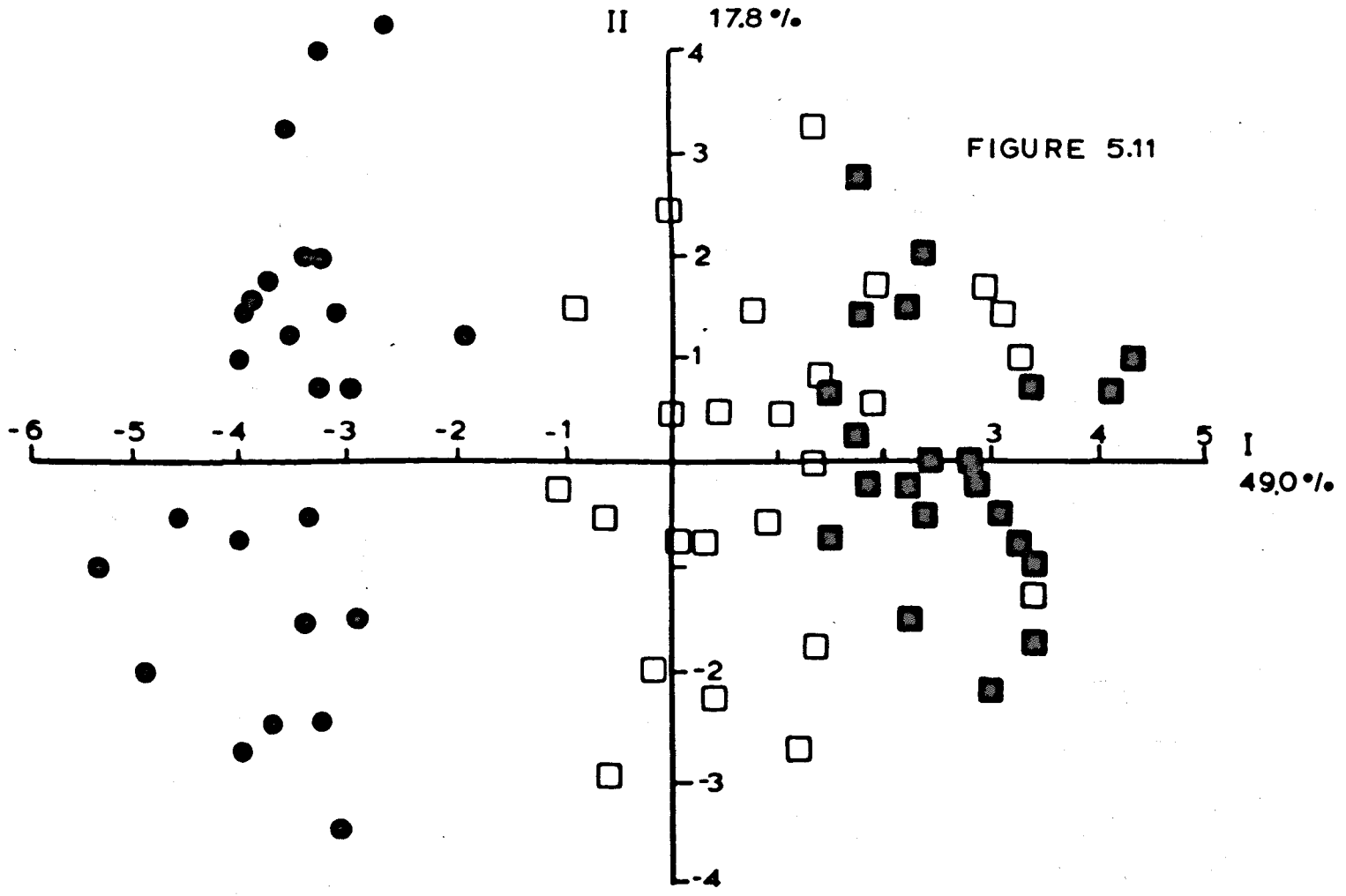


FIGURE 5.11 PCA of individual trees of the reference populations A_2 (pure Q. robur ●), W_5 (pure Q. petraea ■) and the test population AO (a suspected introgressed Q. petraea population □) The first two Components of the correlation matrix are shown, together with the amount of variance accounted for by each Component.

FIGURE 5.12

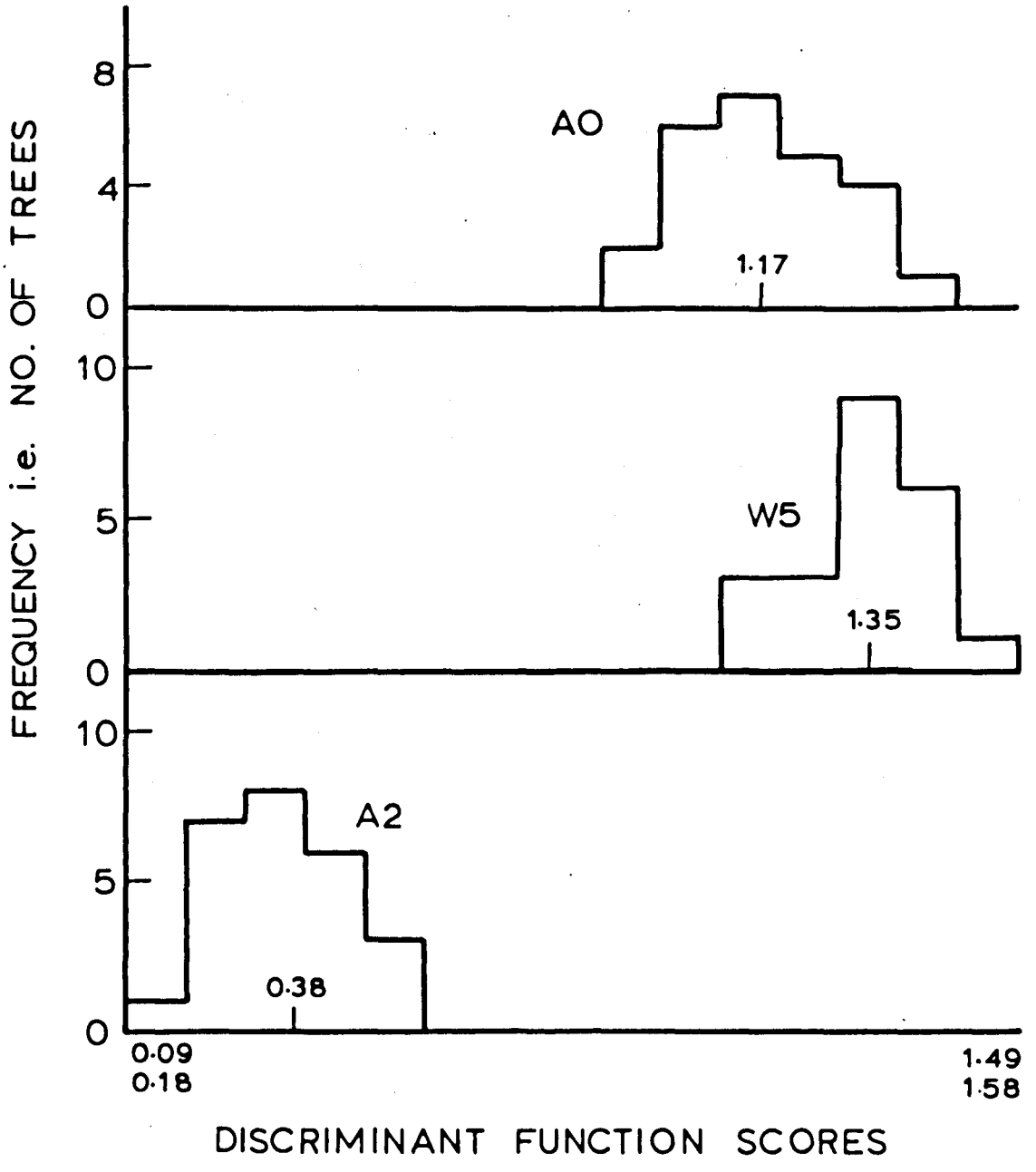


FIGURE 5.12 Frequency of Discriminant Function Scores of the two reference Populations A_2 and W_5, and the test Population AO, a suspected introgressed *Q. petraea* woodland. (The figures for each population refer to the population mean Discriminant Function Score.)

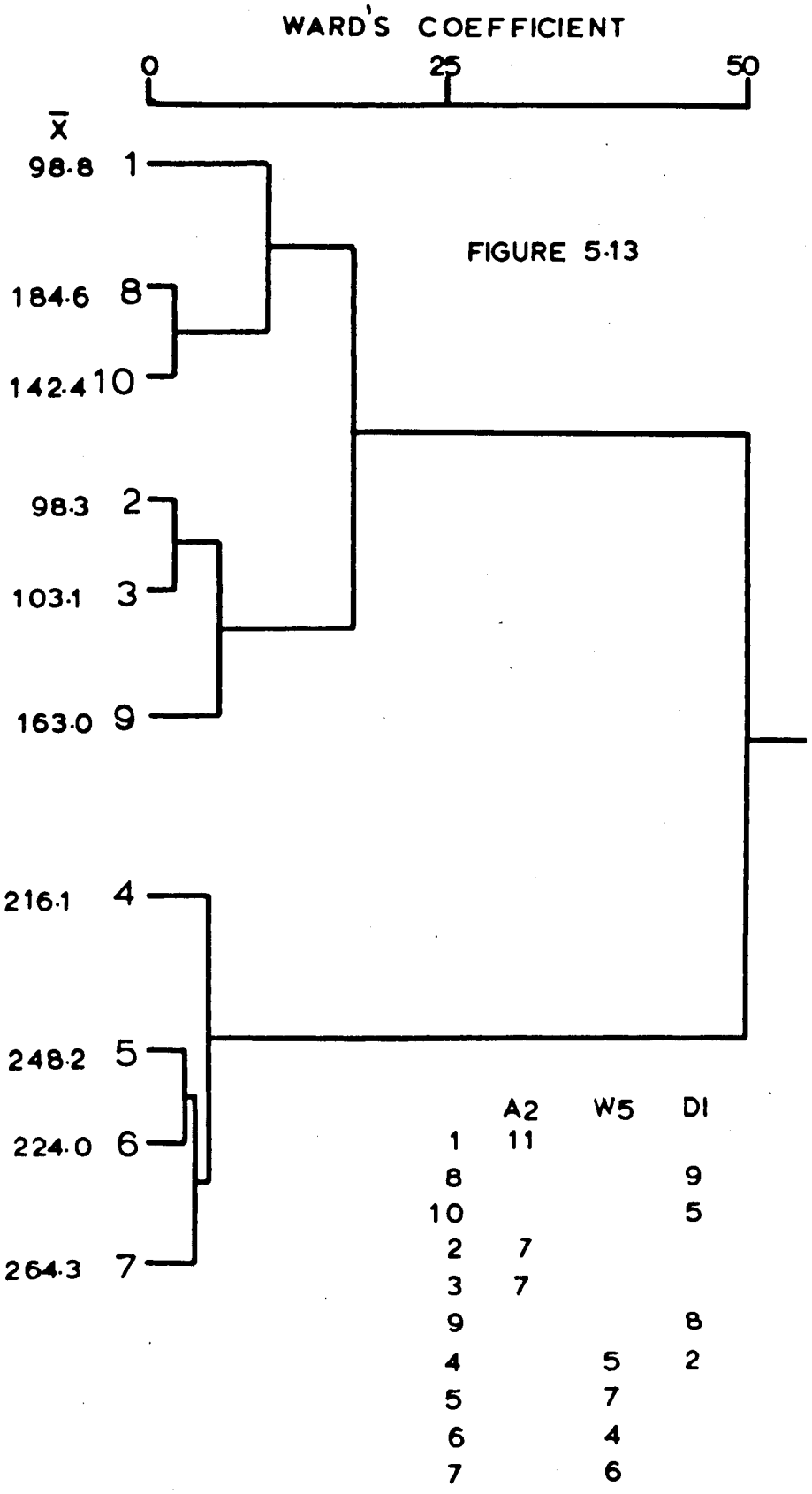


FIGURE 5.13 Ward's Error Sums of Squares Cluster Analysis on the reference Populations A_2 and W_5, and the test Population DI, a woodland composed of large number of hybrids. The table at the bottom of the figure gives cluster membership; $\bar{x}$ refers to the Hybrid Index mean of each cluster.

fusion cycle with the W_5 clusters 4, 5, 6 and 7 suggesting some affinity with Q. robur rather than Q. petraea. PCA (Figure 5.14 and Tables 5.3 and 5.4) also positioned DI in the intermediate zone between A_2 and W_5. DFA (Figure 5.15) positioned the population in the intermediate zone, although slightly more towards A_2 than W_5 (mean discriminant scores being A_2 -0.628, DI -1.040, W_5 -1.717). The significance of the variational patterns shown by this type of population are fully discussed below.

Population AAA

The Hybrid Index frequency histogram for this population was very much as expected for a 'pure' population. However, on closer examination of the subsequent analyses, it became obvious that far from being 'pure' it showed very divergent behaviour from that expected of a 'pure' population. Using PCA (Figure 5.16 and Table 5.3) on the first component AAA grouped very close to the W_5 population, with a slight movement of the population away from W_5 into the intermediate zone. All but two of the trees were within the bounds of the W_5 population. On the second Component, however, the population diverged almost entirely from W_5, W_5 showing only a very small overlap, i.e. the two populations W_5 and AAA could be distinguished on the second Component, but not the first. On Component 1, all characters were important in differentiating the groups with the exception of lamina width, lamina length to the widest part, and also this time lamina length (Table 5.4). On Component 2, the following characters were important: number of sinuses with veins, petiole length, lamina + petiole length, petiole ratio, lamina length to the widest part, lamina length, sinus depth and lamina width, i.e. seven of the characters were the 'size' characters. CA (Figure 5.17) emphasised this division, forming four exclusive AAA clusters, 7, 8, 9 and 10, with no overlap between A_2 or W_5. The four AAA clusters remained together and only fused with the W_5 clusters at the penultimate fusion cycle. DFA (Figure 5.18)

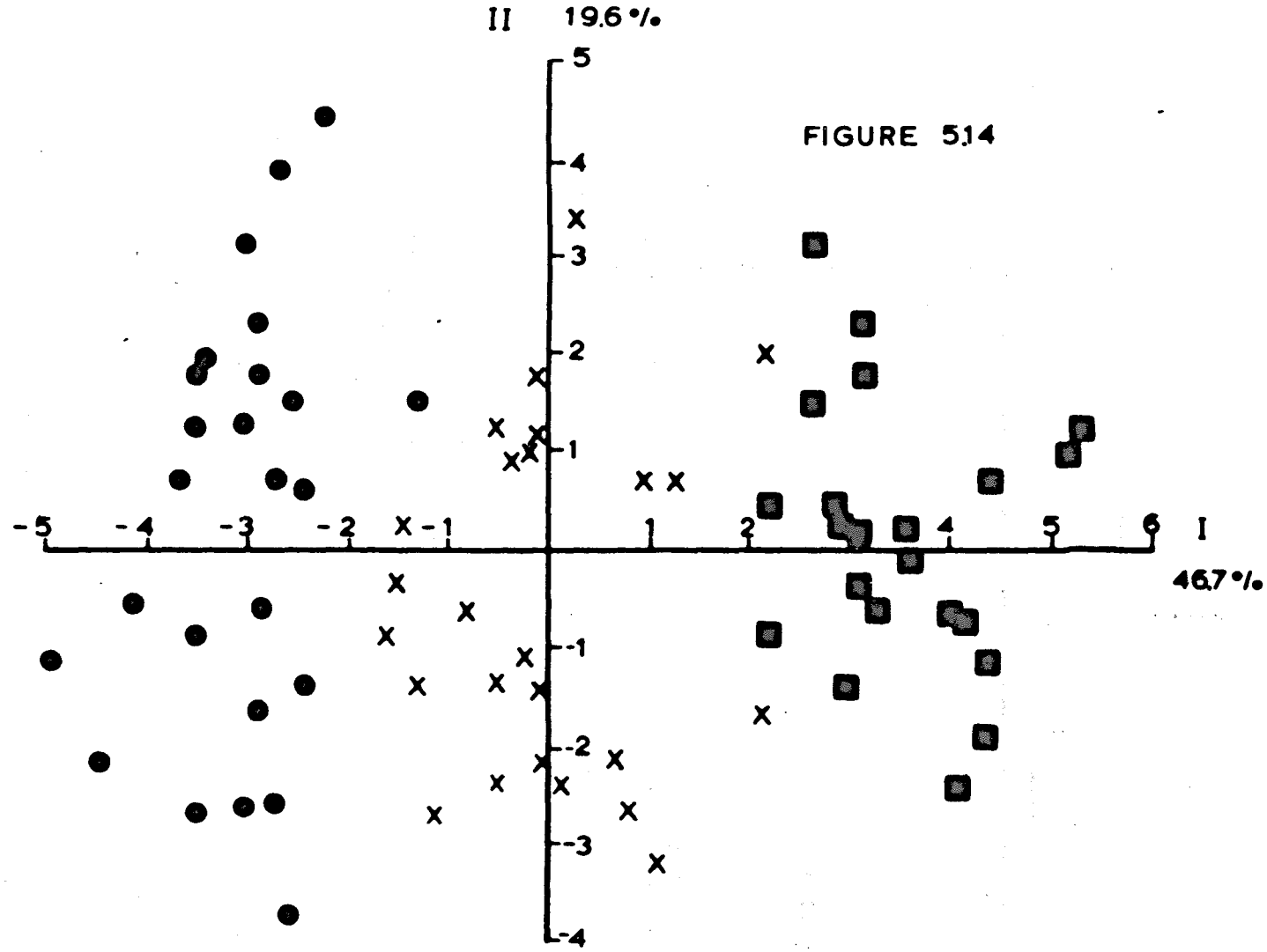


FIGURE 5.14

FIGURE 5.14 PCA of individual trees of the reference populations A_2 (pure Q. robur ●), W_5 (pure Q. petraea ■) and the test population DI (a population of intermediate trees X)
The first two Components of the correlation matrix are shown, together with the amount of variance accounted for by each Component.

FIGURE 5.15

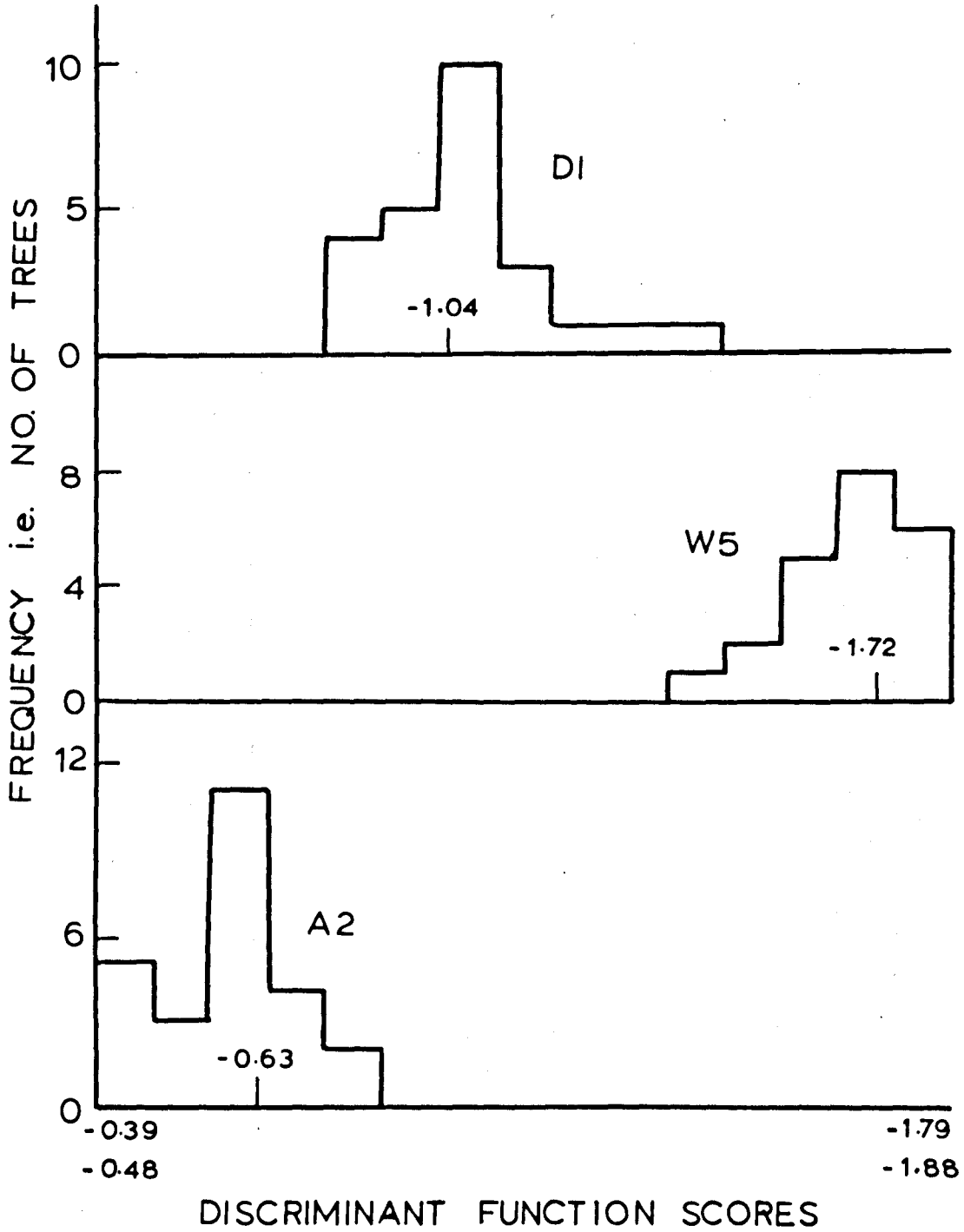


FIGURE 5.15 Frequency of Discriminant Function Scores of the two reference Populations A_2 and W_5 and the test Population DI, a population of completely intermediate trees. (The figures for each population refer to the population mean Discriminant Function Score.)

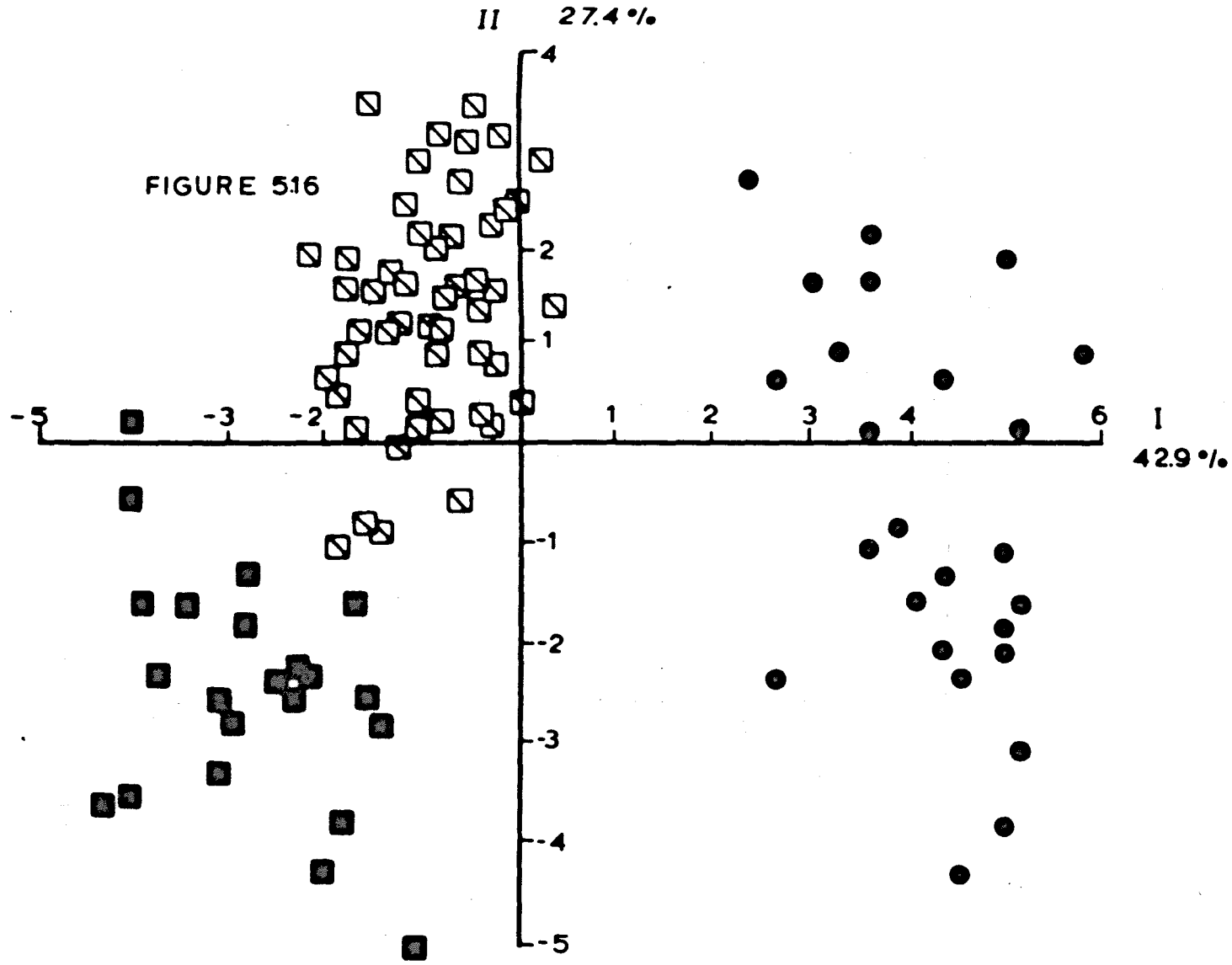


FIGURE 5.16 PCA of individual trees of the reference populations A_2 (pure Q. robur ●), W_5 (pure Q. petraea ■) and the test population AAA (a pseudo-pure Q. petraea population ⊠)
The first two Components of the correlation matrix are shown, together with the amount of variance accounted for by each Component.

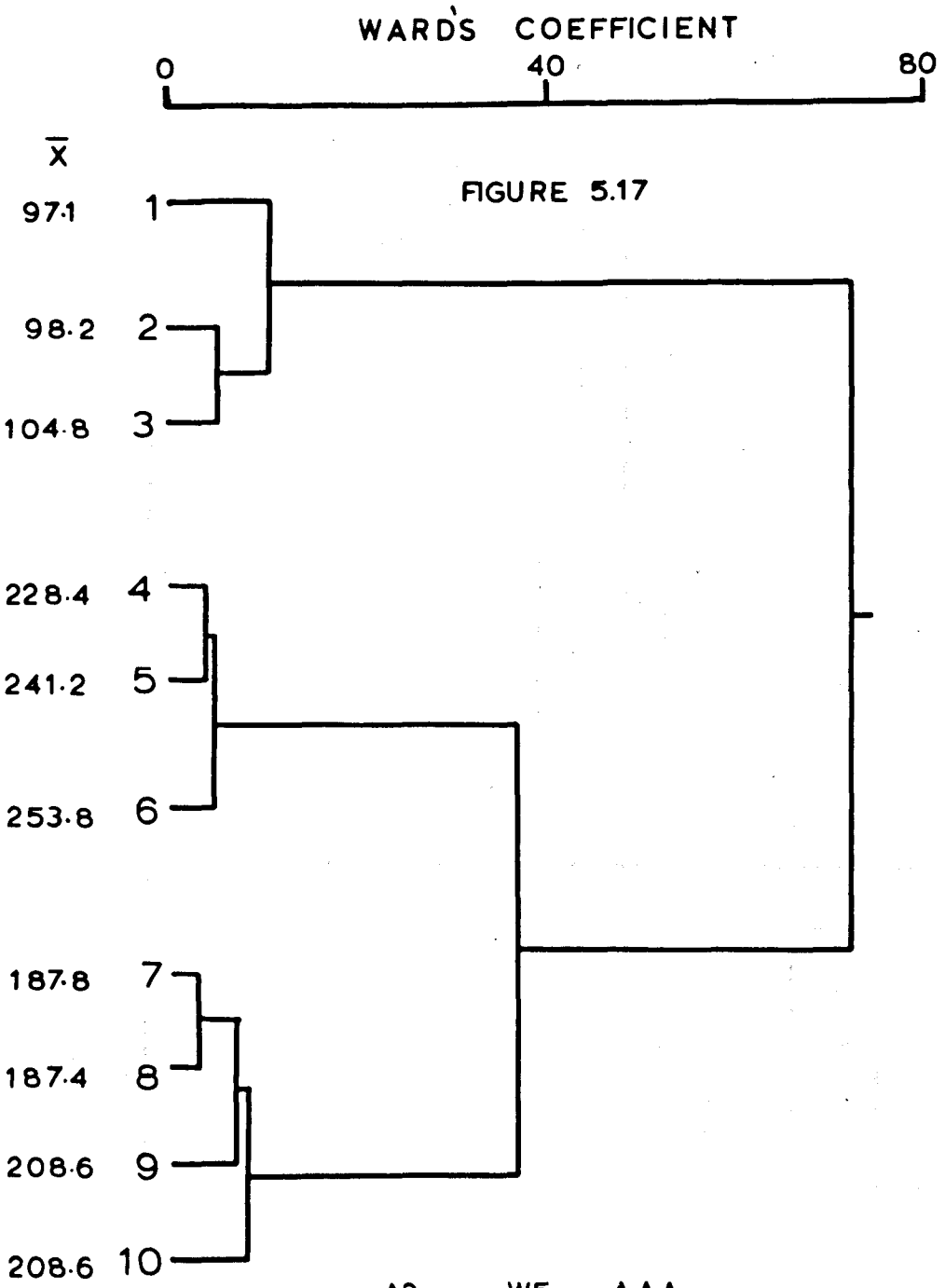


FIGURE 5.17

	A2	W5	AAA
1	10		
2	7		
3	8		
4		3	
5		8	
6		11	
7			12
8			8
9			14
10			16

FIGURE 5.17 Ward's Error Sums of Squares Cluster Analysis on the reference Populations A_2 and W_5, and the test Population AAA, a pseudo-pure Q. petraea woodland. The table at the bottom of the figure gives cluster membership; $\bar{x}$ refers to the Hybrid Index mean of each cluster.

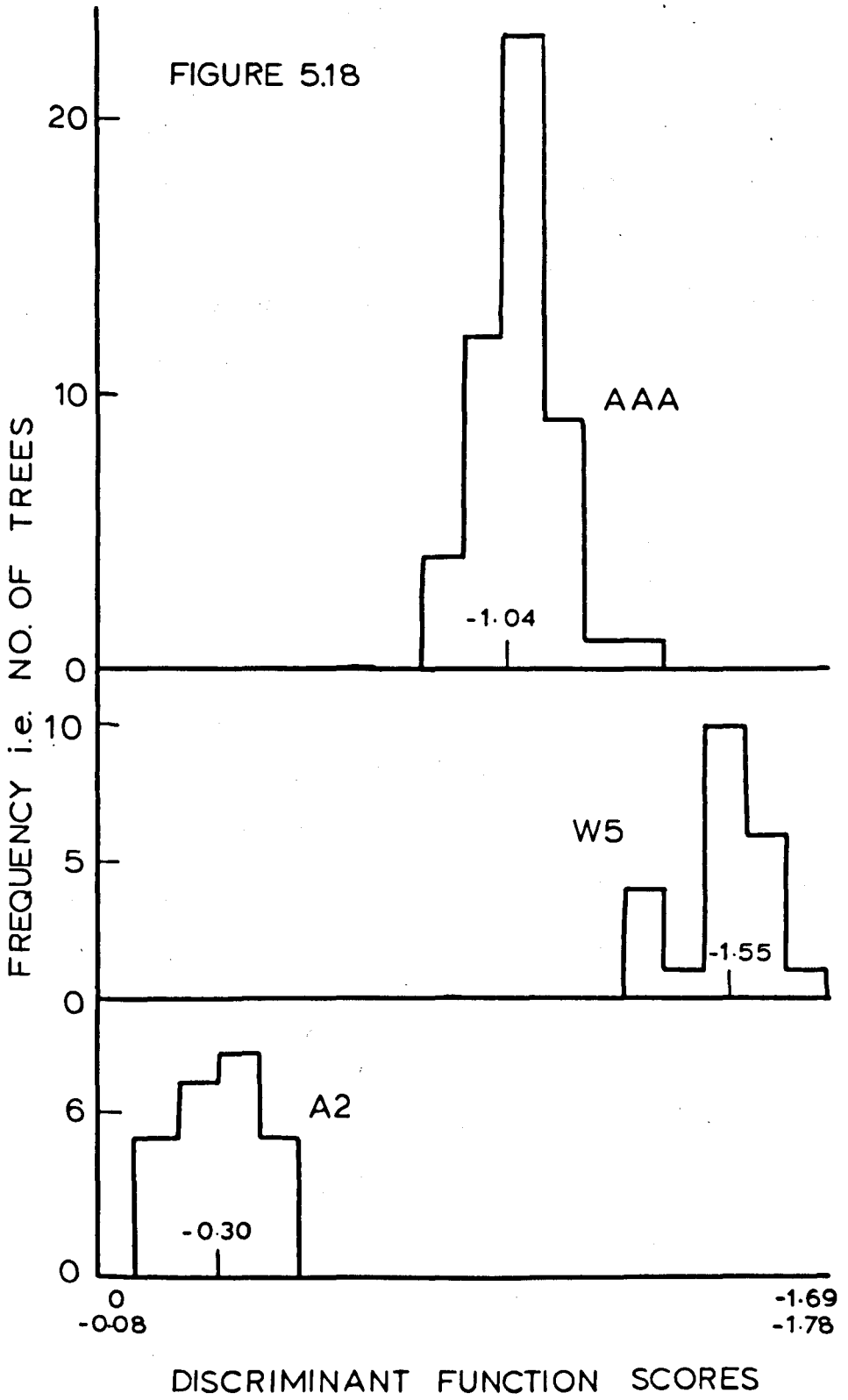


FIGURE 5.18 Frequency of Discriminant Function Scores of the two reference Populations A_2 and W_5 and the test Population AAA, a suspected pseudo-pure *Q. petraea* woodland. (The figures for each population refer to the population mean Discriminant Function Score.)

also showed the same trend, the mean discriminant score for AAA being -1.036 contrasting with A_2 -0.301 and W_5 -1.545, the test population being slightly closer to W_5. Consequently all three analyses picked out this population from the reference populations, although Hybrid Index frequency histograms showed it to be 'pure' Q. petraea. Normally, 'pure' populations failed to separate from the corresponding reference population, so that populations of type AAA represent an interesting and possibly significant departure from normality. The suspected origin of these populations is discussed below.

An assessment of the techniques

Before discussing in detail the different types of population found, it would seem appropriate to attempt to evaluate the usefulness or otherwise of the analyses used here, particularly as they compare with the Hybrid Index frequency histogram and the PSD.

The three analyses rely either on similarity, PCA and CA, or dissimilarity, DFA. CA produces clusters of 'similar' individuals, PCA defines the position of individuals in their relationship to others and DFA identifies groups of similar individuals and differentiates them from other groups by their dissimilarity. It is not perhaps surprising that they produce reasonably equivalent results. The PSD is also concerned with similarity, but similarity defined mainly by the subjective choice of the two axes of the PSD and with the ability of the taxonomist to use the final PSD to detect similar and dissimilar metroglyphs. PCA removes the subjective element of choosing axes; these are derived as being inherent in the data. In addition, the positions of the individuals in the component space are based on the overall consideration of all characters, so that individuals lying close in component space can be considered similar, i.e. the examination of distance between individuals

in PCA scatters is a similar process to the examination of metroglyphs on a PSD scatter, but obviously a much easier process for the human mind. CA and DFA are useful supplementary analyses, particularly as they deal with classifications. The Hybrid Index frequency histogram, although a useful summary is not good for understanding population structure as evidenced by, for example, population AAA.

Population types

The use of the analyses above suggested that the following different types of population could be detected:

'Pure' Q. robur

'Pure' Q. petraea

Intermediate populations, eg. DI

Mixed populations of both 'pure' Q. robur and 'pure' Q. petraea (these normally had some intermediate trees also)

Suspected introgressed Q. robur and Q. petraea populations (it was generally possible to divide each of these into one of three categories depending on the number of intermediates - suspected slight, medium and heavy introgression)

Pseudo-pure Q. robur and pseudo-pure Q. petraea (i.e. those appearing pure but behaving like population AAA in the analyses)

The character of each population accompanies the list of population names, grid references, etc. in Appendix 4.

Of the 135 populations sampled, 58 showed the same pattern of variation as population A_2, and in all analyses were indistinguishable from it. These are thought to represent 'pure' Q. robur populations - population A_2 was originally chosen as conforming to a pure population type, and as noted earlier, evidence from pollen viability will be presented in Chapter 7 in support of this view. Six populations of this 'pure' Q. robur type also had a small proportion of Q. petraea trees, although these populations showed no evidence of intermediate trees. Two 'pure' Q. robur populations, CG and AAE, were of the pseudo-pure type in that they divided from population A_2 in the PCA and CA.

Seventeen populations were of the pure Q. petraea type, and seven of these showed signs of the presence of Q. robur trees. Four populations were of the pseudo-pure Q. petraea type, W_7, AAA, CD and CE. Both CD and CE are subpopulations of Padley Wood, a woodland used for much of the growth analysis work on Q. petraea in the 1960's (Jarvis, 1964). Seven populations were of mixed status, and these frequently had large numbers of intermediate trees. Eight populations exhibited variation typical of the intermediate type of population, with a majority of intermediate trees.

Twenty-one Q. robur populations produced variational patterns typical of the suspected introgressed type. These could be divided into seven slightly introgressed, ten medium introgressed, and four heavily introgressed populations. Many of these populations also contained alien Q. petraea trees, and many of the suspected introgressed Q. petraea also contained alien Q. robur trees. There appeared to be correlation between the different degrees of introgression, and the presence of alien trees (see Table 5.5) - of those populations showing slight introgression, four out of ten showed the presence of alien trees, of those showing more signs of introgression, 26 out of 35 showed the presence of alien trees. Twenty-four Q. petraea populations produced variational patterns expected of a suspected introgressed type of which three were slight, eleven were medium and ten were heavily introgressed.

There are considered to be three possible explanations for the presence of the pseudo-pure populations, provided that sampling and measurement errors are discounted. The first explanation is that these populations, although divergent from populations A_2 or W_5, still represent part of the natural distribution of the species. This is an attractive theory, since in many 'pure' populations, individual trees do stand apart from the reference populations, but it is thought unlikely that a whole

	Number of Populations	Number with alien trees	Percentage of populations with alien trees
Slight introgressed Q. robur	7	3	42.8
Slight introgressed Q. petraea	3	1	33.3
Medium introgressed Q. robur	10	9	90.0
Medium introgressed Q. petraea	11	8	72.7
Heavily introgressed Q. robur	4	3	75.0
Heavily introgressed Q. petraea	10	6	60.0

TABLE 5.5 CORRELATION BETWEEN THE PRESENCE OF ALIEN TREES IN A POPULATION AND THE LEVEL OF INTROGRESSION OBSERVED

population would diverge in this manner unless environmental variables were operative at a population level, modifying the whole morphology of the foliage of all trees. The second is that these populations represent subspecific taxa. Examination of the literature on recorded subspecies would suggest that no described subspecific taxa correspond to the pseudo-pure populations, although one or two of the characters distinguishing the pure from the pseudo-pure population are those used by Weimarck (1947a, 1947b) and Schwarz (1970) for distinguishing subspecies, but these are not the whole range of characters distinguishing the two population types.

The third possibility is that these populations represent old introgressed populations which are not actively hybridising, but have assimilated the alien genes. Support for this view comes from two sources. Examination of the characters differentiating the pure and pseudo-pure populations of Q. petraea on the second Component reveals that six characters, petiole ratio, lamina + petiole length, lamina length to widest part, lamina length, petiole length and number of sinuses with veins were all consistently significantly lower and therefore more Q. robur like in the pseudo-pure populations, whilst only lamina width and sinus depth were higher than the pure population. Similarly with Q. robur populations, seven characters basal shape, auricle development, venation, number of sinuses with veins, shape, sinus depth and lamina width were all significantly higher and therefore more Q. petraea like than those of the pure populations. Only lamina length and lamina + petiole length were lower than the pure population. This would suggest that there is a large amount of variation in these pseudo-pure populations that would be consistent with the view that they are quiescent introgressed populations. Introgressed populations frequently show a shift in the population mean towards the alien species. Cousens (1965) recognised this as the population mean moving down along the Introgression Pathway.

It may also be noted that in some introgressed populations using PCA the main body of the population does not mix completely with the reference population, but lies somewhat to one side. An example of this can be seen in Figure 5.8 in which the main body of population AL lies more towards the base of Component 2, whilst the reference population A_2 lies more towards the top of Component 2. This separation is by no means as clear as that noted for the pseudo-pure populations, but it does indicate the main population shift that could be expected for an old introgressed population.

It is possible to recognise in the oak populations of this study the stages in introgression noted by Cousens (1965) - see Chapter 1. Stage 1, with few hybrids, at the start of backcrossing would be represented by the slightly introgressed populations; Stage 2, showing well advanced introgression, is probably represented by the medium and heavily introgressed populations; Stage 3, after hybridisation has stopped, is possibly represented by some (unknown) populations in the medium introgressed populations, these exhibiting the characteristics of a stage 3 situation, i.e. a contracted introgression 'tail'. Cousens (1965) recognises Stage 4 as the completion of assimilation, after which there is no introgressive trend and it may only be detected if there are data from non-introgressed populations for comparison. The pseudo-pure populations are regarded as belonging to this stage, they being only differentiated from pure populations by multivariate approaches.

Consequently, the results presented here would agree broadly with Cousens (1963, 1965) in terms of the different population types, but not with the level of introgression. In this study, 6673 trees were sampled of which 515 fell into the Hybrid Index range 150-189 (the intermediate zone) or 7.72%. Taking a wider intermediate zone gave 843 trees out of 6673 or 12.63% in the 140-199 range. (The lowest tree found in the W_5

reference population was 200, the highest tree found in the A_2 reference population being 134, suggesting that this last mentioned wide intermediate zone may be unjustified.) A level of hybridisation somewhere between 7% and 12% is very different from the results of Cousens who believed that perhaps 50% of oaks in Scotland are of questionable origin (Cousens, 1963). It should be remembered, of course, that Cousens' work was completed in Scotland, which is at the periphery of the species range, and where barriers between the species may break down more easily. Jones (1959), an advocate of small scale introgression, believed that even in mixed woodland rarely more than 5% of the trees could be regarded as being of hybrid origin. The levels here are slightly in excess of this.

Wigston (1971) describes some oakwoods in his study which were stabilised in an intermediate taxonomic state. Similar populations have been recorded here, although I have no evidence that these intermediate populations are 'stabilised'. These might be looked on as possible hybrid swarms, in which all isolating mechanisms have broken down leading to free gene interchange between the species - in essence possibly the taxonomic position in Scotland on a smaller scale.

The validity of the reference populations

It would be impossible to attempt to objectively assess the validity of the reference populations since the results would be clouded by how well or how badly the taxonomist thought the reference populations had performed. However, it was felt that this was an integral part of the use of the reference populations, and consequently an attempt was made using PCA and CA.

Each population was considered to be a separate OTU, its character values being the mean character values for the population as a whole, to produce a 135 OTU x 17 character matrix. This matrix was subjected to

both PCA and CA using Ward's Error Sums of Squares. The PCA scatter for the first two components (which accounted for 68.96% and 18.22% of the variance) is given in Figure 5.19. Instead of plotting individual anonymous points on this scatter, the points are recorded as belonging to one of the ten groups from the CA. Figure 5.20 records the frequency distribution thus obtained of CA groups along the first PCA axis. Further examination of these data are presented in Table 5.6 which records the occurrence of the population types discussed above in the Ward's clusters.

Cluster 9 contains exclusively pure Q. robur populations, whilst Clusters 1 and 3 similarly are composed of nearly all pure Q. robur populations. Clusters 4 and 5 are predominantly introgressed Q. robur, intermediate and mixed populations, whilst Clusters 2 and 6 are similarly oriented Q. petraea populations. Cluster 8 contains only pure Q. petraea populations, and Cluster 7, although showing some evidence of containing introgressed Q. petraea populations, also has a large proportion of pure Q. petraea populations. Cluster 10, a predominantly pure Q. robur group also contains two possible introgressed populations.

There is good agreement, therefore, between the Wards clustering of populations and the status of populations assessed from the reference populations - circumstantial evidence of the validity of the reference populations. The clustering of populations also show good agreement with the PCA scatter of the same data, the clusters from CA being found in specific areas of the PCA scatter. Differences are apparent between the positioning of the major population types, however, showing structure which was not observed elsewhere. For example, the pure Q. robur Clusters 9, 3, 1 and 10 separate along the second Component; Clusters 7 and 8, the pure Q. petraea populations, separate along the second Component as do the introgressed populations of Clusters 4 and 5, and 6 and 2. The vector loadings for the second Component are given in Table

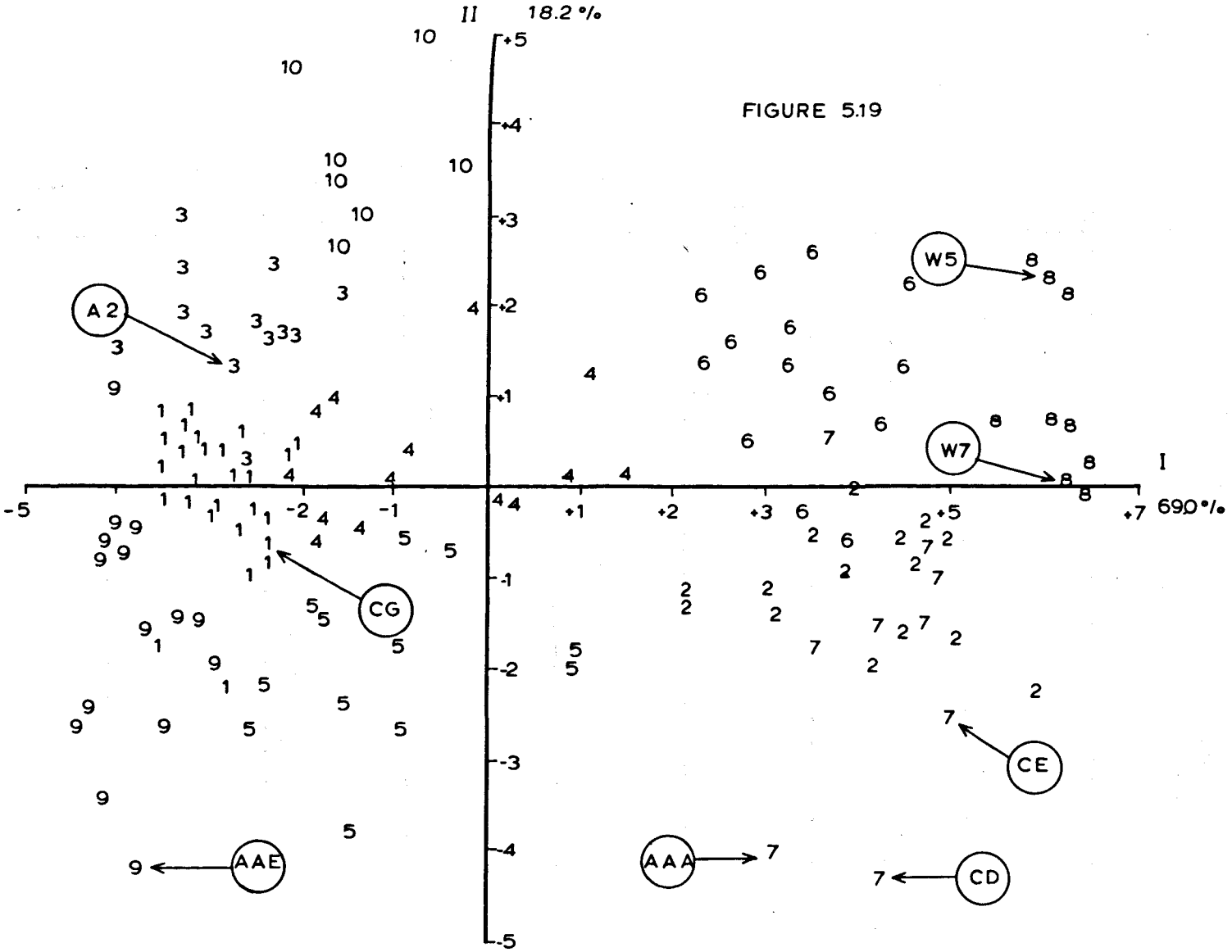


FIGURE 5.19

FIGURE 5.19 PCA of mean Hybrid Index scores for each population. The figure shows the first two Components of the Correlation matrix, and each population classified according to membership of the Ward's Cluster analysis of the same data. The reference populations and 'pseudo'-pure populations are individually labelled.

FIGURE 5.20

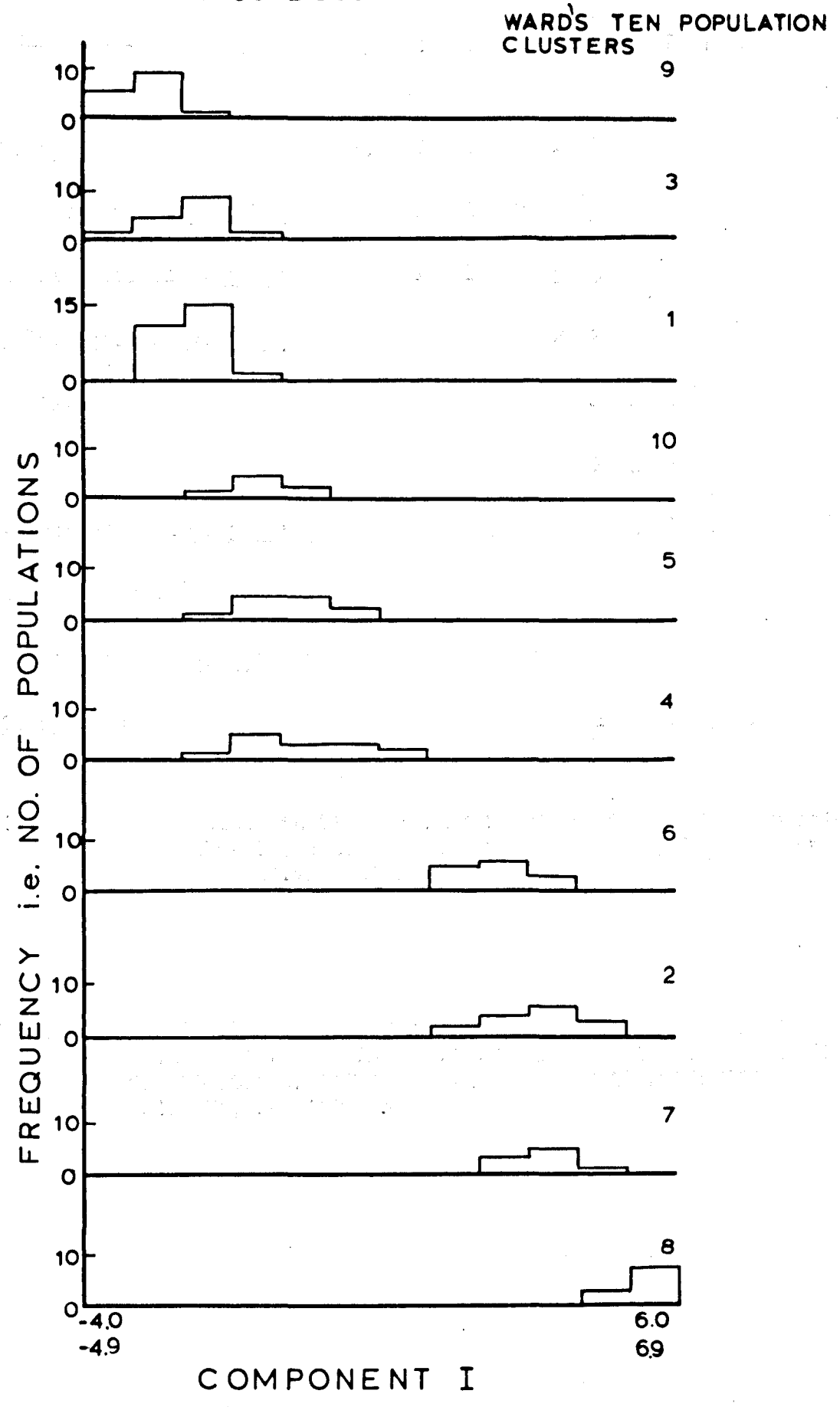


FIGURE 5.20 Frequency of the ten Ward's cluster groupings along Component 1 of a PCA of mean Hybrid Index scores for each population (from the Correlation matrix)

Population Types	Clusters									
	9	3	1	10	5	4	6	2	7	8
Pure Q. robur	15	12	25	4	1	1				
Slight Introgressed Q. robur		1	1	2		3				
Medium Introgressed Q. robur		1	1		5	3				
Highly Introgressed Q. robur					2	2				
Intermediate					2	2	2	2		
Mixed					1	2	3	1		
Highly Introgressed Q. petraea							3	5	2	
Medium Introgressed Q. petraea							7	4		
Slight Introgressed Q. petraea				1					2	
Pure Q. petraea						1		2	5	9

Example: Five populations in Cluster 5 of the CA of the whole population data were classified as medium introgressed Q. robur populations when examined individually.

TABLE 5.6 CORRELATION BETWEEN THE COMPOSITION AT THE TEN CLUSTER LEVEL OF A WARD'S ERROR SUMS OF SQUARES CLUSTER ANALYSIS OF ALL POPULATIONS AND THE ASSESSMENT OF POPULATIONS USING CA, PCA AND DFA

5.7 - the main characters separating these groups being as before the 'size' characters of lamina + petiole length, lamina length to widest part, lamina length, sinus depth and lamina width.

Population A_2 lies in Cluster 3 towards Cluster 1 on the PCA and occupies a central position in the four pure Q. robur clusters (9, 1, 3 and 10). W_5 on the other hand falls well away and at an extreme position from Cluster 7, the other pure Q. petraea cluster. Cluster 8, of which W_5 is a member, is a tight group due to its being composed almost entirely of subsamples of the large Wyre Forest population. Its extreme position would argue for an over-estimation of Q. petraea introgression rather than an under-estimate, but since it cannot be distinguished from individual populations of Clusters 7 and 8 when analysed with them as individual populations (except the four introgressed populations of Cluster 7), its taxonomic position would appear to be close to the expected pure type.

The validity of A_2 and possibly W_5 as reference populations would appear to be established.

The position of the pseudo-pure populations in this analysis is of interest - these are individually marked on Figure 5.19. CD, CE and AAA all separate clearly from W_5 on the second Component, but more importantly from all other pure Q. petraea populations as well. AAE similarly separates from all other pure Q. robur populations. Only CG and W_7 of pseudo-pure group fall close to the centre of the pure species distribution suggesting possible differences between members of the pseudo-pure grouping.

Character correlation and the validity of the hybrid index

Table 5.8 records the correlations between the 17 characters expressed as hybrid indices, calculated from the mean values for the 135

Eigenvectors - standardised so that the sum of elements squared equals the latent root

	Component 1	Component 2
Leaf regularity	0.87	- 0.23
Basal shape	0.93	- 0.02
Auricle development	0.94	- 0.04
Simple hairs	0.94	- 0.21
Stellate hairs	0.94	- 0.20
Lobe number	0.84	0.08
Sinus number	0.94	- 0.14
Venation	0.96	- 0.11
Petiole length	0.95	0.07
Lamina + petiole	0.81	0.56
Petiole ratio	0.94	- 0.05
Lamina to widest part	0.17	0.90
Lamina length	0.68	0.71
Obversity	0.78	- 0.15
Lobe depth	0.57	- 0.74
Lamina width	- 0.49	- 0.82
Lobe depth ratio	0.92	- 0.19

TABLE 5.7 EIGENVECTOR LOADINGS FOR THE FIRST TWO COMPONENTS OF THE PCA OF THE TOTAL POPULATION HYBRID INDEX DATA

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.00																
0.85	1.00															
0.86	0.99	1.00														
0.84	0.84	0.85	1.00													
0.84	0.83	0.85	0.98	1.00												
0.73	0.81	0.80	0.76	0.76	1.00											
0.80	0.87	0.87	0.90	0.89	0.76	1.00										
0.82	0.89	0.89	0.91	0.90	0.82	0.99	1.00									
0.76	0.82	0.83	0.88	0.89	0.72	0.88	0.89	1.00								
0.56	0.72	0.72	0.64	0.65	0.68	0.67	0.70	0.84	1.00							
0.77	0.81	0.82	0.90	0.91	0.70	0.90	0.89	0.99	0.75	1.00						
-0.04	0.14	0.14	-0.04	-0.03	0.27	0.05	0.10	0.21	0.64	0.10	1.00					
0.43	0.62	0.61	0.49	0.50	0.60	0.53	0.56	0.69	0.97	0.59	0.75	1.00				
0.69	0.74	0.73	0.77	0.77	0.52	0.71	0.70	0.75	0.59	0.75	-0.21	0.48	1.00			
0.65	0.53	0.55	0.67	0.68	0.41	0.66	0.63	0.48	0.06	0.56	-0.45	-0.12	0.42	1.00		
-0.24	-0.42	-0.41	-0.30	-0.30	-0.43	-0.36	-0.38	-0.52	-0.85	-0.42	-0.75	-0.91	-0.35	0.37	1.00	
0.83	0.83	0.84	0.89	0.89	0.75	0.91	0.91	0.84	0.64	0.85	0.06	0.50	0.66	0.76	-0.30	1.00

Significance Levels: 1% 0.505
5% 0.396

1. Leaf regularity
2. Basal shape
3. Auricle development
4. Simple hairs
5. Stellate hairs
6. Lobe number
7. Sinus number
8. Venation
9. Petiole length
10. Lamina + petiole
11. Petiole ratio
12. Lamina to widest part
13. Lamina length
14. Obversity
15. Lobe depth
16. Lamina width
17. Lobe depth ratio

TABLE 5.8 CORRELATION OF LEAF CHARACTERS FROM THE RESULTS OF 25 RANDOMLY CHOSEN POPULATIONS

populations. All show very high positive correlations with the other characters, with the exception of lamina length to widest part of the lamina which shows a variety of significant negative and significant positive but mainly non-significant correlations with other characters; and lamina width which shows negative correlations with all other characters of which eight were significant. Since these were computed on hybrid index data, using all populations, i.e. both Q. petraea and Q. robur, it would be expected that all characters should show high positive correlations with each other if the hybrid index ranges have been satisfactorily chosen. All but two characters conform, and it must be concluded from this that the hybrid index ranges are reasonably based. This is supported by ordination of the characters in component space (Figure 5.21). It is difficult to compute a PCA for character ordination using all populations since this involves storage of a 135 x 135 correlation matrix, and so the PCA for Figure 5.21 was completed on a subset of 25 populations, randomly chosen, but representing the different population types in the same ratio as the full data set. Again, it must be expected that the characters would ordinate together, showing signs of similar behaviour, i.e. all characters being high in Q. petraea populations and low in Q. robur populations. Twelve of the characters ordinate very closely together, with another three quite close - of the two remaining characters lamina width separates on the first Component, whilst lamina length to widest part of the lamina separates on the second Component. The lack of agreement between these two characters and the others both in simple correlation and PCA indicates a poor delimitation of the original hybrid index scale from the raw data. The large number of significant negative correlations between lamina width and the other characters suggests that the original range might require to be completely reversed to obtain positive correlation between this

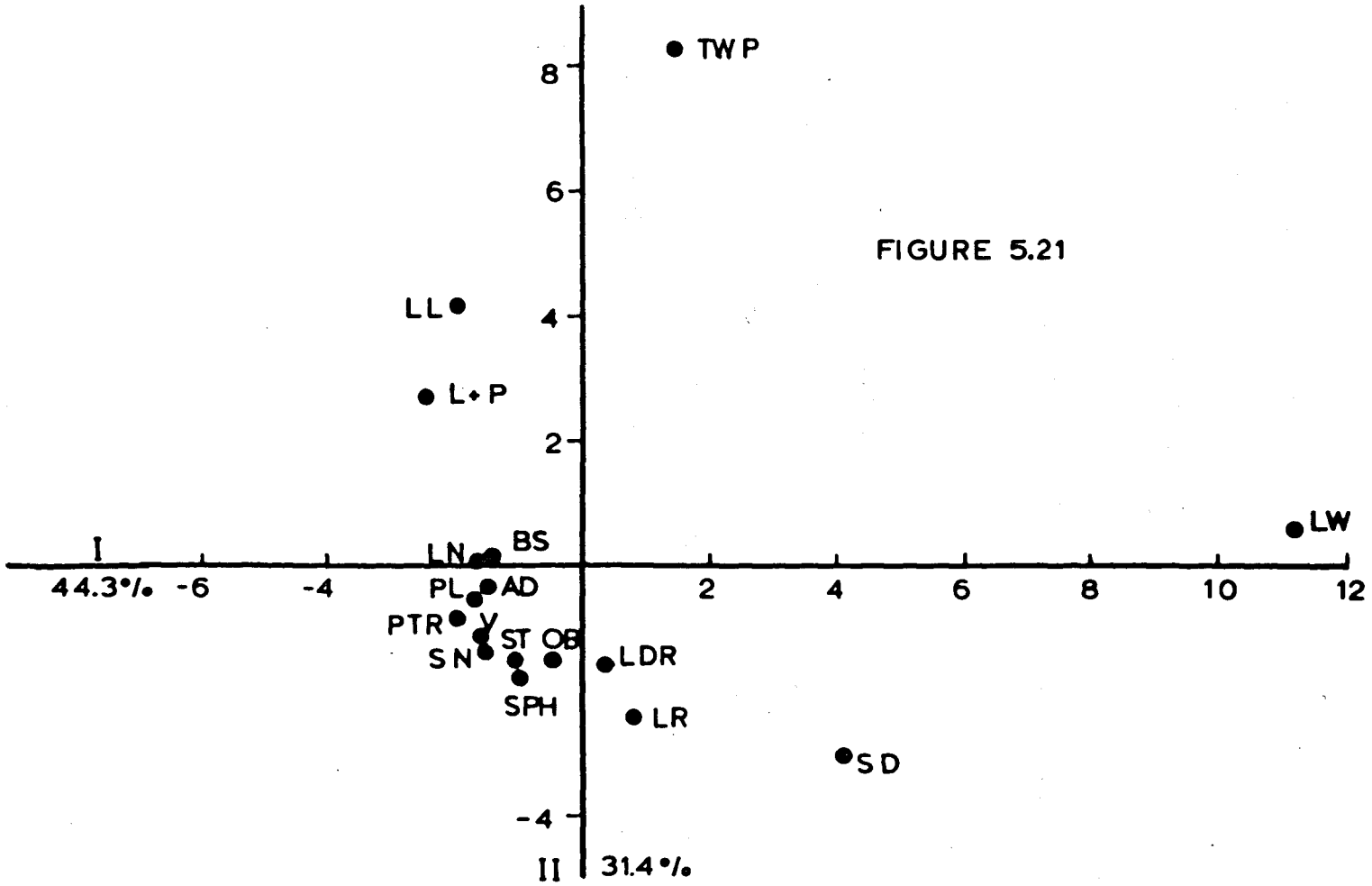


FIGURE 5.21 PCA of characters used in the populational survey using 25 randomly chosen populations. The scatter shows the first two components from the correlation matrix. (See Table 5.1 for a listing of the characters)

and other characters. The general lack of any correlation between lamina length to the widest part would indicate it is a poor character to differentiate the species.

The abnormal behaviour of these two characters is not thought to have influenced the results of the population assessment.

In conclusion, it may be reaffirmed that although it is possible to recognise populations showing varying stages of suspected introgression, it is not possible to support the view of wide scale introgression noted by Cousens (1963, 1965) in Scotland. Evidence must, however, be presented of a genetical nature before definite conclusions can be drawn from the taxonomic status inferred for the populations discussed here.

CHAPTER SIX

ENVIRONMENTAL VARIABLES AND THE DISTRIBUTION OF OAK POPULATIONS

Introduction

Anderson (1948) has speculated on the importance of the habitat as a factor in controlling hybridisation under natural conditions. He argues that the F_1 generation should be uniform in its ecological requirements, and that these may be expected to be intermediate between those required by the two parents, but the segregation and recombination occurring in the F_2 generation would indicate that this generation will have a series of habitat requirements, each individual requiring its own peculiar habitat. As evidence he cites the segregation and recombination of many physiological characters including length of flowering season, disease resistance, light tolerance, cold tolerance etc. each of which would indicate that recombinant types would require particular habitat features. Anderson and Hubricht (1938) give an ecological example of this form of hybrid behaviour. They reported that two Tradescantia species, T. subaspera and T. canaliculata grow in adjacent habitats which differ in, amongst other things, shade, soil type and leaf mould cover. Under artificial conditions, the two species hybridise freely, but under natural conditions, the hybrids are rarely found. The reason would appear to be the lack of a hybrid habitat, i.e. a habitat intermediate between that of the two parental habitats, in this case, a gravelly soil, light leaf mould cover and partial shade. Anderson (1948) recognises that the F_2 generation, for only these three 'habitat characters', would require six different habitat types to accommodate all possible recombinant types, and for twenty basic habitat differences over a million different habitat types would be required. Such considerations would account for the apparent lack of naturally occurring

hybrids between the two Tradescantia species, and the lack of hybrids in other similar situations. In areas disturbed by man, the creation of new and possibly hybrid habitats might lead to the promotion of hybridisation, and examples have been reported (Anderson, 1949) where this would appear to be the case.

Examples of the control of hybridisation by ecological factors have been reported in the genus Quercus by Muller (1952). He recognised two possible restrictions to hybrid establishment, edaphic restriction and climatic restriction. Q. Mohriana is a species of limestone areas, either growing on limestone itself or in shallow soils overlying limestone, whilst Q. Havardi is confined to coarse sands. In hilly limestone areas overlain with sand, the two species meet at the boundary between the sand plateau and the limestone slopes, and at this boundary where edaphic conditions are intermediate between the two parental preferences, the species hybridise and the hybrids survive. Q. Harvardi also hybridises with Q. stellata but only in areas where their distributions overlap do hybrids survive, Muller (1952) recognising this as climatic restriction of hybridisation. Benson et al.(1967) recorded similar phenomena to those of Muller (1952) on a much smaller scale, the different recombinant types occurring on slopes with differing degrees of exposure suggesting ecological selection operating on the F_2 generation.

Q. robur and Q. petraea are not geographically, climatically or edaphically separated in the British Isles - they show instead different 'preferences', there being no apparent delimiting line between the habitat types of the two species. These preferences and geographical differences within the British Isles were discussed in Chapter 1. It would seem reasonable to suppose, from evidence of the rest of the genus, that hybrids between Q. robur and Q. petraea would show habitat preferences different, and in some way intermediate, between the two

parents. This chapter investigates the geographical distribution of the population types recorded in the previous chapter, with reference to the influence of environmental parameters in determining their occurrence.

The geographical distribution of population types

The sampling of the 135 populations could be considered to be a random sample, although the use of 'known' populations would have caused some departure from a purely random situation. The distribution of the different population types is given in Figure 6.1, and the distribution of the population types as they were classified using Ward's Error Sums of Squares Cluster Analysis (i.e. the results from Table 5.6) is shown in Figure 6.2.

With few exceptions, the pure Q. robur populations were found to lie in the east of the country encompassing the Lincolnshire Wolds, East Anglia, Essex, Cheshire, The Midlands, Buckinghamshire, Oxfordshire, Gloucestershire, Shropshire and Worcestershire. The two exceptions were populations AH and AG in the Cardiff area of South Wales, but otherwise pure Q. robur populations were completely absent from Wales. Indeed, in the eastern half of the area, with the exception of populations CD and CE (pure Q. petraea), four mixed populations CCE, BF, BBB and BBC, and one introgressed Q. petraea population CX, all populations were either pure Q. robur or introgressed Q. robur. Nottinghamshire, Lincolnshire, East Anglia and Essex also appeared to consist entirely of pure Q. robur populations, the exception being the mixed population CCE in Norfolk.

The distribution of the introgressed Q. robur populations lies to the west of these populations, but still mixed very intimately with the pure Q. robur distribution. Such populations do extend much further west, however, reaching the coastal part of Wales, with populations in Denbighshire, Caernarvonshire, Carmarthenshire and Monmouthshire. Within

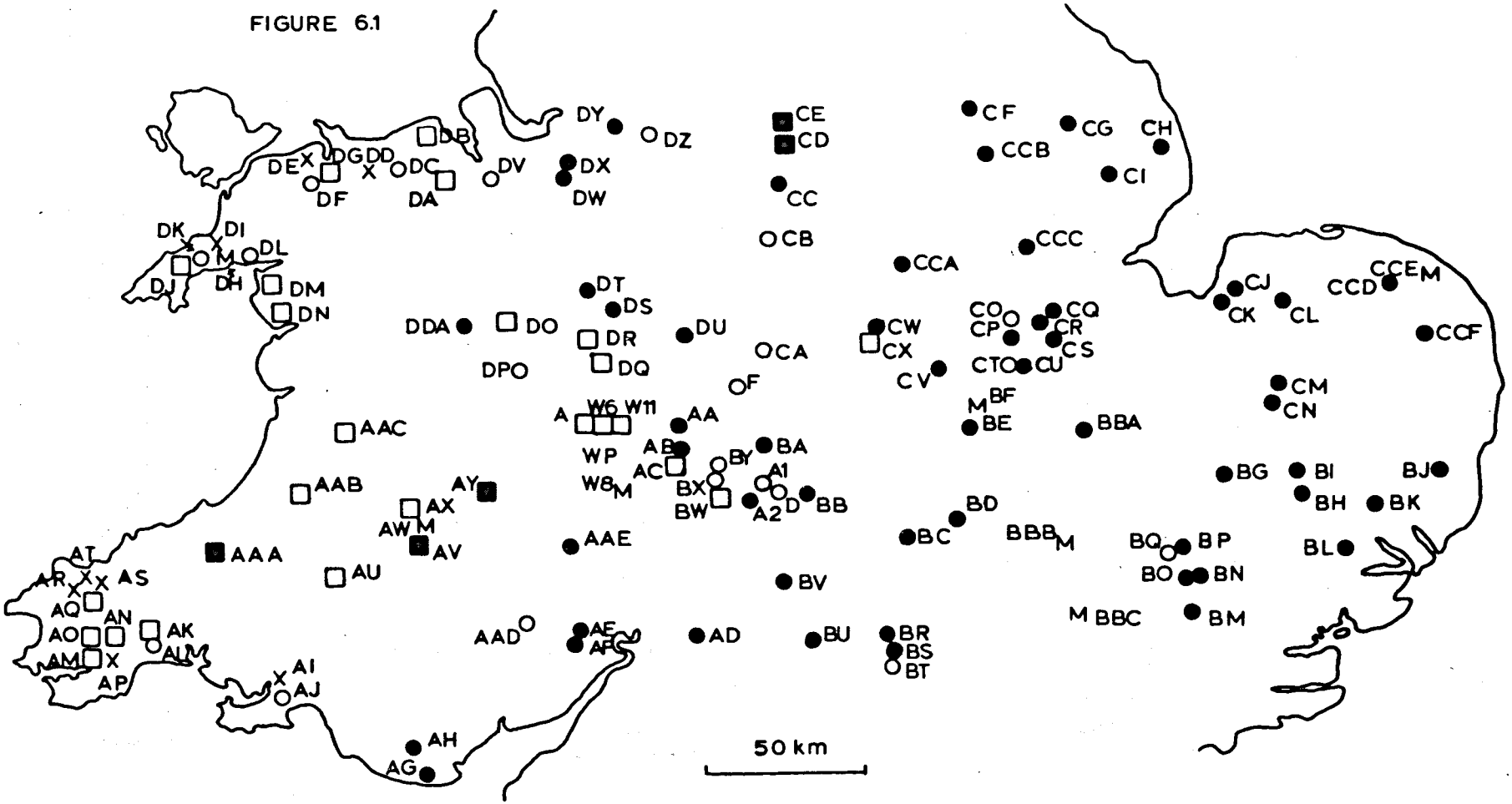


FIGURE 6.1 Geographical distribution of population types
(● pure Q. robur; O introgressed Q. robur
■ pure Q. petraea; □ introgressed Q. petraea
X intermediate populations; M mixed populations)
WP = 12 pure Q. petraea Wyre Forest sub-populations

FIGURE 6.2

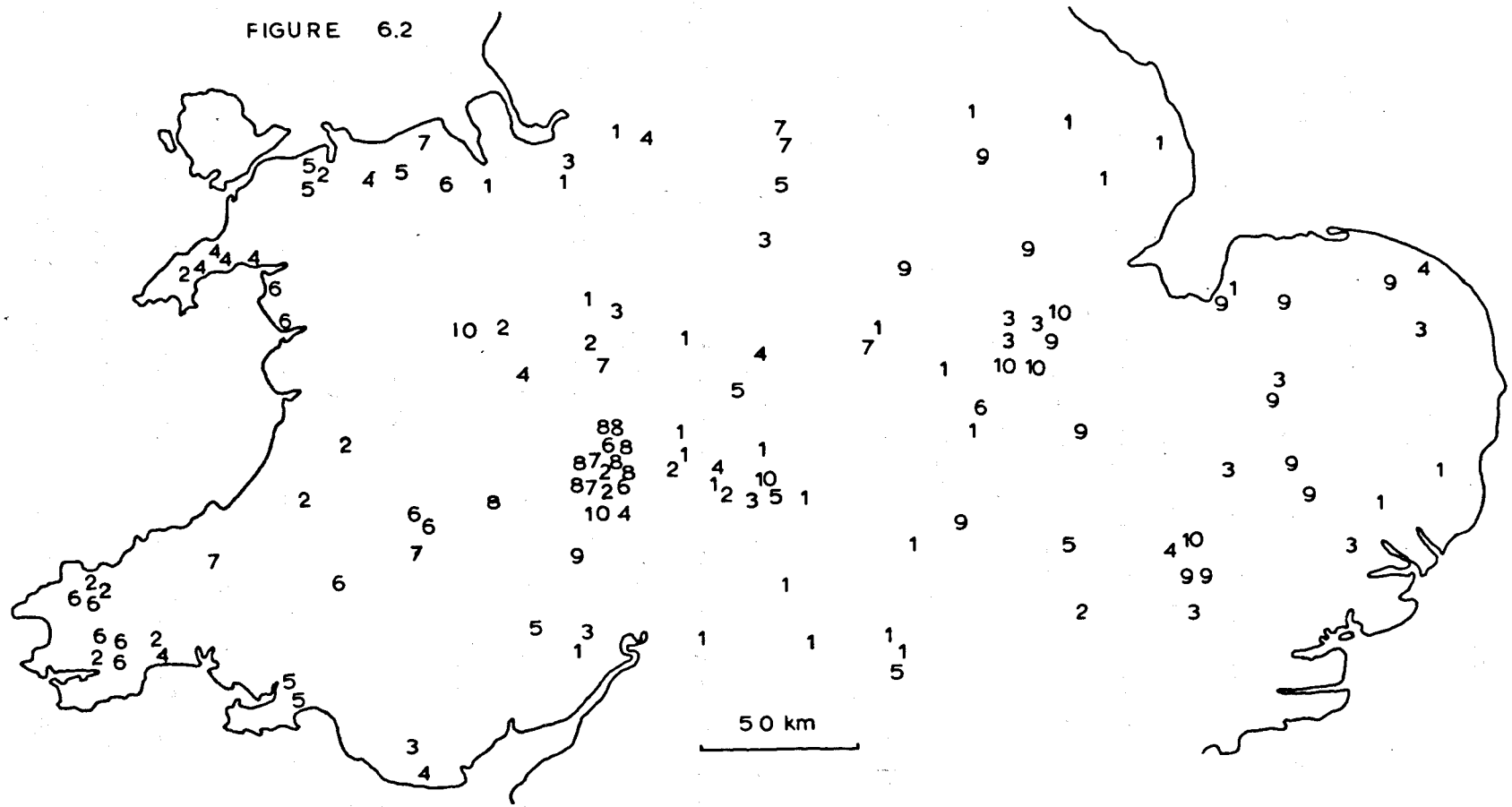


FIGURE 6.2 Geographical distribution of the ten Ward's cluster groupings using mean Hybrid Index data for each population

the West Midlands area, there are at least six introgressed Q. robur woodlands (CA, F, BY, BX, Al and D), and this must represent a concentration centre for hybridisation.

The pure Q. petraea populations were mainly represented by the Wyre Forest sub-populations (W_1, W_2, W_3, W_4, W_5, W_7, W_9, W_{10}, H, B, C and G), the others being CD, CE, AY, AV and AAA. These all lie to the west of the main pure Q. robur distribution, populations CD/CE proving the exceptions; the Wyre Forest populations lying just on the dividing between the Q. robur and Q. petraea areas.

It should be remembered that CD, CE, W_7 and AAA were all shown to be examples of the 'pseudo'-pure Q. petraea population type, and certainly the positioning of CD/CE in a geographical sense would appear to be rather anomalous for a pure Q. petraea population, although edaphically its position would appear logical since it is located in the Pennine Range.

The introgressed Q. petraea populations, with the exception of CX, all lie in the west of the area, particularly along the Welsh coastline from Flint to Pembroke. Other groups of introgressed Q. petraea populations occur, namely the AAB, AAC, AX, AU group in the Radnor area, the DO, DR, DQ group in Shropshire and the BW, AC, A, W_6 W_{11} group in Warwickshire/Worcestershire, suggesting again the importance of hybridisation in the Midlands area.

The lack of relatively few pure populations of either species in Wales, particularly along the Welsh coastline, is also reflected in the distribution of the intermediate populations, i.e. those containing only apparently hybrid intermediate individuals. Of the eight populations of this type, all occur along the Welsh coastline, usually in close association with introgressed populations of both species. Wales generally consists therefore of a mixture of intermediate and introgressed

populations, with very few recognisably pure populations of either species. From a comparable point of view, Wales is at the edge of the distribution of both species (if Ireland is discounted), and would appear to show the same pattern of variation as that recognised by Cousens in Scotland (Cousens, 1963) also at the limits of the distribution, although individual populations of Wales are by no means as heavily introgressed as those in Scotland.

The distribution of mixed populations varies widely, and may possibly be due to planting rather than natural occurrence.

The two pseudo-pure Q. robur populations CG and AAE are widely separated geographically, but they also separated in the PCA of the whole population data, CG grouping with the main pure Q. robur group, AAE being well separated from it and the pure Q. robur populations. CG is a Lincolnshire population, and consequently within the distribution range of the pure Q. robur populations, whilst AAE falls at the very west of the distribution of the pure Q. robur range, this agreeing with their positioning in the PCA.

The distribution of the members of the ten clusters of the CA of the population data is shown in Figure 6.2, and although broadly following the same pattern as that discussed above, there are some interesting differences.

In Figure 5.19, the pure Q. robur groupings from the PCA of the total population data separated into the four Wards clusters of the same data, Clusters 9, 1, 3 and 10 on the second Component. Geographically, these clusters do not remain distinct (Figure 6.2) but some generalisations may be made concerning their distribution. Cluster 9, the one occurring at the base of Component 2 in Figure 5.19, centres in the East Anglian and Hertfordshire regions, eleven populations out of the fifteen in the group being found in this area. Another three

lie in the Nottinghamshire/Lincolnshire/Leicestershire border areas. Cluster 1, however, falls more towards the Northamptonshire, Oxfordshire, Gloucestershire and The Midlands area, i.e. more towards the west, but also to the north with populations in Cheshire and North Lincolnshire. Clusters 3 and 10 appear to be more widespread, but 3 has a centre of distribution (8 out of 14 populations) in the area of the main Cluster 9 group.

Clusters 4 and 5, the main introgressed Q. robur clusters, tend to have their centre of distribution in North Wales, whilst Clusters 2 and 6, the main introgressed Q. petraea clusters, have their centre of distribution in South Wales. This emphasises a point not immediately evident from the distribution of population types (Figure 6.1) that although the situation in Wales is very confused, with nearly all populations in some way deviating from the pure types, there would appear to be more influence of Q. robur in the populations of North Wales than those in the south.

Cluster 8, the cluster of pure Q. petraea populations containing only Wyre Forest populations and AY, and Cluster 7, the other similar cluster, both have their centres of distribution in The Midlands, spreading into Central Wales and the North Midlands.

Environmental variables and the distribution of population types

The above discussion would suggest that the distribution of population types follows, although by no means closely, a geographical gradient. The apparent preponderance of Q. robur populations in the east would clearly suggest that in this area, although Q. petraea occurs (see Figure 1.2), it rarely forms populations of any size - if it did, then at least some of these would be expected to have been chosen by the approximately random sample. Planting cannot be discounted as a possible

influence on the apparent distribution of population types, but populations that were obviously planted were not included in the survey. However, some small error due to the possible effects of planting must be accepted.

In order to investigate further this relationship between population type and geography, environmental variables of an edaphic, geographical and climatic nature were obtained for each population and the data subjected to a PCA. The variables were:

1. Altitude - above sea level, measured in feet.
2. Soil pH - during the collection of the population samples, soil samples were also removed. pH measurements were completed on five subsamples of each fresh sample.
3. Base status of the soil - since only comparative measurements were required, it was decided to use the pH method of determining total exchangeable bases using normal acetic acid (Brown, 1943). Although a slightly inaccurate measure, it has the advantages of being very rapid and giving consistent results - milli-equivalents.
4. East-West geographic position - measured from a baseline running along the East Anglian coastline, through Norwich and parallel to the line of longitude.
5. North-South geographic position - measured from the 52° $30'$ N line of latitude.
6. February minimum temperature - $^{\circ}C$
7. January mean temperature - $^{\circ}C$
8. July mean temperature - $^{\circ}C$
9. Rainfall - inches
10. Humidity

Temperatures and rainfall figures were derived from local weather stations closest to each population or, if a population was a reasonable distance from the nearest weather station, then from published weather maps, interpolating the values for the population. Values for humidity were derived from the ratio Precipitation/Saturation deficit as described by Perring and Walters (1962). Each population was assessed for the ten variables, and the data matrix subjected to a PCA. This raw data matrix is filed in Appendix 4. Figure 6.3 shows the resultant scatter diagram

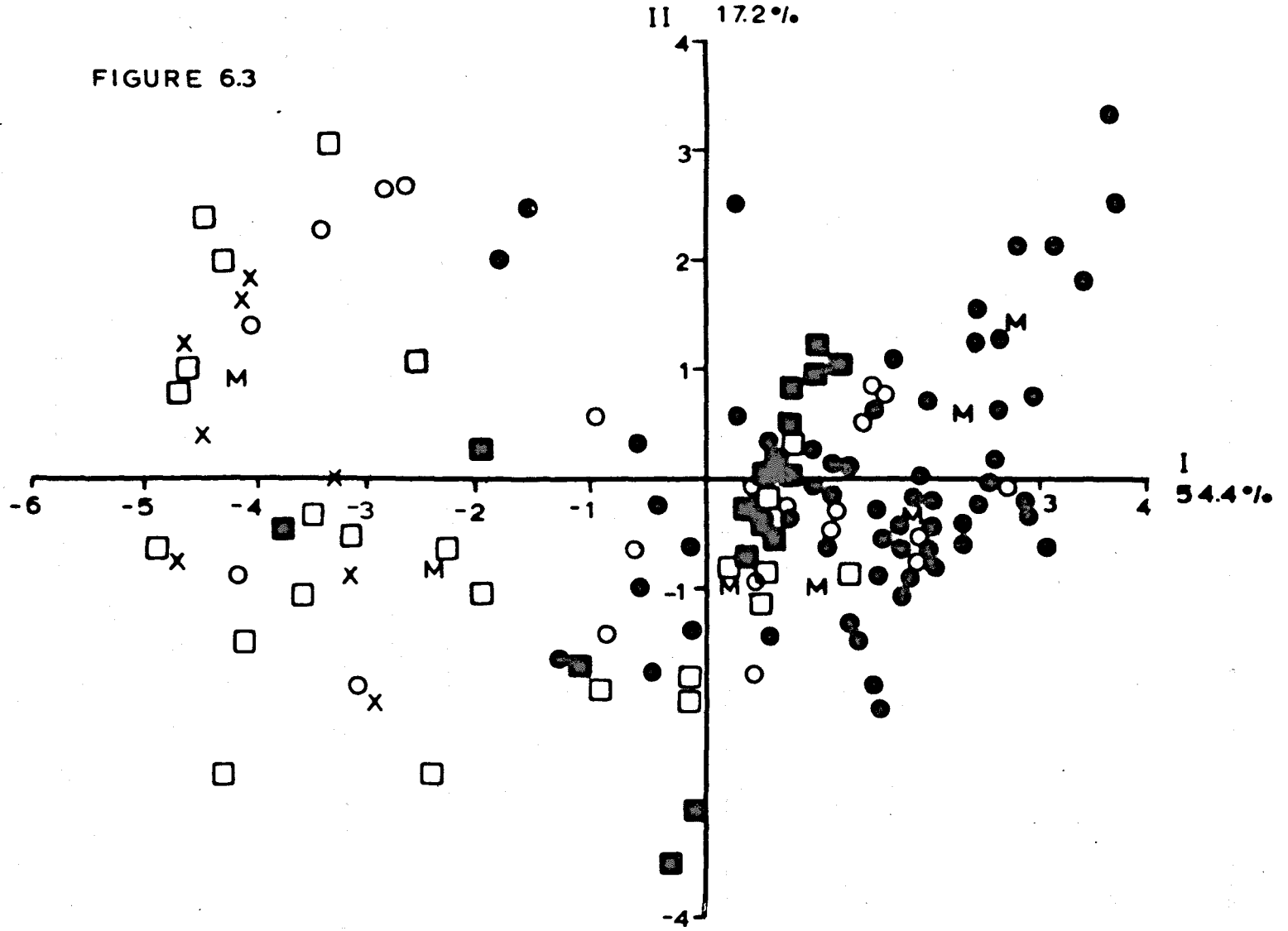


FIGURE 6.3

FIGURE 6.3 PCA of all populations using environmental variables
(● pure Q. robur; O introgressed Q. robur
■ pure Q. petraea; □ introgressed Q. petraea
X intermediate populations; M mixed populations)

for the first two components of the correlation matrix which accounted for 71.58% of the variance.

Since the majority of the variables used in this analysis might be expected to be correlated with geographical position, eg. temperature, rainfall, etc. then it might be expected that the resultant scatter diagram reflects geographical position. This is in essence the underlying feature of Figure 6.3 - the left-hand side represents Wales, the right-hand side East Anglia, the top represents Oxfordshire, Gloucestershire, and the bottom Derbyshire, Lincolnshire, etc.

However, when the eigenvectors are examined (Table 6.1), although the first component is seen to have high loadings for temperature, rainfall, humidity and east-west position variables, the soil factors also show a high positive loading. The second component is a north-south component, but the highest loadings are soil pH and soil base status.

The distribution of population types estimated from individual analysis and the population groupings derived from Ward's Error Sums of Squares CA along the first Component are shown in Figures 6.4 and 6.5 respectively. The pure Q. robur populations fall to one end of Component 1 (see Figure 6.4) and this is also reflected in the position of Clusters 1, 3 and 9, the pure Q. robur clusters, which also occur at the extreme part of Component 1 (Figure 6.5). Introgressed Q. robur Clusters 4 and 5 although showing a small peak at the approximate position of Clusters 1, 3 and 9 extend right across the first Component. This is emphasised in Figure 6.4, where again the introgressed Q. robur populations extend across Component 1 but peak in the pure Q. robur zone. The introgressed Q. petraea populations and the corresponding Clusters 2 and 6 show a completely opposite distribution pattern, a peak on the extreme of Component 1 from Q. robur, and spreading along the Component. The intermediate populations similarly fall at the extreme end of Component 1, reflecting their

	Component 1	Component 2
Altitude	- 0.20	- 0.36
Soil pH	0.43	0.74
Base status	0.52	0.71
East-West position	- 0.91	0.12
North-South position	- 0.01	0.59
February minimum temperature	- 0.92	0.29
January mean temperature	- 0.93	0.30
July mean temperature	0.82	0.05
Rainfall	- 0.90	0.00
Humidity	- 0.96	0.14

Eigenvectors, standardised so that sum of elements squared equals the latent root

TABLE 6.1 EIGENVECTORS FOR FIRST TWO COMPONENTS OF THE PCA ON ENVIRONMENTAL VARIABLE DATA

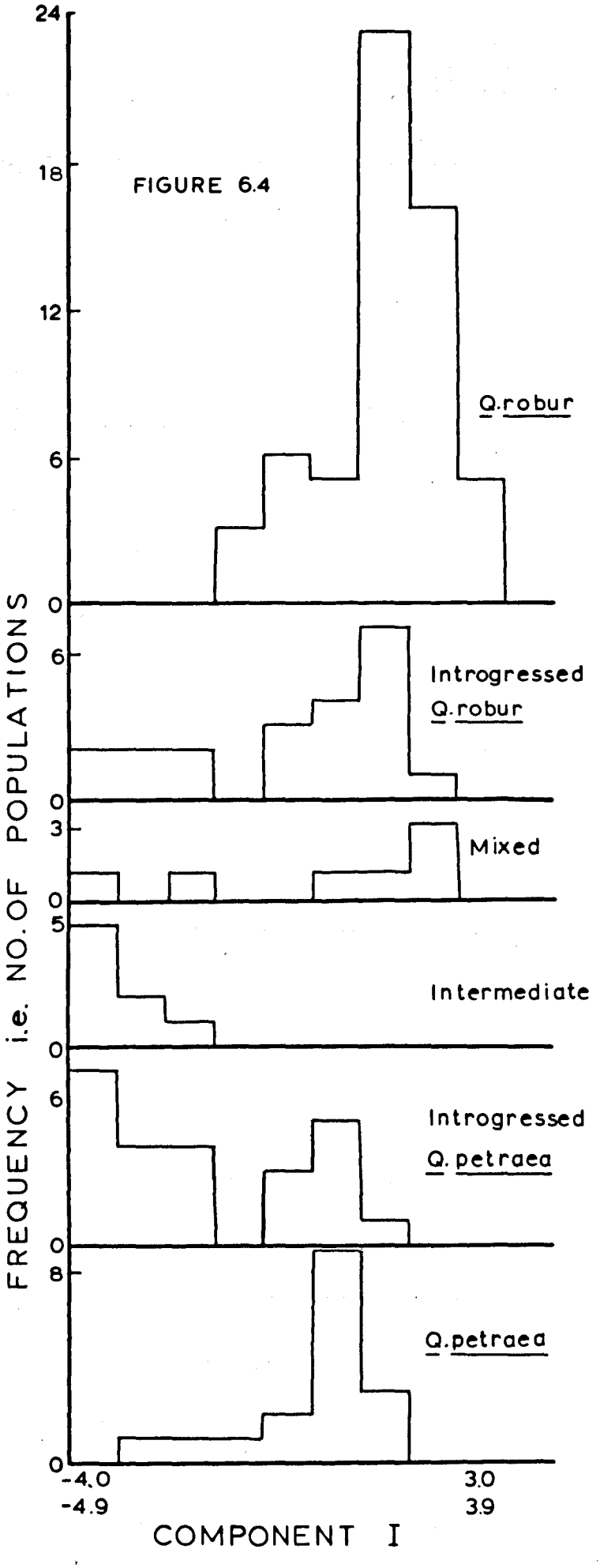


FIGURE 6.4 Frequency of population types along Component 1 of a PCA of environmental data recorded for each population (from the Correlation matrix)

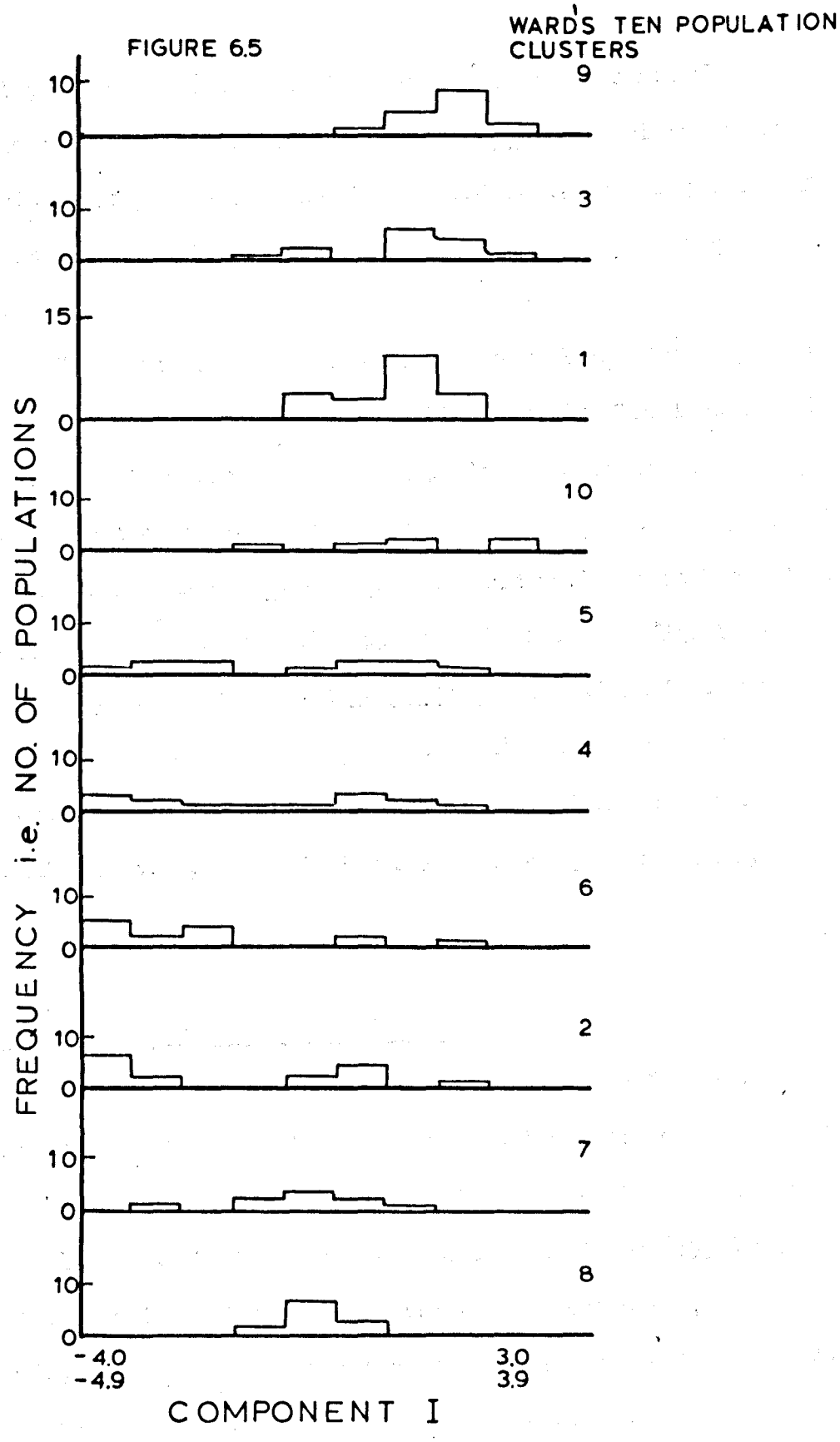


FIGURE 6.5 Frequency of the ten Ward's cluster groupings along Component 1 of a PCA of environmental data recorded for each population (from the Correlation matrix)

geographical position, whilst the mixed populations occur all along the first Component. The position of the pure Q. petraea populations reflects also the position of populations in the sampled area, as being some way between the introgressed Q. petraea populations and the Q. robur populations.

The first Component is not, however, a purely climate/geographical one, since the two soil factors also have high positive loadings on this Component. This would agree in part with published accounts of the preferences of the two species for soil type - Q. robur, a basic/neutral soil with a high base status; Q. petraea, an acid soil low in base status. The pure Q. robur populations would appear to be found in high base status, high pH soils, and the introgressed Q. petraea populations in low base status, low pH soils. The pure Q. petraea populations form something of an anomolous group, due probably to a lack of sufficient different populations of this type, the majority being sub-samples of the Wyre Forest.

Floristic characteristics of oak populations

The analysis presented above may be taken one stage further. Since much preliminary work has been completed on the floristic composition of oakwoods, it has been possible to prepare lists of species associated with particular soil types. Such an exercise is that of Jones (1959) who recognised three basic soil types - nutrient deficient soils, usually podsols or gley-podsols on highly siliceous rocks; base-deficient soils, but better supplied with nutrients than the podsols, and usually well-aerated (the Brown Forest Soils); and basic soils, generally rich in nutrients. Each soil type developes a characteristic ground flora under an oak canopy, but this becomes modified by the drainage pattern. Characteristic species are, therefore, found on, for example, wet podsols.

During the course of the population sampling, lists of associated species were prepared in presence/absence format. To analyse this data, it was decided to ignore all species except the more common species recorded and those mentioned by Jones (1959) as occurring on different soil types under oak canopies. This gave a list of 37 species, and the soil types of which they are characteristic are given in Table 6.2. The use of such a reduced data list is easily justified. The rare species add greatly to computer time, but although it may be argued as rare species, they probably have a well defined niche, and are therefore ecologically important in defining habitats, rarity may be a function of sampling time. Vernals would be rare if sampled in autumn, and consequently rarity cannot always be considered an important ecological property. Other species were excluded, since during the sampling, the 37 species were specifically sought after, other species were noted when found, and as this possibly introduced some bias into the data collection, it was thought best to restrict analysis to the shortened data set.

The data was analysed in three different ways - by Association Analysis (Williams and Lambert, 1959, 1960) to provide a grouping of populations, by Inverse Association Analysis (Williams and Lambert, 1961) to provide a grouping of species and by PCA to provide an ordination of populations. Pielou (1969) has argued that the use of PCA with qualitative data although formally possible is not illuminating, and has suggested instead the use of Principal Coordinate Analysis. The experience of the present author not only with the data of this thesis but other data sets representing a wide range of situations is that PCA produces good results with qualitative data and more importantly good correspondence with the results from Principal Coordinate Analysis on the same data, and for these reasons PCA was used on the floristic data. The results of the Association Analysis are given in Figure 6.6, those of the Inverse Analysis in

	Dry soils	Damp soils	Wet soils
Nutrient deficient soils	1. Betula spp. 2. Sorbus aucuparia 3. Ilex aquifolium 4. Vaccinium myrtillus 5. Deschampsia flexuosa 6. Melampyrum pratense 7. Galium hercynicum 8. Potentilla erecta	1. Betula spp. 2. Sorbus aucuparia 3. Ilex aquifolium 9. Molinia caerulea 10. Blechnum spicant + species of dry soils	1. Betula spp. 2. Sorbus aucuparia 3. Ilex aquifolium 9. Molinia caerulea 11. Juncus acutiflorus 12. Viola palustris 13. Lotus uliginosus 14. Sphagnum palustre
Base deficient soils but not as impoverished as above	15. Holcus mollis 16. Pteridium aquilinum 17. Digitalis purpurea 20. Endymion non-scriptus 37. Luzula multiflora	19. Anemone nemorosa 21. Rubus fruticosus agg. 22. Salix spp.	23. Deschampsia caespitosa 24. Agrostis stolonifera
Basic, or nutrient rich soils	25. Mercurialis perennis 26. Arum maculatum 27. Circaea lutetiana 28. Geranium robertianum 29. Sanicula europaea 30. Zerna ramosa	31. Allium ursinum 32. Rumex spp. 33. Angelica sylvestris	18. Carex riparia 34. Mentha aquatica 35. Viburnum opulis 36. Epilobium hirsutum

The numbers refer to the code numbers used for each species during recording.

TABLE 6.2 SOIL TYPES OF BRITISH OAKWOODS AND THEIR ASSOCIATED FLORA (after Jones, 1959)

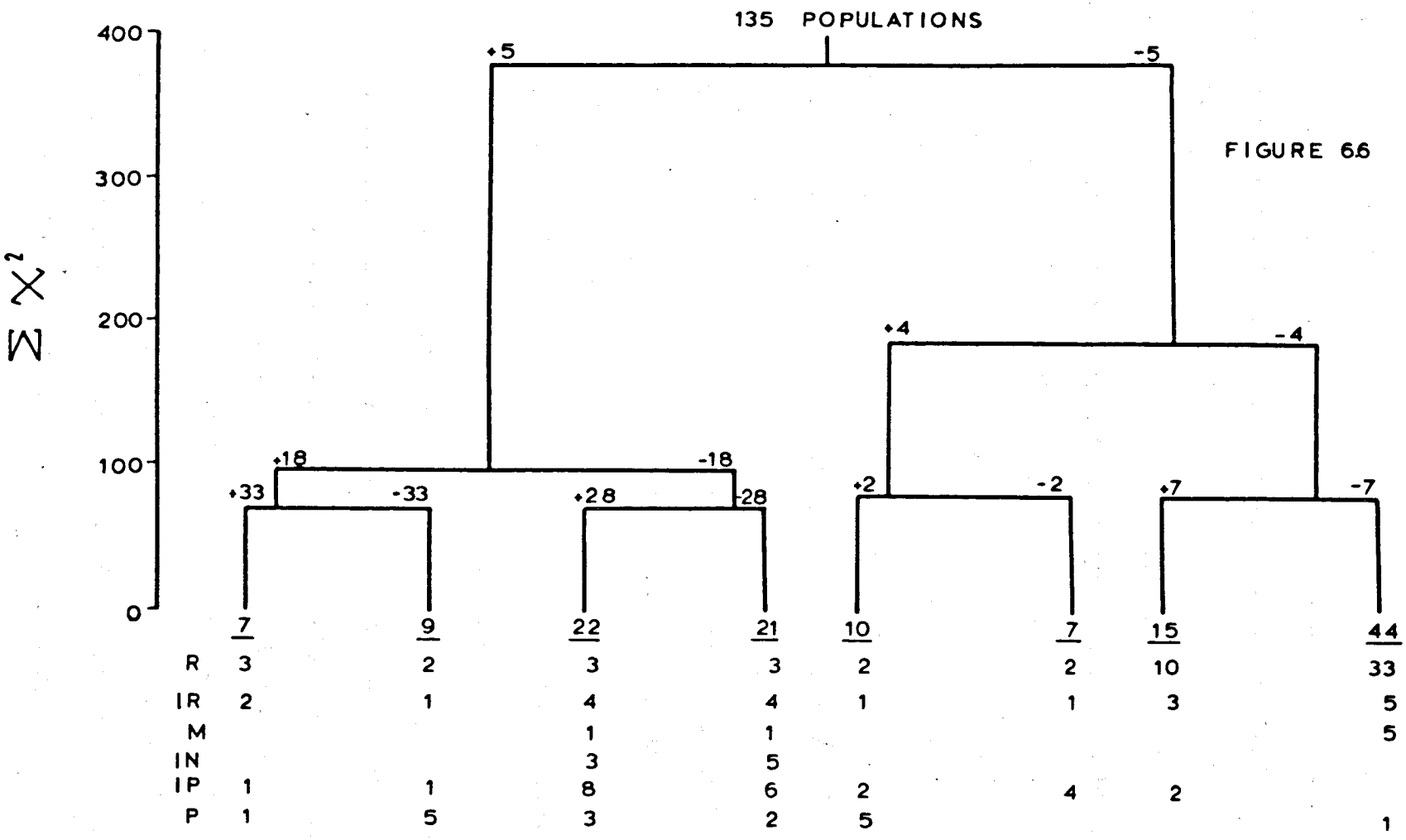


FIGURE 6.6

FIGURE 6.6 Association Analysis using presence and absence data for 37 species recorded in 135 populations. The figure shows the membership of the Association Analysis groupings classified into population types - R, pure Q. robur; IR, introgressed Q. robur; M, mixed populations; IN, intermediate populations; IP, introgressed Q. petraea; P, pure Q. petraea. The code numbers for division species are listed in Table 6.2.

Figure 6.7, and the PCA ordination in Figure 6.8. The original data set on which these analyses is based is given in Appendix 4.

The Inverse Analysis (Figure 6.7) produced groups of species reasonably consistent with the groupings expected for different soil types, with one exception, that while species divided well into the three base status types, they did not on the whole divide into soil moisture groups. For example, Group 1 (Figure 6.7) contained six species characteristic of the basic soil type, of which three were 'dry' species, one a 'damp' species, and two were 'wet' species. Similarly, Group 11 was a nutrient deficient grouping with three 'dry' species, one 'damp' and one 'wet' species, together with two species covering the range of moisture levels for this soil type. The analysis recognised the nutrient deficient group of species (Groups 4 and 11), the base rich group of species (Groups 1, 5 and 12) but the middle grouping of species of Brown Forest Soils was represented only by the small aggregate group 2/10/6 which contained three of the Brown Forest Soils species. The other species of this group appear to have grouped with the other two major soil types - species 21, 22, 23, 24 and 37 with the base rich species and species 17 and 19 with the nutrient deficient soil species.

Association Analysis, although showing some of the expected divisions, failed to clearly separate many groups. Groups 4 and 8 (Figure 6.6) defined as being without Deschampsia flexuosa and Vaccinium myrtillis (i.e. base deficient, dry soil species) contained a large proportion of the pure Q. robur populations (43 out of 58), but few other population types appeared to show much correlation with the Association Analysis groups.

PCA proved much more successful. From the scatter diagram of the population types (Figure 6.8) the frequency of each population type has been calculated along the first Component. This is shown in Figure 6.9. The distribution of population types was much clearer, pure Q. robur

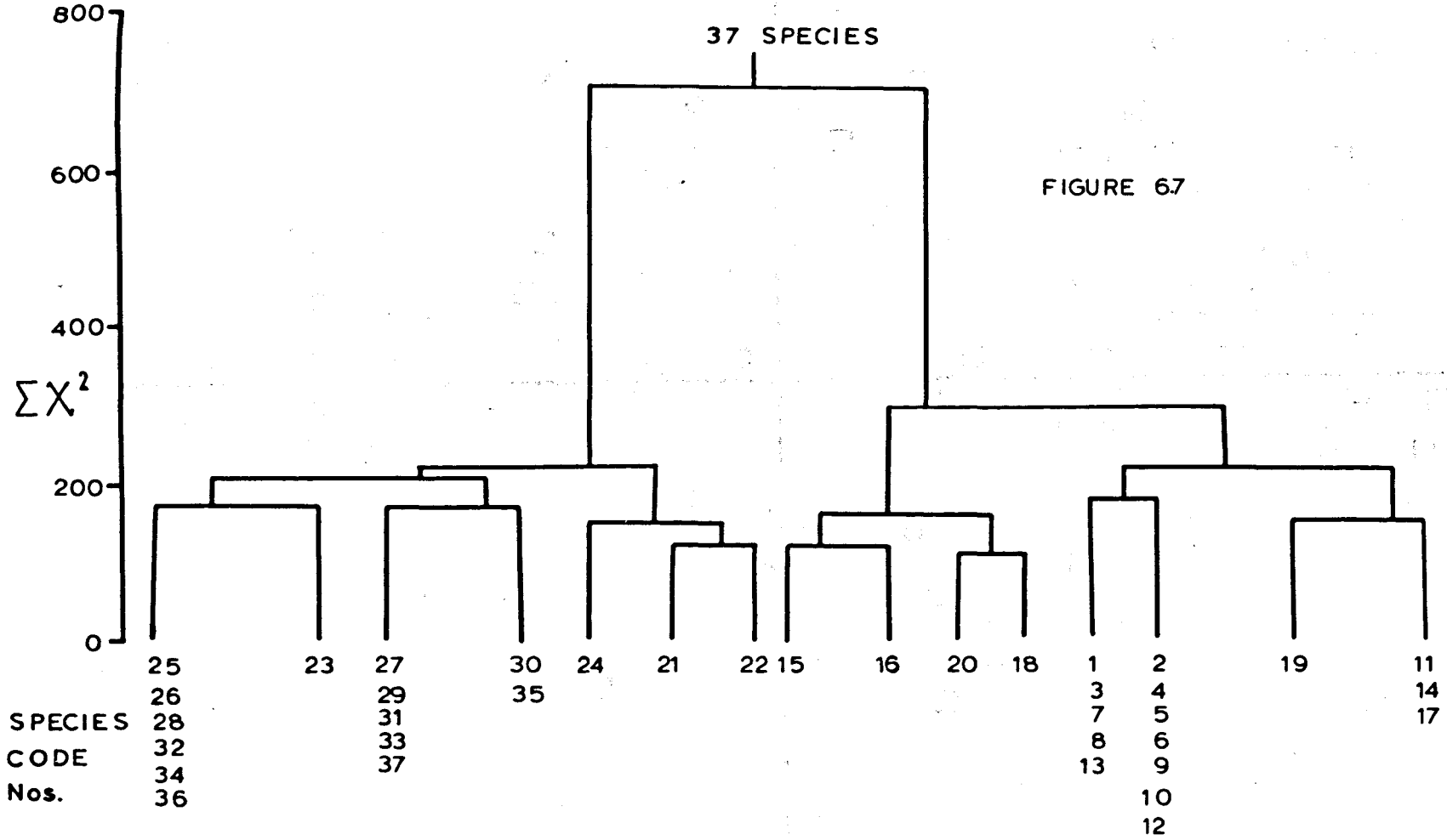


FIGURE 6.7

FIGURE 6.7 Inverse Association Analysis using presence and absence data for 37 species in 135 populations. Table 6.2 lists the species and their code numbers.

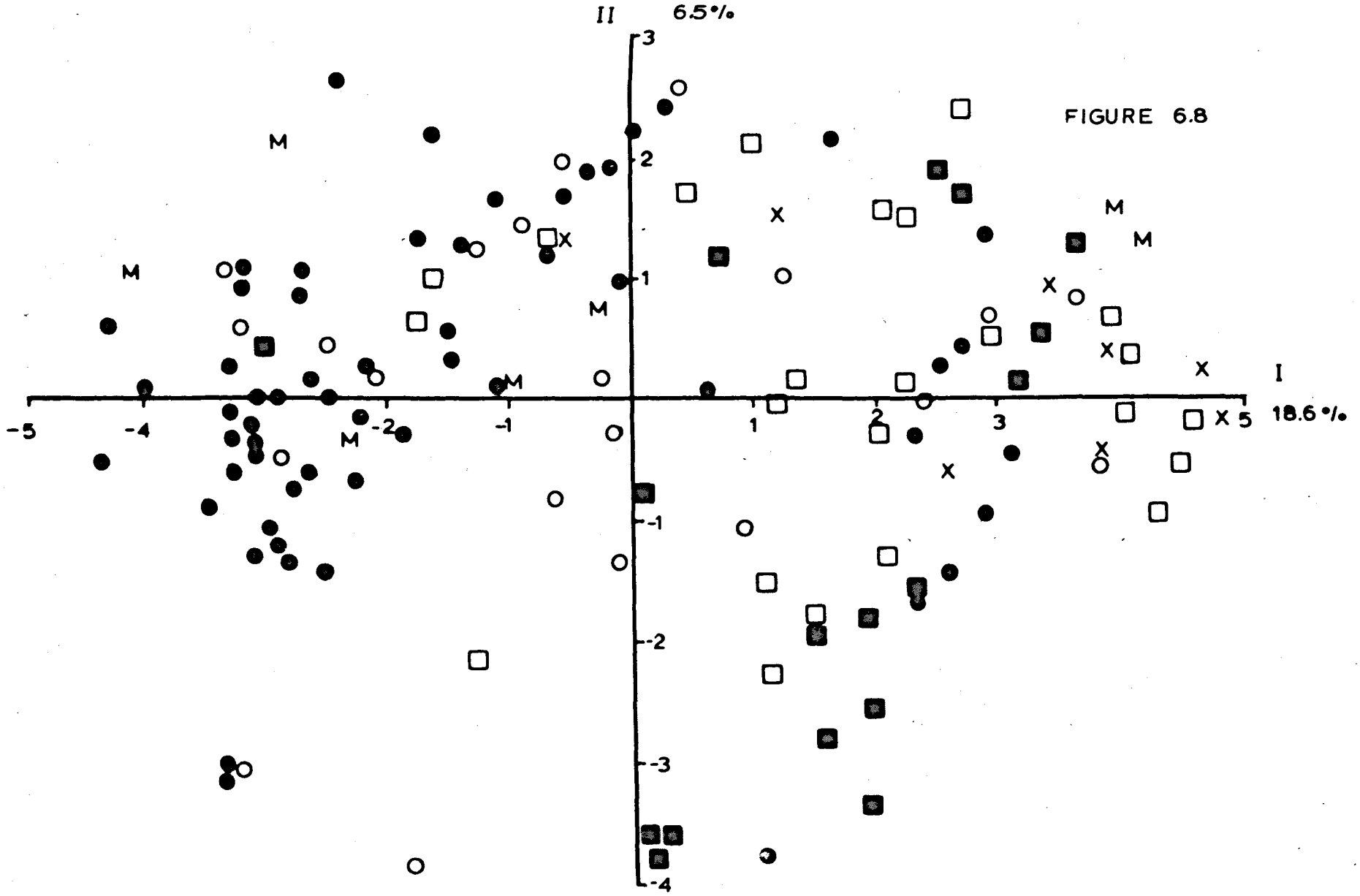


FIGURE 6.8 PCA of all populations using presence and absence floristic data for 37 species
(● pure Q. robur; ○ introgressed Q. robur
■ pure Q. petraea; □ introgressed Q. petraea
X intermediate populations; M mixed populations)

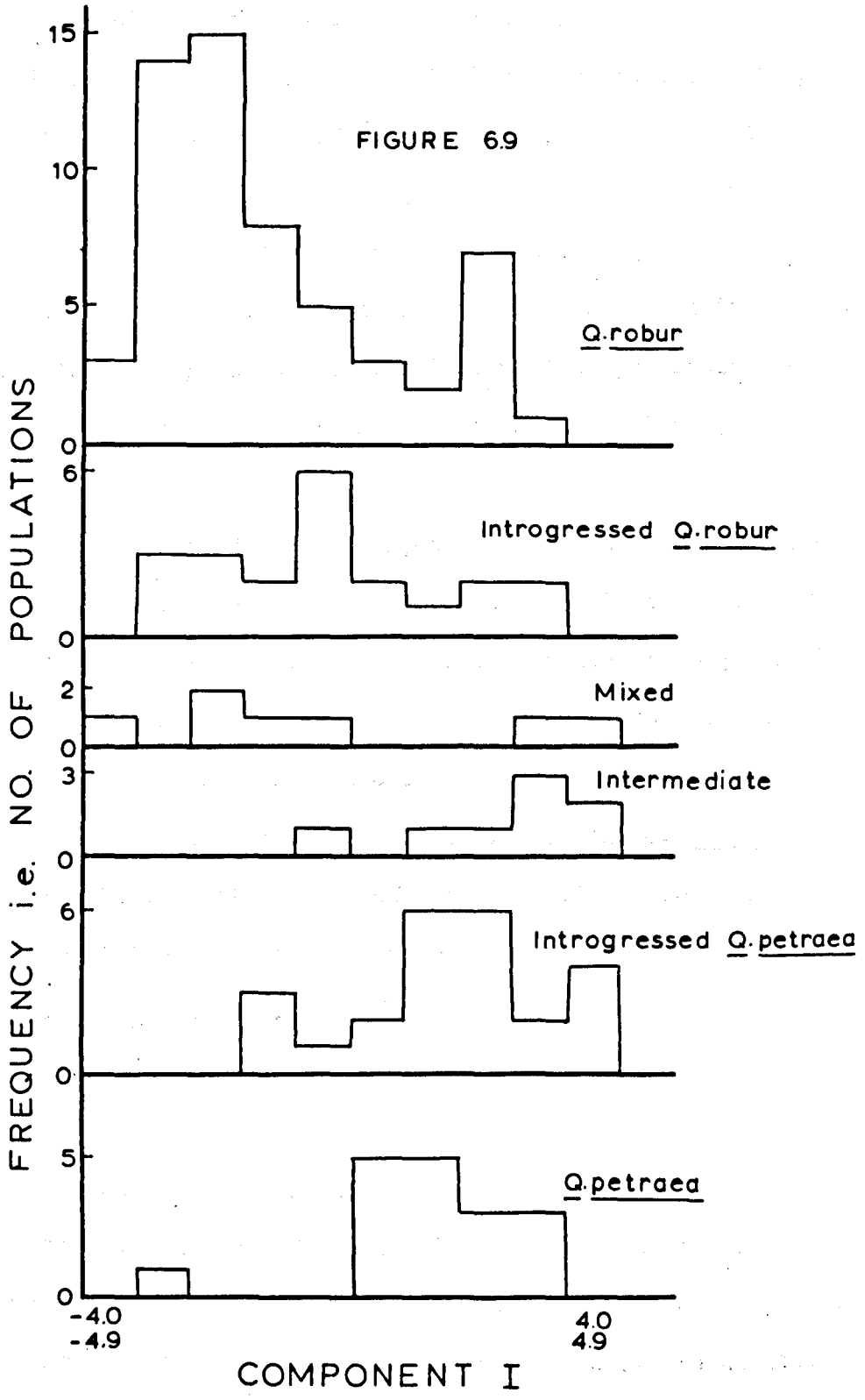


FIGURE 6.9 Frequency of population types along Component 1 of a PCA of presence and absence floristic data recorded for each population

populations being found to the left of the first Component, introgressed Q. robur populations although showing a similar range to the pure species peaked much closer to the centre of the Component, and the mixed populations occurred throughout the range. The pure Q. petraea populations showed a much narrower range than the introgressed Q. petraea populations, but this might be due to the influence of the large number of sub-samples from one population. The intermediate populations again formed a tight grouping at the extreme end of Component 1 away from the Q. robur populations.

The eigenvectors showed that the species of the nutrient deficient soils had a high positive loading for Component 1, whilst the base rich species had a high negative loading. The intermediate group generally showed low positive loadings. Consequently, the nutrient deficient group of species were to be found associated with populations at the far right of Component 1, the centre of distribution of the Q. petraea populations, and the base rich group of species could be found associated with populations at the far left of Component 1, the centre of distribution of the Q. robur grouping. (See Table 6.3)

Discussion

The geographical position of the population types would argue for areas of stability - the east of the sampled area, areas of partial breakdown of isolating barriers - The Midlands, and areas of comparatively large amounts of hybridisation - Wales. Measurements of the habitat either directly by climatological variables, soil factors, etc. or indirectly with associated flora would indicate that this gradient of hybridisation follows reasonably closely a habitat gradient.

Such habitat preferences have been recorded in similar situations, but more usually on a smaller scale. Carlisle and Brown (1965) in

1.	Betula spp.	0.64	19.	Anemone nemorosa	0.14
2.	Sorbus aucuparia	0.58	20.	Endymion non-scriptus	0.02
3.	Ilex aquifolium	0.47	21.	Rubus fruticosus agg.	-0.06
4.	Vaccinium myrtillus	0.67	22.	Salix spp.	-0.14
5.	Deschampsia flexuosa	0.71	23.	Deschampsia caespitosa	0.06
6.	Melampyrum pratense	0.66	24.	Agrostis stolonifera	0.09
7.	Galium hercynicum	0.48	25.	Mercurialis perennis	-0.32
8.	Potentilla erecta	0.56	26.	Arum maculatum	-0.48
9.	Molinia caerulea	0.58	27.	Circaea lutetiana	-0.35
10.	Blechnum spicant	0.63	28.	Geranium robertianum	-0.44
11.	Juncus acutiflorus	0.42	29.	Sanicula europaea	-0.32
12.	Viola palustris	0.55	30.	Zerna ramosa	-0.52
13.	Lotus uliginosus	0.60	31.	Allium ursinum	-0.31
14.	Sphagnum palustre	0.00	32.	Rumex spp.	-0.55
15.	Holcus mollis	0.09	33.	Angelica sylvestris	-0.48
16.	Pteridium aquilinum	0.22	34.	Mentha aquatica	-0.26
17.	Digitalis purpurea	0.31	35.	Viburnum opulis	-0.35
18.	Carex riparia	-0.26	36.	Epilobium hirsutum	-0.33
			37.	Luzula multiflora	-0.43

TABLE 6.3 EIGENVECTOR LOADINGS FOR COMPONENT 1, PCA PRESENCE AND ABSENCE FLORISTIC DATA

studying the taxonomic status of sub-populations of Roudsea Wood recorded Q. petraea being found on slate, Q. robur on peat, with a mixture of species and intermediate forms on limestone areas of the same woodland. The peat site also had a number of intermediates. A mosaic of this type in an otherwise uniform woodland must argue strongly for the siting of oak populations of specific types on specific types of soil. Wigston (1971) has noted a parallel situation, but over a larger geographical area -- in the north of the area he surveyed, to the east of Bude (Cornwall), populations were of a Q. petraea type, to the south, both east and west lay pure Q. robur types, whilst intermediate populations fell some way in between, particularly around the margins of Dartmoor where intermediate populations predominated.

The influence of planting on alien soil types cannot, however, be discounted. Efforts were made during collection of population samples to exclude obviously planted stands, but in particularly old populations it becomes very difficult to recognise the signs of planting. The impact of man is only to bring about situations which promote hybridisation, rather than the reverse, and consequently if the impact of man had been to plant alien species, then in the east of the area, i.e. East Anglia, etc. there might have been more evidence of hybridisation among the sampled populations. As it was, the most variable situation was to be found in Wales. Tansley (1939) comments on the Welsh oakwoods:

"The Welsh oakwoods have not been closely studied, but like practically all others on the siliceous hillsides of the west those which have been examined are sessile oakwoods. The few trees of Quercus robur that are sometimes seen are always either near the bottom of a river valley or in situations where they may obviously have been planted."

The small amounts of planting noted by Tansley probably coupled with the presence of Q. robur in the river valleys has possibly been sufficient to bring about the variable, hybrid situation found in Wales. The absence of pure Q. robur populations in Wales is very noticeable, but

the distribution of introgressed Q. robur populations or mixed populations always occur close to introgressed Q. petraea populations, suggesting that gene flow in small areas is taking place between species in both directions, eg. Figure 6.1 - populations DE, DF and DG are close together and consist of an introgressed Q. robur, introgressed Q. petraea and an intermediate population.

The intermediate populations show a very marked distribution throughout Wales, being found mainly on or close to the coast. In detail, they are also restricted in the main to the sides of river valleys, eg. population AR is on the valley side of Afon Gwaun, and they possibly occupy therefore the hybrid habitat between the siliceous areas (the mudstones, sandstones and slates of the higher areas) and the wetter, more poorly drained and conceivably richer valley bottoms. The concept of the hybrid habitat may, therefore, hold true for British oaks in certain geographical areas.

SECTION FOUR

REPRODUCTIVE BIOLOGY

CHAPTER SEVEN

POLLEN AND THE PROBLEM OF HYBRIDITY

Introduction

Hybridity is generally recognised by morphological intermediacy in several characters and indeed as Gottlieb (1972) points out in the absence of such morphological intermediacy, hybridity would rarely be suspected. However, morphology is not and should not be the only criterion used by taxonomists in determining hybridity. Other criteria exist and these have been recently reviewed by Gottlieb (1972). One useful technique is the study of pollen, and this has proved particularly important in hybridisation studies.

a) Pollen grain size: The size of pollen is generally related to the ploidy level of the organism, and may be of use in determining hybrid origin. Majumdar and Riley (1973) found that each additional set of chromosomes in Haworthia species and their hybrids caused incremental increases in pollen grain size up to the tetraploid level. Variability in pollen grain size has also been used in studies of experimental hybridisation in Carex section Acutae (Faulkner, 1973).

b) Surface sculpturing: Scanning electronmicroscopy of pollen grain surfaces has been used in several studies recently. Cribb (1969) used the pattern of surface protuberances of pollen to determine the origin of tetraploid Solanum species.

c) Meiotic behaviour : Chromosome pairing during meiosis in pollen mother cells has been used to determine the hybrid origin of species eg. Iyengar (1944).

d) Pollen viability: Plants of hybrid origin frequently show loss of fertility (Gottlieb, 1972) and this has been used as a criterion of hybridity. For example, Woodell (1965) showed that plants of morphological intermediacy between Primula vulgaris and Primula veris had low

levels (43%) of fertile pollen. Many similar studies exist eg. Bradshaw (1958) - Agrostis, Gottlieb (1972) - Stephanomeria, Ockendon and Walters (1970) - Potentilla anserina L., Majumdar and Riley (1973) - Haworthia.

Taxonomic investigations in the genus Quercus L. have rarely involved researches into pollen characteristics. This chapter reports investigations into the viability, size and sculpturing of the pollen grains of Quercus robur and Quercus petraea.

The measurement of pollen fertility

Three different methods have been used to determine the fertility of pollen grains (the so-called pollen viability).

a) Simple staining techniques: Several stains exist for the intact nuclear and cytoplasmic material found in fertile pollen grains eg. aceto-carmine (Majumdar and Riley, 1973), aniline blue in lactophenol (Hauser and Morrison, 1964). There is, however, no guarantee that stained pollen grains have the ability to germinate, and consequently such staining methods probably overestimate the fertility of the pollen.

b) Germination tests: The ultimate test for pollen viability is to determine whether or not the grains will germinate. Usually sucrose is used as a substrate. However, research work over the last two decades would suggest that so many variables are important in pollen grain germination that although comparative studies are possible, absolute measures of viability which are so important in hybridisation studies are not particularly feasible with this method. Vasil (1960) determined the germination rate of different species of the Cucurbitaceae on different substrates, and with different additive substances. Boric acid (0.005-0.02%) and borax (0.01%) proved important in increasing both germination rate and the length of the pollen tubes, but other growth

substances such as indoleacetic acid, indolebutyric acid, biotin and thiamin also improved germination. More importantly, Brewbaker and Kwack (1963) in a survey of 86 species, showed that the rate of germination was directly proportional to the density of the pollen population. In sparse populations, little germination took place, whilst in dense populations, germination was high. Metallic ions including Ca^{++}, Mg^{++}, K^{+} and Na^{+} (Brewbaker and Kwack, 1963), and pH level (Vasil, 1960) have also been shown to be important in the germination of pollen grains. Similar results have been obtained by Hall and Farmer (1971). The influence of so many variables probably accounts for the erratic response of pollen to in vitro germination.

c) Stains for metabolic activity: The difficulties experienced in determining pollen viability by the above two methods have prompted several authors to suggest alternative methods, particularly those which seek to demonstrate respiratory activity in pollen grains. Such a test has been proposed by Hauser and Morrison (1964). The method relies on the ability of a colourless salt, tetrazolium, to pick up electrons removed from a succinate via succinic dehydrogenase. The tetrazolium salt is reduced to a coloured salt, formazan, which acts as the stain. The development of a colour inside a cell demonstrates a capacity for oxidative metabolism. In the present investigation, nitro-blue-tetrazolium (NBT) was used as the tetrazolium salt after Hauser and Morrison (1964), but other tetrazolium salts have been used, eg. 2, 3, 5 - triphenyltetrazolium (Diakonu, 1968).

Pollen Viability: Preliminary study

Catkins were collected from trees of known morphological character during the flowering season, May 1969. The catkins were placed on netting suspended over petri dishes so that as the pollen was shed it

fell into the dish. Each dish was covered with a plastic 'sandwich' box to prevent contamination. Three different methods of assessing pollen fertility were compared on different samples of each tree's pollen.

1. Germination percentage was estimated from pollen grown on agar containing 0.5M sucrose, a concentration suggested by Jones (1959) at which oak pollen germinates well. Counts were made of pollen grains transferred from the agar to microscope slides after 72 hours. A pollen grain was deemed to have germinated if the pollen tube was at least as long as the diameter of the grain. Although the majority of the grains germinated within the first 24 hours, it was found necessary to score the cultures after 72 hours when they had reached a steady germination percentage. However, such a long period also meant that the first grains to germinate had long pollen tubes after 72 hours and trying to trace these for scoring purposes proved very difficult.

2. Aniline blue in lactophenol was prepared after Hauser and Morrison (1964). Samples of pollen were stained for 24 hours using approximately 1 mg. pollen to 10 ml. stain. Since pollen tends to float, the samples were constantly agitated to ensure complete contact between the grains and stain.

3. NBT was generally prepared fresh in 100 ml. amounts as follows:

33.3 ml. 0.06M Sorensen's phosphate buffer
33.3 ml. 0.2M Sodium succinate
33.3 ml. NBT (1 mg. dissolved in 1 ml. distilled water)
25.3 mgs. Sodium amytal

Although the colour developed in the pollen under conditions of room temperature, to ensure uniformity, all samples were incubated at 35°C for 30 minutes. Again, the samples were agitated and approximately 1 mg. pollen was used to 10 ml stain. The stain faded quickly (after

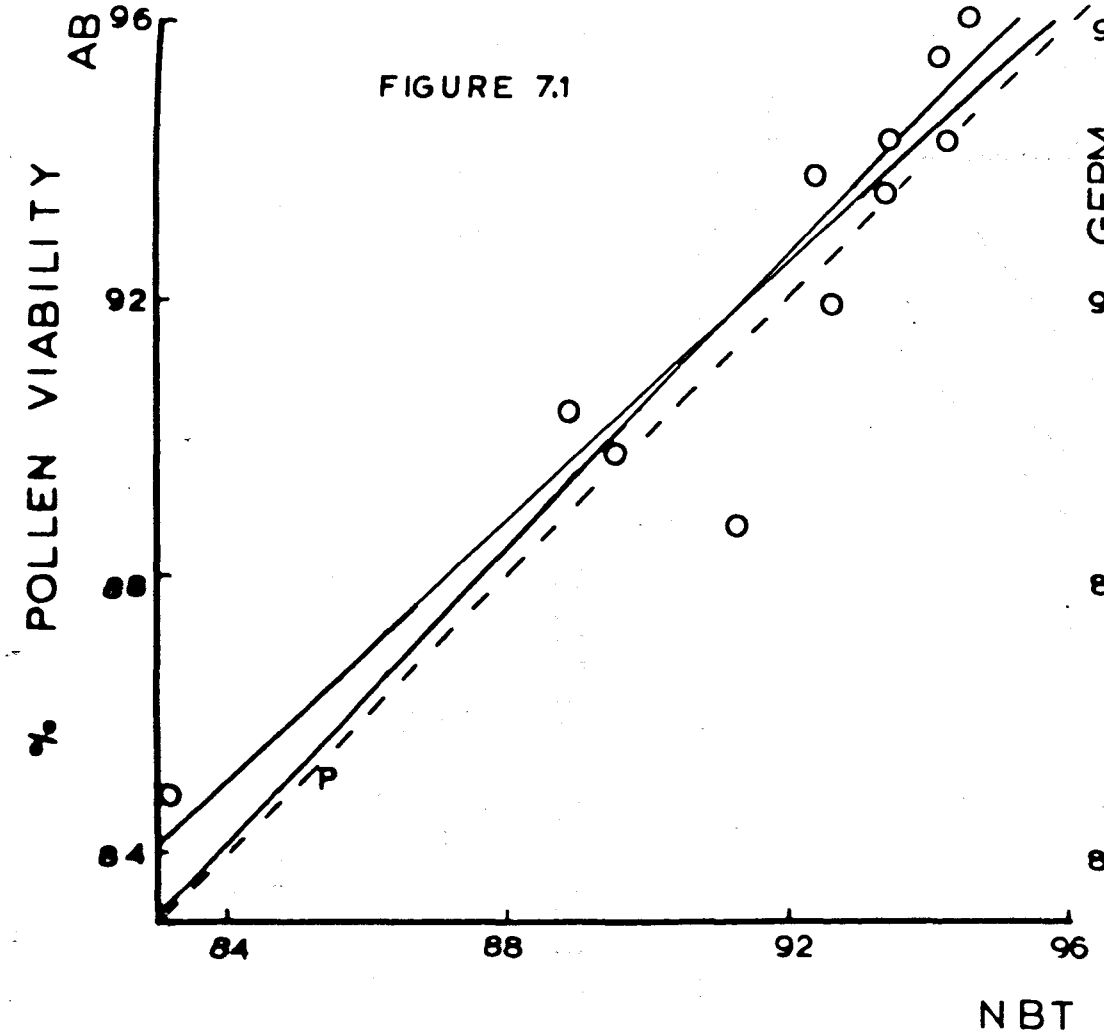
2 or 3 hours, much of the stain was lost) and consequently after incubation, the pollen was fixed in FAA (5% formalin, 5% glacial acetic acid, 90% alcohol). No difficulties were experienced in detecting stained and unstained grains using either the aniline blue or NBT method.

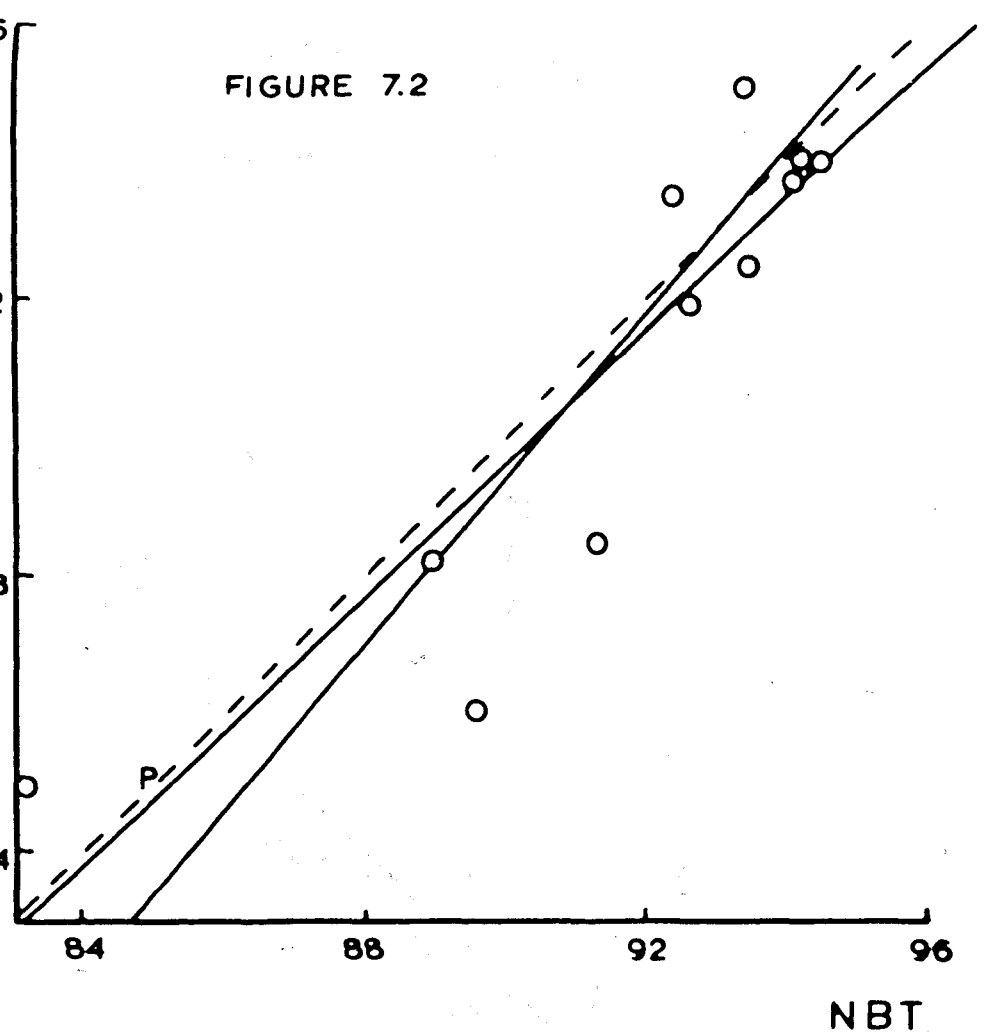
Scoring pollen grains for stainability is difficult since the act of placing a microscope slide coverlip over a drop of liquid containing pollen grains causes differential movement of viable grains and grains without contents due to their different densities - the lighter, non-viable grains moving further to the edge of the coverslip. Since scoring under low power (x100) or under high power (x400) may be influenced by such differential distribution of grains, pollen from one tree was scored under both low and high power. The pollen was divided into five portions and each stained separately with NBT. Five microscope slides were prepared from each sample and each microscope slide scored at x100 and x400. The results are given in Table 7.1. Scoring at x400 gave consistently lower estimates of pollen viability and was much easier to perform. At x100, up to 150 grains were in the field of view - this made counting difficult, and also meant that if only a count of 200 grains was to be made, as was frequently the case, then generally only two 'stations' on the slide were counted. At x400, only four or five grains were in the field of view at any one time, so that in order to count 200 grains, more 'stations' had to be used than a count at x100, thus giving a wider coverage over the slide, and possibly negating the differential distribution of pollen grains mentioned above. Although differences were consistent, scoring at x400 was personally easier and has been used in all pollen viability estimations.

In comparing the three methods, seven Q. petraea and four Q. robur trees were used. The results are graphed in Figures 7.1, 7.2 and 7.3.

	LOW POWER (x100)		HIGH POWER (x400)	
SAMPLE NUMBER	MEAN	STANDARD DEVIATION	MEAN	STANDARD DEVIATION
SAMPLE 1	88.52	2.77	86.44	3.36
SAMPLE 2	88.30	1.15	87.02	0.66
SAMPLE 3	87.72	1.46	86.56	1.78
SAMPLE 4	87.70	1.54	86.76	2.69
SAMPLE 5	87.28	2.40	87.46	1.32
TOTAL	88.10	1.86	86.85	1.92

TABLE 7.1 VARIATION IN SCORING THE SAME POLLEN UNDER DIFFERENT MAGNIFICATIONS (NBT stain)





% POLLEN VIABILITY

FIGURE 7.1 Correlation between pollen viability measured using aniline blue and nitro-blue tetrazolium. P = line of parity. Also shown are the linear regression lines of X on Y and Y on X.

FIGURE 7.2 Correlation between pollen viability measured using nitro-blue tetrazolium and actual pollen germination P = line of parity. The linear regression lines of X on Y and Y on X are also shown.

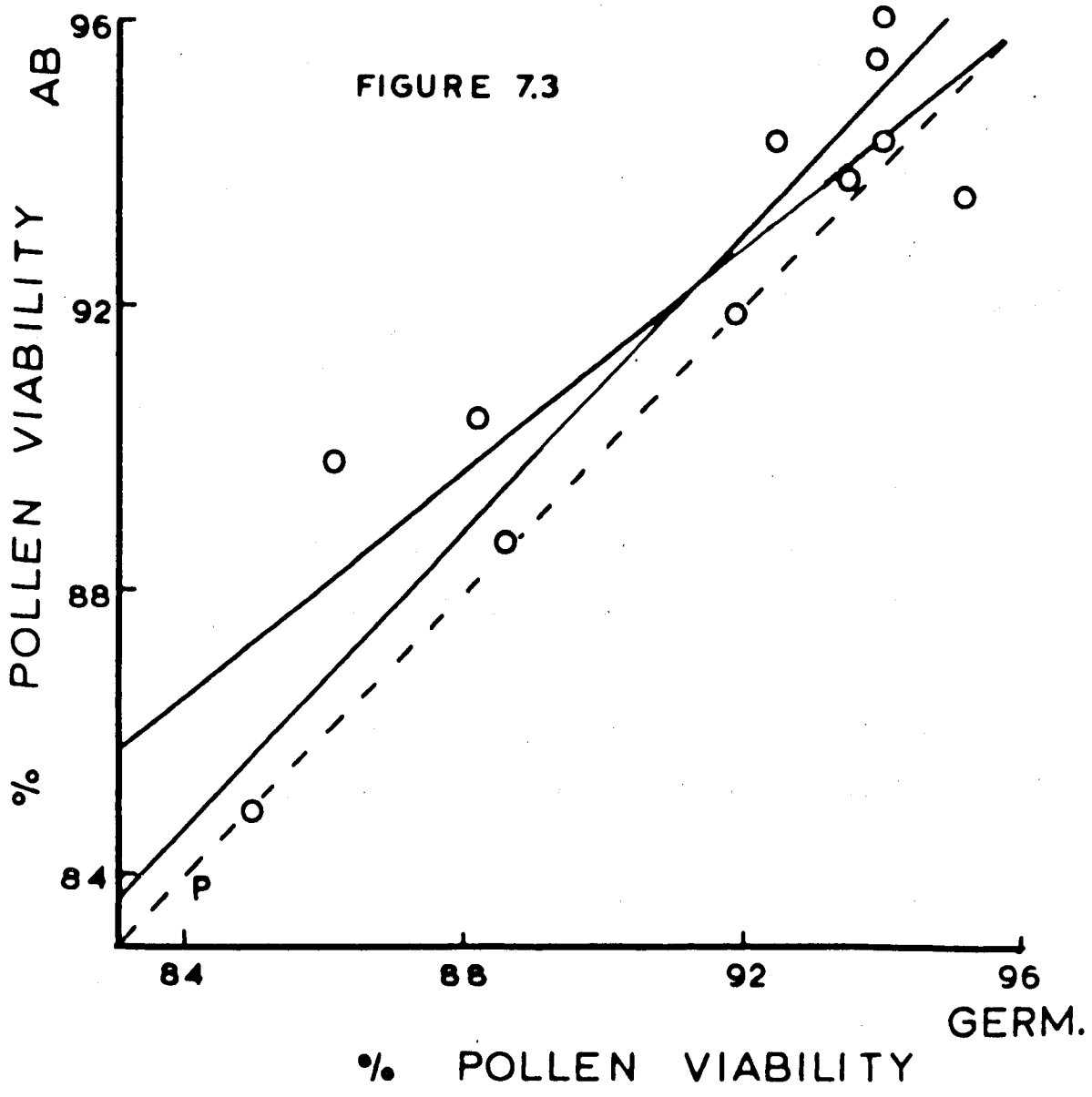


FIGURE 7.3 Correlation between pollen viability measured using aniline blue and actual pollen germination P = line of parity. The linear regression lines of X on Y and Y on X are also shown.

Both aniline blue and NBT produce levels of viability in excess of that shown by the germination method (as seen by the regression lines in Figures 7.3 and 7.2 which lie to one side of the line of parity). Two possible reasons would account for such a result. Firstly, stainability does not necessarily guarantee that such a pollen grain would have germinated - many barriers could come into play between an ungerminated pollen grain and its production of a pollen tube. Secondly, as discussed earlier, so many variables are important in in vitro culture of pollen grains, that non-availability of certain substances such as calcium (Brewbaker and Kwack, 1963) or pollen population density might be responsible for depressing the germination percentage and not allowing it to reach a maximum. Figure 7.1 relates the viability scored by aniline blue and NBT. The regression lines lie to the top of the line of parity suggesting that aniline blue scores higher viabilities than NBT. A similar result was obtained by Hauser and Morrison (1964) - in 31 species and hybrids tested, 22 showed higher pollen viabilities measured by aniline blue. It is perhaps not surprising that some grains which have contents (and therefore stain with aniline blue) are not respiring.

The difficulties in scoring viability directly by germination, and the differences between aniline blue and NBT detailed above persuaded the present author that for estimates of pollen viability in large-scale population samples, NBT was easier and conceivably the better method.

Pollen Longevity

Pollen has a finite life, and viability falls rapidly in storage (Alam and Grant, 1971). It is important to know the length of time pollen may be stored under given conditions if large-scale determinations are being carried out, since in such circumstances it is difficult to

deal with samples as they are collected, and so storage may be necessary. Several storage techniques are known to improve retention of viability. For example, Birch pollen stored at 2-5°C falls to only 20-40% after 105 days whilst at room temperature it falls to 1% after 60 days (Alam and Grant, 1971). Storage in liquid nitrogen does not affect the viability of some tree pollen even after eight years, eg. Pinus spp., Larix leptolepis (Ichikawa and Shidei, 1972). The Forestry Commission use storage at room or refrigerator temperatures in low humidity (Gardiner, 1968, pers. comm.). Jones (1959) notes that oak pollen may be stored for up to eight weeks if kept at 60% relative humidity and 0°C. Maintenance of viability is of paramount importance when artificial hybridisation is being attempted between species of widely differing flowering times. Since flowering in oaks takes place over a period of three or four weeks in any given area (Hyde, 1950a, 1950b), it is important to determine whether pollen collected at the beginning of the period can still be used at the end. Tkačenko and Vlasova (1968)suggest that in several trees and bushes, viability might only be retained for 10 to 15 days after collection.

In order to determine pollen longevity, large numbers of catkins were collected from four Q. petraea and three Q. robur trees. The pollen was collected over petri dishes, as described earlier, and determinations of pollen viability made over a period of ten weeks using NBT. The pollen was stored in open vials in a closed 'sandwich' box in the base of which was 1" of silica gel. The silica gel was replaced each time the box was opened. The box was stored at room temperature in the dark.

Results are presented in Table 7.2 and graphed in Figure 7.4. In all seven trees, viability remained relatively constant for the first fifteen days after collection, but fell off rapidly over the next twenty days (up to Day 35) to about 10%. After ten weeks, no viable pollen

DATE - 1969	TREE TYPE						
	PETRAEA	PETRAEA	PETRAEA	PETRAEA	ROBUR	ROBUR	ROBUR
DAY 1 (1st June)	94.7 *	93.6	94.7	89.8	90.1	83.9	97.5
	2.2 **	1.9	1.8	2.3	2.2	2.5	1.2
DAY 8 (8th June)	94.3	94.3	93.8	89.4	90.4	83.6	96.1
	3.7	1.7	1.0	1.3	2.6	4.1	1.2
DAY 13 (13th June)	94.7	93.9	93.9	88.9	89.3	84.2	95.0
	2.7	2.5	1.9	1.1	3.0	2.2	1.9
DAY 15 (15th June)	91.1	93.9	89.7	85.9	89.8	81.1	90.1
	2.1	2.4	2.7	4.4	2.5	2.7	2.1
DAY 22 (22nd June)	63.5	88.8	85.5	76.2	82.7	72.2	77.8
	3.8	3.2	2.2	3.9	2.9	3.8	4.1
DAY 29 (29th June)	57.0	80.5	76.1	60.8	81.3	63.1	64.2
	2.1	1.8	5.3	6.2	2.6	1.9	6.3
DAY 35 (5th July)	4.3	9.7	29.0	9.5	33.2	10.1	1.6
	3.1	2.1	10.1	1.6	4.1	1.1	0.3
DAY 43 (13th July)	1.9	2.8	8.4	3.2	8.9	3.8	0.0
	1.2	2.1	4.7	1.3	1.2	1.8	0.0
DAY 49 (19th July)	0.7	0.5	4.0	0.6	4.3	1.6	0.0
	1.2	0.5	2.1	0.9	0.8	0.5	0.0
DAY 56 (26th July)	0.0	0.0	2.4	0.0	2.7	0.8	0.0
	0.0	0.0	0.9	0.0	1.1	0.8	0.0
DAY 62 (1st August)	0.0	0.0	0.9	0.0	1.1	0.0	0.0
	0.0	0.0	0.0	0.0	0.5	0.0	0.0
DAY 70 (9th August)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 7.2 POLLEN LONGEVITY - THE CHANGE IN % POLLEN VIABILITY WHEN STORED AT ROOM TEMPERATURE AND LOW HUMIDITY (SEVEN TREES)

* Mean Percentage Pollen Viability
** Standard Deviation of the Mean

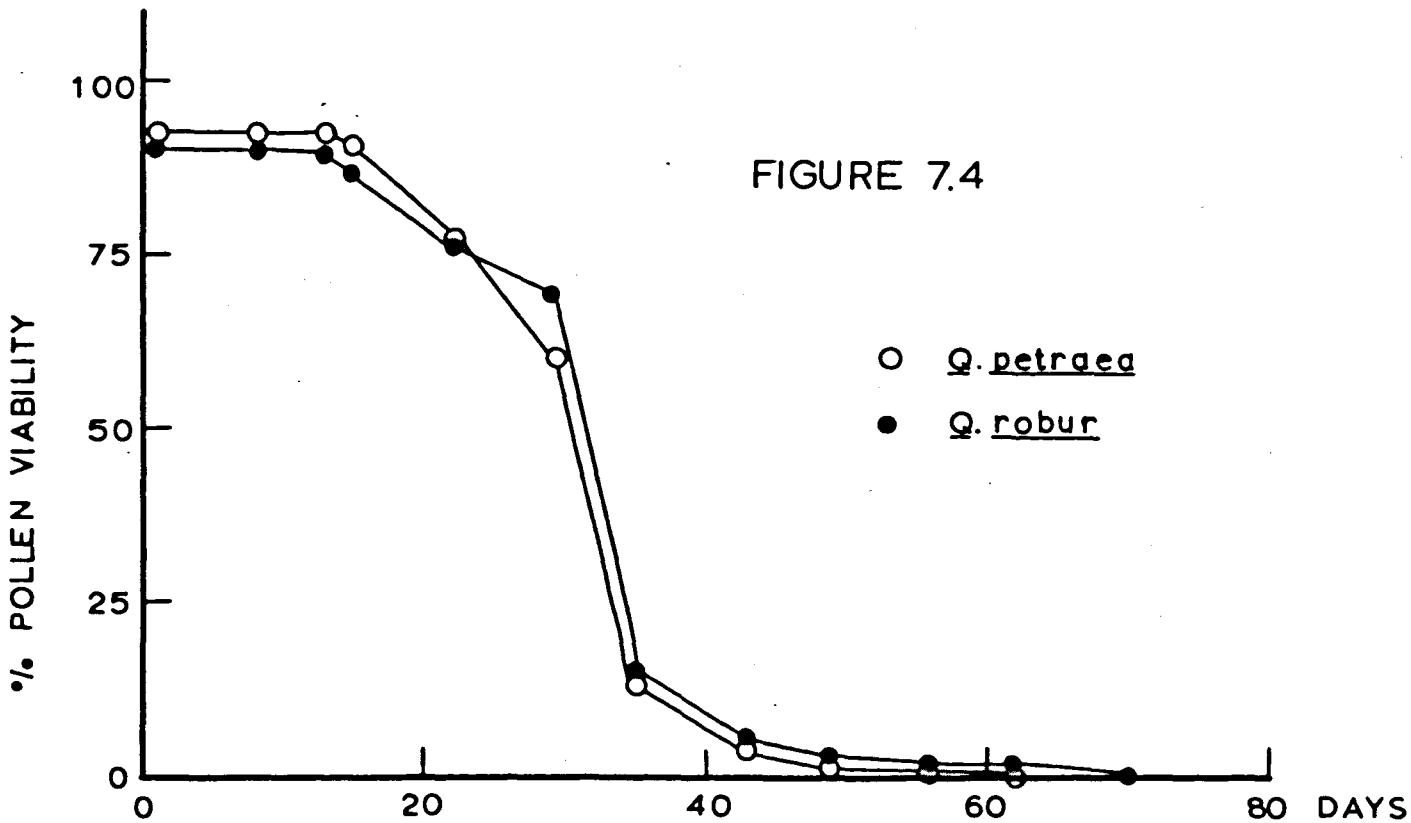


FIGURE 7.4 Decline in pollen viability of stored pollen (Storage conditions: Sandwich box, with silica gel, room temperature, darkness)

grains could be detected. For all practical purposes therefore, it was necessary to score viability or use the pollen for artificial hybridisation within about twelve days of collection.

Pollen viability: Population studies

Bradshaw (1958) and Woodell (1965) have shown that plants in hybrid populations produce pollen of low viability. Woodell (1965) recorded twenty plants in Boarstall Wood, which on morphological grounds were hybrids between Primula veris and Primula vulgaris, as having a mean pollen viability of 43.45% and a range from 9-70%. Bradshaw (1958) was able to show a low pollen viability (40%) in plants at the mid-point of a hybrid index score between Agrostis tenuis and Agrostis stolonifera. These intermediate plants were assigned to F_1 hybrid status. With the exception of individual records of pollen viability in certain oak species (eg. Pyatnitski , 1947; Sax, 1930), pollen viability has never been measured on a large scale population basis for oak populations. The only work of relevance concerns observations made by Jones (1959) on 25 trees in Bagley Wood. Three trees produced a high proportion (25-50%) of abortive pollen and were of morphological intermediacy. Since pollen viability studies have been so useful in the past in elucidating the hybrid origin of species, it was argued that studies on the viability of pollen from individual trees in pure and suspected introgressed populations might prove worthy of study.

Initially, five populations were chosen - these had in previous years been shown to be of pure, mixed or suspected introgressed status. Approximately fifty trees from each population were sampled - catkins were removed from each tree, suspended over petri dishes and the pollen collected. The trees were individually marked so that they could be

sampled later in the season for leaf material. Pollen viability was determined using NBT, as described earlier. Since large numbers of samples were involved, pollen had frequently to be stored for four or five days before scoring - this should have had little effect on the final measured viability (see previous section). The pollen sample from each tree was divided into five portions and each stained separately. Two slides were prepared from each portion giving ten slides for each tree. A count of at least 200 grains was made on each slide using x400. Later in the season, five leaves were collected from each tree and scored on a hybrid index scale from 0-340 as described in Section 3. The results are graphed in Figures 7.5 to 7.9.

Generally, the results would suggest that trees of intermediate morphology have lower viabilities than 'pure' trees. Hetchell Wood (Figure 7.5) is a mixed woodland, dominated by Q. robur trees - in the sample of 50 trees, 43 are Q. robur. The trees show morphological indices typical of the 'pure' types - Q. robur 50-110 and Q. petraea 225-300, and all have viabilities greater than 80% (indeed 47 trees have viabilities over 85%). Population B (Figure 7.6) is a pure Q. petraea population with little morphological evidence of Q. robur trees or hybrids. The viabilities are generally high, with all but three trees having pollen viabilities over 85%. Mad Brook (Figure 7.7) shows a mixed population dominated by Q. robur trees, but with at least one Q. petraea tree and several trees of suspected hybrid status. The pollen viabilities of the morphologically 'pure' trees is generally high (above 85%), whilst several trees in the range 110-225 have pollen viabilities in the 60-80% range. Sutton Park (Figure 7.8) shows a similar situation, with a predominantly Q. robur population, a few Q. petraea trees and a large proportion of morphological intermediates. These intermediate forms also show low pollen viabilities - one as low

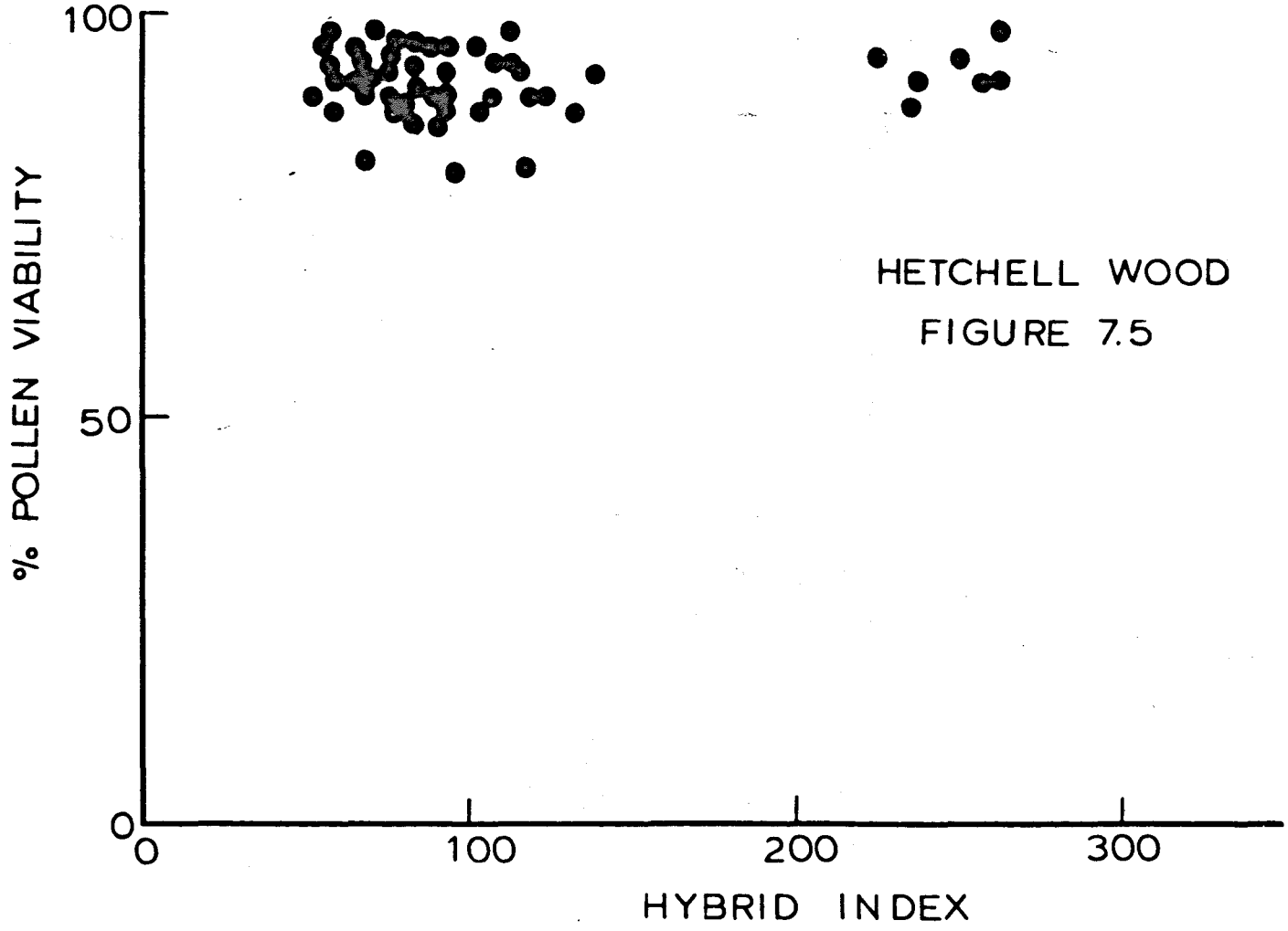
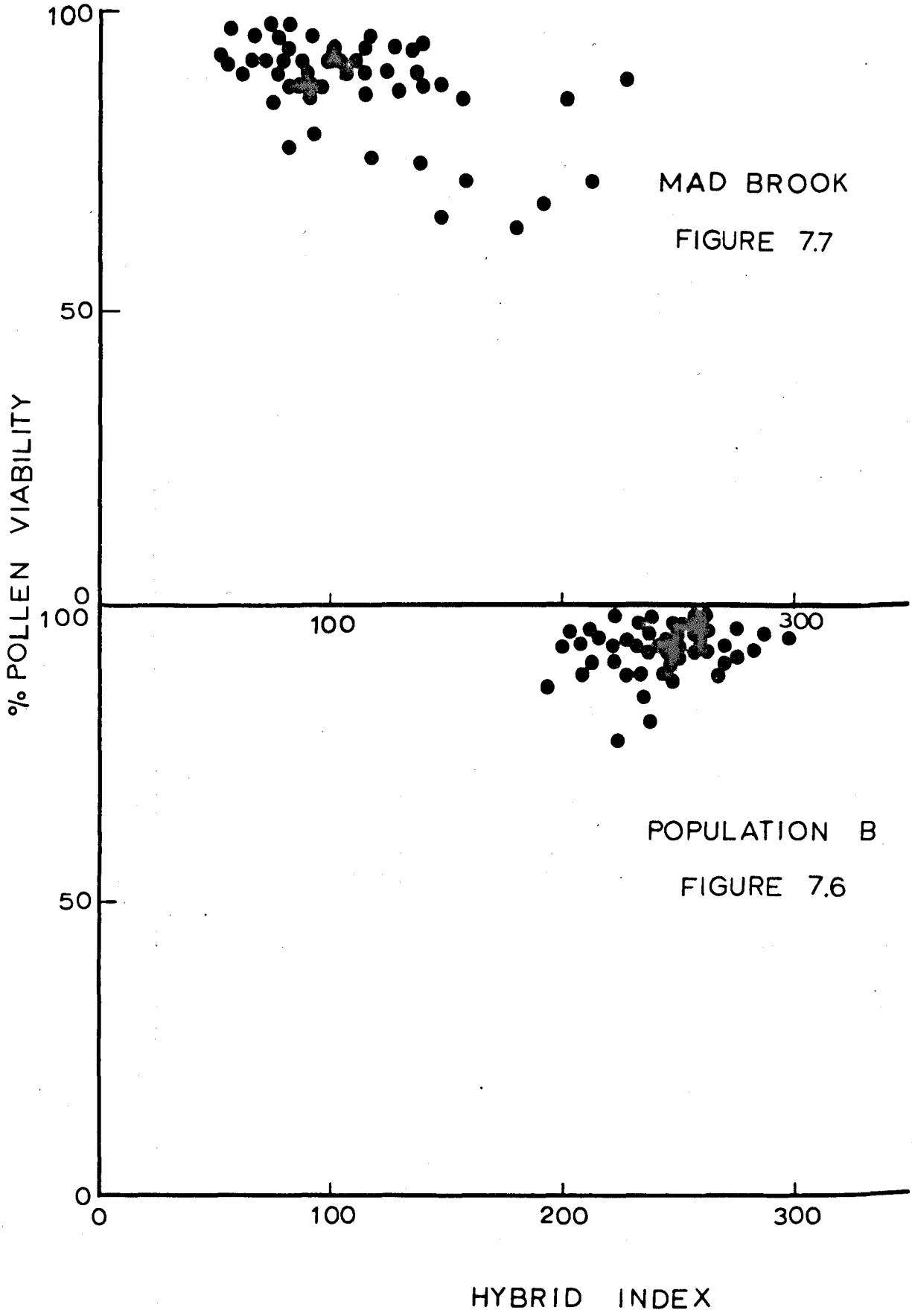
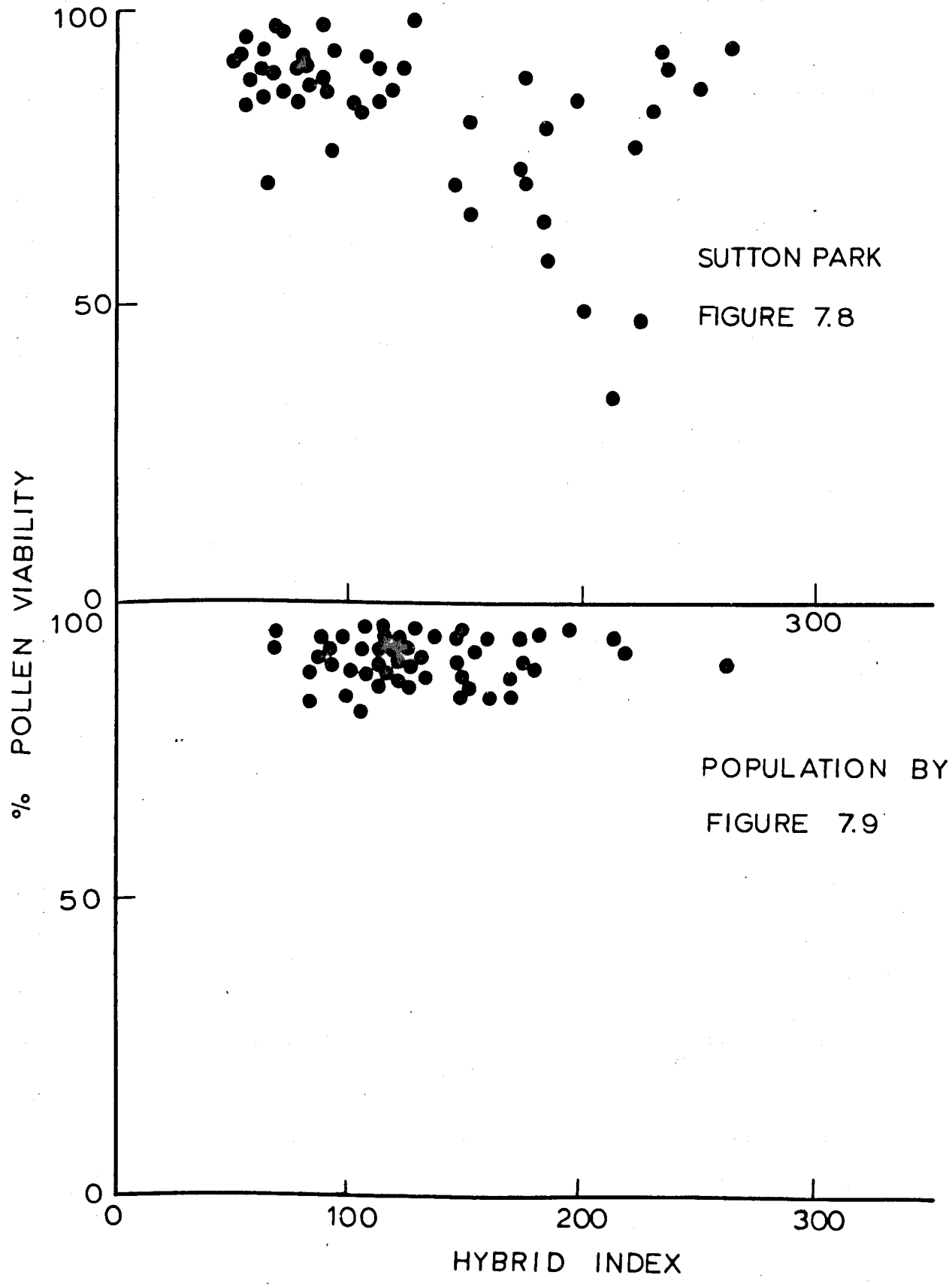


FIGURE 7.5 Percentage pollen viability (measured using NBT) and Hybrid Index of 50 trees of Hetchell Wood



FIGURES 7.6 and 7.7 Percentage pollen viability (measured using NBT) and Hybrid Index of 50 trees of Population B and 50 trees of Mad Brook respectively



FIGURES 7.8 and 7.9 Percentage pollen viability (measured using NBT) and Hybrid Index of 50 trees of Sutton Park and Population BY respectively

as 34%, the others between 47% and 77%, although there are one or two exceptions. Population BY (Figure 7.9) is an interesting population. It consists of a Q. robur woodland with at least one Q. petraea tree; the rest of the trees being morphologically intermediate. However, all trees have pollen viabilities above 80%. No explanation is readily forthcoming to account for this situation, but it is thought that such a result could be due to sampling error, experimental error or that it is valid phenomena and is due either to the trees of morphological intermediacy (and therefore of suspected hybrid origin) never losing their pollen fertility or alternatively that they have regained their pollen fertility.

Of course, restoration of fertility is one of the important steps to be overcome by hybrid progeny if they are to be successful in an evolutionary sense (Stebbins, 1966). Bradshaw (1958) recognised individuals that could have been F_2s and backcross derivatives of hybrids between Agrostis tenuis and Agrostis stolonifera. These showed pollen fertilities greater than those shown by F_1 individuals (F_1 fertilities - 15%, 13%, 17%; Derivatives - 92%, 8%, 64%, 48%). Doroszewska (1965) estimated pollen fertility in Trollius chinensis, Trollius europaeus, their F_1 hybrids and F_2 hybrids formed under conditions of 'free pollination'. The fertility of the pure species was generally above 90%, and that of the F_1 hybrids between 40% and 76%. The fertility of the F_2 hybrids was generally much higher than that of the F_1 hybrids, ranging from 80-90%; indeed several individuals had viabilities equal to those of the parental species.

Results from the other oak populations would suggest that oak hybrids have a lowered pollen viability and consequently the trees of morphological intermediacy in population BY that show high pollen viabilities are more likely to have restored pollen viability rather than

never having had low viabilities. The work of Bradshaw (1958) and Doroszewska (1965) would suggest that F_2 and backcross derivatives may regain viability. This might explain the high viabilities in the trees of suspected F_2 backcross status, but certainly not the trees in the middle of the morphological range which are suspected F_1 hybrids. It can only be concluded that, although these trees produced an intermediate score, they were not F_1 hybrids or, alternatively, they are F_1 hybrids with high viabilities. The Sutton population shows the tree with the lowest recorded viability - 34%, but this tree had a morphological index score of 212 and consequently perhaps it is also not valid to try and sub-divide the intermediate zone between 110 and 225 into F_1, and F_2 backcross zones. Sax (1930) recorded that interspecific hybrids within Quercus frequently produce no more abortive pollen than do pure species. Jones (1959) also notes that oak trees considered hybrid on morphological grounds may in fact be fully fertile. Only population BY would seem to support this point of view, the other populations studied having morphologically intermediate trees with low pollen viability. Weimarck (1947b) recorded the Q. petraea x Q. robur hybrid has being almost sterile.

The sampling procedures used in this investigation require, however, closer scrutiny. The sample, although in one sense random, is also biased in favour of those trees that are reproductive at the time of sampling, i.e. only trees that were reproductive were chosen for sampling. The non-reproductive trees might well have been trees that had already produced catkins or more importantly trees that were incapable of producing catkins or even trees that had reproduced the previous year. (Ovington and Murray, (1964), in recording acorn production during years 1960, 1961 and 1962, found that, during 1961, virtually no acorns were produced in the wood under investigation, whilst in 1960 and 1962, acorn production was prolific, suggesting that reproductive capacity fluctuates from year to year.) One further point concerns the length of the flowering period. Hyde (1950a, b) estimated

that flowering in any one area might spread over three or four weeks. Since all samples from any one population were collected together, for a given population the sample time might have been at either the beginning, middle or end of the flowering period - unfortunately during the sampling, no notes were made as to indications of the state of the flowering of the population, eg. dead catkins, fully expanded leaves, etc.

In the case of populations that showed trees with low pollen viabilities, the non-randomness of the sampling techniques would appear to have had little effect - the non-reproductive trees might have only increased the trees in the 'low fertility' class. Populations regarded as being of 'pure' status are also unlikely to have been influenced by such sampling - comparison of the morphology of population B for reproductive trees (Figure 7.6) agrees very closely with the morphology of the same population when sampled randomly for leaves on both reproductive and non-reproductive trees (see Appendix 4). The sampling of the populations for leaves in Section 3 did not take into account whether or not the trees were reproductive and consequently would have included both potentially reproductive and potentially non-reproductive trees.

Experimental error is unlikely to have influenced the result for population BY since pollen from other populations was stained and counted at the same time.

The initial use of five populations was regarded as a preliminary investigation. In all, another fifteen populations were studied over a period of two years - these results are presented in tabular form (Table 7.3). The fifteen populations were chosen so as to present a representative cross-section of the different population types that had been found during the population analysis (Section 3). The 'mixed' category contained two different types of population, population BBC with few suspected hybrids and approximately equal numbers of the 'pure'

Pop. No.	Pop. Type	Sample Size	Morphology of Population R	H	P	Morphological Index Range	Mean Morphological Index	Pollen Viability
A_2	R	25	25	0	0	52-131	99.9	All trees above 85% viable pollen
AD	R	50	50	0	0	63-143	100.2	All trees above 85% except two trees at 79% (109) and 81% (114)
AAE	R	50	50	0	0	64-131	92.7	All trees above 82% viable pollen
H	P	35	2	0	33	94-276	226.7	All trees above 85% except three trees at 78% (224), 78% (224), 81% (219)
AY	P	50	0	0	50	179-284	235.5	All trees above 85% viable pollen
AV	P	50	0	0	50	189-267	223.5	All trees above 85% viable pollen
CA	INT R	50	35	11	4	63-241	122.2	Population very like Mad Brook. Four trees with very low viabilities - 48% (154), 49% (163), 52% (147)
AAD	INT R	50	39	10	1	60-224	131.1	Generally high pollen viabilities, over 80%, but with three trees somewhat lower - 67% (133), 74% (128), 75% (119). To be compared with population BY.
DC	INT R	50	35	15	0	53-209	139.9	Population very like Mad Brook. No trees below 55% viable pollen.

Pop. No.	Pop. Type	Sample Size	Morphology of Population R	H	P	Morphological Index Range	Mean Morphological Index	Pollen Viability
AU	INT P	50	35	15	0	132-246	196.2	Population very like Mad Brook. No trees below 53% viable pollen.
DO	INT P	50	1	15	34	84-261	197.8	Population very like Mad Brook. No trees below 56% viable pollen.
DJ	INT P	50	3	16	31	114-241	194.8	Population very like Mad Brook. No trees below 62% viable pollen.
BBC	MIXED	50	19	4	27	64-274	177.2	Generally high pollen viabilities, the suspected hybrids producing somewhat lower values - 63% (143), 56% (149), 32% (163), 75% (158)
CCE	MIXED	50	23	16	11	51-266	147.5	Generally high pollen viabilities, with three trees somewhat lower - 74% (154), 63% (199), 58% (116). To be compared with population BY.
DE	MIXED (intermediate)	50	14	28	8	89-222	163.4	Population very like Sutton, with a large proportion of the suspected hybrids with low pollen viabilities.

N.B. Population H represents a second sample of population W_5 and as such can be considered here to be the pollen viability profile of the Q. petraea reference population used in Chapter 5.

TABLE 7.3 POLLEN VIABILITY AND LEAF MORPHOLOGY IN FIFTEEN OAK POPULATIONS

species and populations CCE and DE which contained very high proportions of suspected hybrid trees - 32% and 56% respectively.

The populations chosen as being representative of 'pure' populations all showed high levels of pollen viability, with very few trees below 80% viability. These populations follow the pattern found in populations B and Hetchell earlier. The populations chosen as being representative of introgressed populations, with one exception, behaved very much like the Mad Brook population with generally high levels of pollen viability, but with some much lower values particularly in trees of suspected hybrid status. The exception, population AAD, will be discussed below. Population BBC, a mixed population with approximately equal numbers of Q. petraea and Q. robur trees, produced high pollen viabilities, with the exception of three of the four suspected hybrid trees which scored values of 75%, 63% and 56% viable pollen. Population DE, a population with a very large number of suspected hybrid trees, behaved very similarly to the Sutton population, with a large proportion of the suspected hybrids having low pollen viabilities. Population CCE was chosen as a mixed population with a large proportion of suspected hybrid trees (32%). The population produced a distribution of pollen viabilities very close to that seen in population BY, the anomalous population from the initial survey. The main difference between the two populations, BY and CCE, was that CCE produced three trees with lower viabilities than any of the trees in BY, but these bore no correlation with morphological status - 74% (154), 63% (199) and 58% (116). Population AAD, originally chosen as an introgressed Q. robur wood, also produced high pollen viabilities with three trees somewhat lower - 74%, 75% and 67%. Both populations CCE and AAD could probably be regarded as the same type of population as BY, that is, populations containing morphologically intermediate trees with high pollen

viabilities. Since three populations have been detected in twenty populations studied, the occurrence of such populations would appear to be reasonably frequent.

Pollen Morphology

Conflicting reports occur in the literature concerning differences between the pollen morphology of Q. robur and Q. petraea. Erdtman et al. (1961) reported that two different pollen types could be distinguished in the Scandinavian pollen flora, both of which could be ascribed to the genus Quercus L.. They referred these types rather tentatively to a Quercus petraea-type pollen and a Quercus robur-type pollen. The exine and sexine were both described as thinner in the Q. petraea-type (Exine, Q. petraea-type 1.5 μ thick, Q. robur-type 2 μ thick; Sexine, Q. petraea-type 1.2 μ thick, Q. robur-type 1.6 μ thick). The tegillum of the Q. petraea-type was shown to have short, blunt, irregularly-spaced processes of intermediate size (in comparison with the Q. robur-type). These processes were less densely spaced in the Q. robur-type, and psilate areas occurred between them, whilst these psilate areas appeared to be absent in the Q. petraea-type. Size differences were also noted in the bacula supporting the tegillum. These extensive differences, particularly as regards exine thickness, have been confirmed in part by Campo and Elhai (1956).

However, more recent studies have failed to distinguish between two pollen types in the Quercus robur/petraea complex. Smit (1973) employed scanning electron microscope techniques to study the pollen morphology of several species in the genus Quercus. She was able to recognise three distinct groups of pollen - the Quercus robur/petraea-type including Q. robur, Q. petraea, Q. pubescens, Q. pyrenaica, Q. dentata and Q. pontica, the Quercus ilex/coccifera-type, and the

Quercus suber-type, and there appeared to be good correlation between the pollen groupings and subgenera. Smit did not, however, sub-divide the Q. robur/petraea group further. A similar grouping has been derived by Spoel-Walvius (1963) using phase contrast microscopy. Wigston (1971) failed to record any differences between the pollen of the two species. Tutin (1967) and Hibbert (1967) in personal communications cited by Wigston (1971) also expressed difficulties in distinguishing the pollen types.

Pollen was obtained from trees of known morphological type in May 1969, and prepared for examination using the method suggested by Faegri and Iversen (1964) of indirect acetolysis. The pollen was examined under both light and phase contrast microscopy at x1000. The thickness differences between the Q. robur-type and Q. petraea-type exine and sexine noted by Erdtman et al. (1961) and Campo and Elhai (1956) were confirmed:

	Q. robur	Q. petraea
Exine	2.07 μ ± 0.16 μ	1.64 μ ± 0.19 μ
Sexine	1.54 μ ± 0.17 μ	1.23 μ ± 0.14 μ

(Sample size: 150 grains of each species)

The morphological details described by Erdtman et al. (1961) were not, however, substantiated. The general structure of the tegillum was confirmed, but the closer packing and intermediate size of the processes in the Q. petraea pollen could not be detected. Both species appeared to be devoid of psilate areas.

The difficulty experienced in this investigation in distinguishing the pollen of Q. robur and Q. petraea is unfortunate since all Quercus pollen found in pollen deposits can only be ascribed to the generic level. Since one of the important debates in palynological research is the relative migrations of Quercus robur and Quercus petraea

(Godwin, 1956), the difficulty in distinguishing the pollen of these two species would seem to hamper rather than aid this debate. Perhaps the small differences in exine and sexine thickness, since these have been substantiated by at least three workers (Erdtman et al., 1961; Campo and Elhai, 1956; and the present investigation), may prove useful in future researches.

Pollen Size

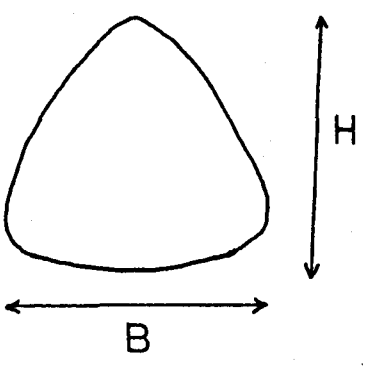
Pollen grain size has been used extensively in the detection of hybrids, eg. Majumdar and Riley (1973), but little information exists on pollen grain sizes in the genus Quercus, and such information as exists has never been used in taxonomic studies. Campo and Elhai (1956) are the only authors to record pollen grain sizes in Quercus spp., the diameter of the pollen grains being:

Quercus robur	Range 24-32 u	Median 26.15 u
Quercus petraea	Range 23-36 u	Median 29.30 u

Although the ranges overlapped, the median values were different.

During extensive studies of Quercus pollen in this investigation, observations suggested that the size of Q. robur pollen differed from that of Q. petraea. Pollen grain size was thus investigated as follows.

Pollen was collected from a number of trees in four oak populations. Twenty grains from each tree were measured using an eyepiece micrometer at x400. The pollen grain as seen in polar view can be treated for all practical purposes as a triangle, and two measurements were made on the grain (see Diagram 7.1 below). The 'area' of the grain was calculated as (B x H)/2.



The results for the different populations are shown in Figure 7.10. The two populations, Wyre 1 and Wyre 2, are both 'pure' Q. petraea populations; in populations Uffmoor and Sutton only trees known to be of Q. robur affinity were used. Wyre 1 and Wyre 2 both showed pollen grains larger than those of Uffmoor and Sutton. However, Figure 7.10 shows that although these differences exist, standard deviations are large and a degree of overlap occurs. Analysis of variance (see below) shows Wyre 1 and Wyre 2 populations to be significantly different from the Sutton and Uffmoor populations.

The above researches suggest significant differences between the pollen grain size of Q. robur and Q. petraea. Measurement of pollen grains from trees of morphological intermediacy during the following flowering season did not, however, produce pollen grains of intermediate size, the grains being either of Q. robur or Q. petraea size. It is possible that the differences between the species are so small, and the degree of overlap so large, that any such intermediate grains would not be easily recognised. As can be seen from Figure 7.10, the smallest Q. petraea grains are the same size as the largest Q. robur grains.

Item	Degrees of Freedom	Sums of Squares	Mean Square	Variance Ratio
Between species	1	2,000,194	2,000,194	445 ***
Within species	2458	11,046,745	4,494	
Total	2459	13,046,939		

The variance ratio is significant at the 0.1% level.

An assessment of the use of pollen studies in oak hybridisation

Both pollen grain size and the thickness of the exine and sexine have been shown to differentiate Q. robur and Q. petraea but at present it has been possible to assess only pollen grain size in suspected hybrid trees and this has not proved intermediate. It has not been found possible

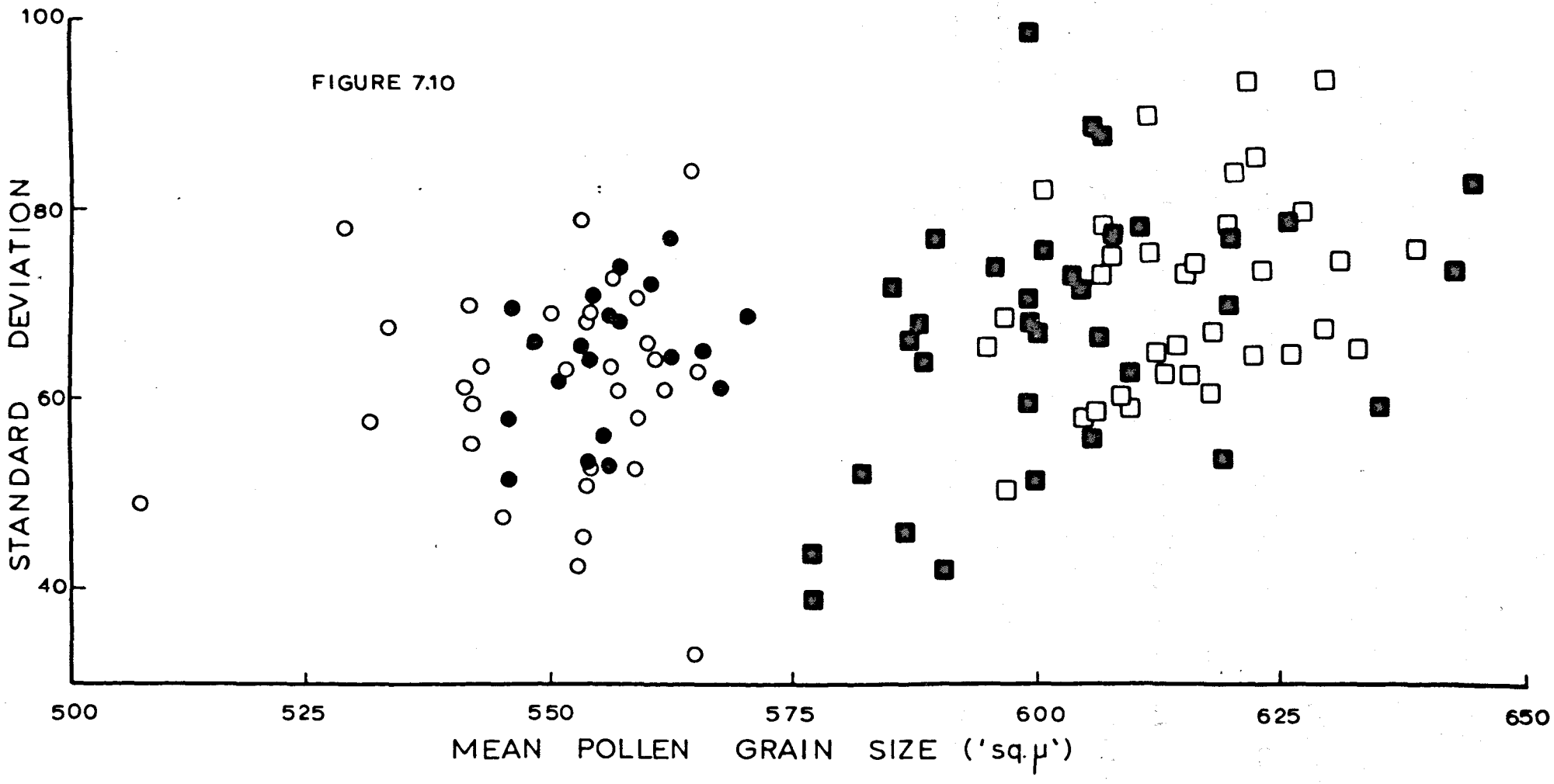


FIGURE 7.10 Pollen grain size in four oak populations
□ and ■ Two sub-samples of Wyre Forest (Q. petraea)
○ Uffmoor Wood (Q. robur)
● Sutton Park (only Q. robur trees)

to differentiate the species using the sculpturing of the pollen grain as seen under both light and phase contrast microscopy.

Pollen viability has proved useful in distinguishing the suspected hybrid trees from the 'pure' species. This is particularly important since the work of Cousens (1963, 1965), Carlisle and Brown (1965) and Wigston (1971) all assumed that trees of morphological intermediacy are of hybrid origin. The observations by Jones (1959) and Sax (1930) suggested that the reverse was true. The present extensive series of measurements on a wide range of populations would support the contention that morphologically intermediate trees are of hybrid origin, and that the interpretations concerning such intermediate trees in oak populations in Section 3 are valid.

CHAPTER EIGHT

ARTIFICIAL HYBRIDISATION

Introduction

The occurrence of hybrids between Quercus robur and Quercus petraea in nature has been noted by many authors (see Section 1 for a full discussion of these records), but there have been few attempts made to raise the hybrid by artificial means.

The earliest account of attempted crossing of the two species is given by Klotzsch (1854). Klotzsch prepared only a short account of his work and indeed gave no details of the method of pollination or the number of successful crosses. The hybridisation would appear to have been successful, however, since the progeny were recorded as exhibiting heterosis when eight years old (ex Dengler, 1941). Another early account is that of Geschwind (1876). His attempts produced six acorns of which four later germinated. Two of these plants were planted out in a forest, but these were not detectable after a few years. Dengler (1941) has criticised this work particularly from the point of view of the contamination possible in an anemophilous species. Pyatnitski (1939, 1954, 1960) has attempted an extensive breeding programme between many oak species, but has failed to make the cross Q. robur female x Q. petraea male. Generally, in all his attempts within the genus, he has reported only very small percentage success rates. The most successful attempts at producing artificial oak hybrids have been made by Dengler (1941). Dengler's crossing experiments were both extensive and more importantly carried out over several years (ten flowering seasons), but generally they produced fertile acorns in less than 2% of the attempted crosses. Such a result is, however, very deceptive since differences between years were very large. For example, in 1936, 15% (46 acorns)

of the Q. robur female x Q. petraea male crosses were successful and 6% of the reciprocal crosses were successful. Importantly, Dengler's experiments were carried out in carefully controlled conditions which would probably meet modern-day tree breeding requirements. Dengler (1941) also notes that artificially produced hybrids were more vigorous than the parental types.

There appears to be little work in progress on producing hybrids between Q. robur and Q. petraea. Krahl-Urban (1959), in the latest 'monograph' on European oaks, mentions very briefly preliminary results of his own investigations into artificial hybridisation between Q. robur and Q. petraea. He records that although his results are preliminary, they tend not to support the findings of Dengler. Harkai (1966) has work in progress on breeding Q. robur and Q. petraea but, as yet, no results are available.

This chapter reports the attempts to hybridise the two species and the subsequent use made of the acorns formed to study acorn characters and the karyotype of the two species.

Artificial Hybridisation

Attempts to hybridise the species were carried out over three seasons - the first summer, 1969, at Wyre Forest and Uffmoor Wood, near Birmingham, and at Hetchell Wood and the surrounding small copses during summer 1970 and summer 1971. The methods employed were identical for all three years.

During the previous growing season, trees of each species were identified and labelled. Only trees that were unmistakably one species or the other were used. In April, the following year, pollination bags were attached to branches at approximately 6' from the ground. The pollination bags were supplied by Duraweld Ltd., Scarborough. Each bag

was 18" x 9" with a transparent window to allow in sufficient light for the leaves to expand and photosynthesise properly. The material of the bag allowed gaseous exchange to take place between the inside and the bulk air. The bags were attached to the branch with either nylon wool or wire around a non-absorbent cotton wool collar.

It was necessary to 'bag-up' the branches reasonably early, since during the flowering season, there is a constant pollen 'fall-out'. Semerikov and Glotov (1971) have estimated that pollen may travel up to 100 metres and spread over an area of 3 hectares from a single tree. The stigmas on any one particular tree probably become receptive before the anthers on the same tree discharge (Pyatnitski, 1947), and consequently although catkins have to be removed from inside the pollination bags, the removal of catkins is not critical. Nevertheless, the bags were checked each day and catkins removed when they were large enough (which was always before they ripened). By 'bagging-up' as soon as the buds began to swell, and removal of the catkins before they ripened, it was hoped that the female flowers produced in each bag were uncontaminated.

Since variation in time of flowering occurs, it was necessary to devise a crossing plan that would compensate for such variation as both resources (numbers of pollination bags) and manpower were limited. It was important that comparative results were obtained, i.e. it is desirable to obtain not only success rates between species, but also, for comparative purposes, the success rates within species. For these reasons, the following plan was employed.

Five branches were 'bagged' on each tree. As the trees came into flower, they were divided into groups of four trees, two Q. robur and two Q. petraea. Large quantities of pollen were collected from each tree (as described in Chapter 7). Of the five bags on each tree, the

flowers of the first were pollinated by pollen from the same tree, the flowers of the second by the other tree of the same species, the flowers of the third and fourth by the two trees of the other species in the group, and the fifth bag was opened and closed along with the others and catkins removed; the last bag acting as a control. The viability of the pollen of each tree was also measured using NBT - this provided a check in case of anomalous results.

At the time of pollination, the number of female flowers in each bag was noted - this varied between five and forty-eight. Several different pollen applicators are available, and these have been shown to influence the result of controlled pollination. For example, Alfjorden (1973) showed that a 'hekto-spray' which blows pollen increases the number of seeds produced over a 'vibrator pistol' from which the pollen simply falls. Sindelar and Musil (1969) give details and performances of nine such pollinating devices. Alfjorden (1973) and Šindelář and Musil (1969) were all interested in pollinating conifers, where the cone structure requires injection-type methods; it was decided that provided care was taken to avoid contamination, camel-hair paint brushes could be used, since the stigma is very accessible. Pyatnitski (1947) notes that the stigmatic surface of oaks have a short period of maximum receptiveness (up to six days), but that they remain receptive for a period of 10-14 days. The female flowers were pollinated as they became receptive and pollen was transferred to the stigmatic surface every other day until eight days after the first pollination. The pollination bag was replaced after each 'pollination'. The controls were 'pollinated' with a camel-hair brush which was not charged with pollen.

The pollination bags were left in place until mid-July, when all danger of contamination had passed; the bags also provided some protection

during the early development of the acorns. The bags were replaced towards the middle of September, before the acorns had ripened to protect them from the many birds and mammals which use the acorns for food, so that the mature acorns could be studied. The acorns were finally harvested in mid-October. Deliberate vandalism throughout the three years of these experiments proved exceptionally fierce and accounted for some 15% loss of all pollinated material.

The results for the three years are given in Table 8.1, and analysis of the results is presented in Appendix 6. Differences are very evident between the success rates of different years - 1970 appears to be a particularly poor year for the production of acorns. Such variations between years are fairly common, eg. Ovington and Murray (1964) noted a particularly poor year in 1961, preceded and followed by two good years; Sharp and Sprague (1967) recorded similar differences between 1961 and 1960 and 1962 in Pennsylvania whilst studying flowering and fruiting in white oaks. Such differences are probably related to climatic factors. Jones (1959) believes there to be evidence that long, warm summers favour the growth of acorns. In interpreting these results, due regard should be given to the fact that the results for 1969 are from a different woodland than those for 1970 and 1971. However, since the results for 1969 and 1971 are very similar, and the results for 1970 and 1971 so dissimilar, the low success rates recorded for 1970 would appear to be due to climatic differences between years rather than differences between forests. Analysis of variance (Appendix 6) for differences between years shows clearly that for every type of cross except selfed Q. petraea there is a significant difference between years.

In comparing the different types of cross, there are obvious differences. Dengler (1941) regarded Q. robur as self-sterile and indeed Pyatnitski (1947) showed that under natural conditions the pollen

Type of Cross	1969 No. of Trees	1969 No. of Flowers	1969 Mean Success %	1969 Standard Deviation	1970 No. of Trees	1970 No. of Flowers	1970 Mean Success %	1970 Standard Deviation	1971 No. of Trees	1971 No. of Flowers	1971 Mean Success %	1971 Standard Deviation
Q. robur selfed	17	298	22.9	11.9	25	307	10.3	10.6	25	264	25.7	12.6
Q. petraea selfed	24	392	47.4	12.9	20	215	26.7	15.3	17	297	40.3	10.3
Q. robur intra specific crosses	20	415	58.7	8.5	20	354	31.4	14.7	22	364	62.3	17.9
Q. petraea intra specific crosses	14	321	55.2	17.5	27	541	25.9	10.6	27	404	58.1	15.4
Q. petraea female x Q. robur male	49	681	0.58	0.33	45	767	0.13	0.10	55	653	0.76	0.90
Q. robur female x Q. petraea male	53	640	0.62	0.49	50	594	0.33	0.24	51	599	1.00	0.87

The control bags all failed to produce mature acorns.

All trees used had pollen viabilities greater than 82%.

TABLE 8.1 RESULTS OF ARTIFICIAL CROSSING EXPERIMENTS IN Quercus L.

of Q. robur did not germinate so well on the stigmas of the pollen parent as on those of other trees, but these differences were marginal - 38% germination compared with 52% after 3 hours and 75% and 83% after 24 hours. However, there is good evidence from the results presented here that, although not self-sterile, the success rate of selfed Q. robur trees is significantly different from that of intraspecific Q. robur crosses for all three years (see analysis in Appendix 6). Generally, in such selfed Q. robur crosses, the success rate is depressed by about 50%. The same is not true for selfed Q. petraea and intraspecific Q. petraea crosses, although in two years out of the three, there is a reasonable discrepancy between these types of crosses, the selfed crosses producing a poorer performance than non-selfed crosses, and in one year, 1971, this difference became significant (see analysis in Appendix 6). There is, therefore, perhaps some indication here that although by no means self-sterile, Q. petraea responds better to intraspecific crossing.

Between Q. robur and Q. petraea, there appears to be little difference - both intraspecific crossings for the two species show no significant differences (see analysis in Appendix 6). The selfed crossings do, however, show significant differences in years 1969 and 1971 but not in the poor year 1970.

One important point concerns the low levels of success obtained in the intraspecific crosses. With the exception of the 1970 results, only about 50% of the flowers pollinated actually produced acorns. The probable reasons for this would appear to lie either with the pollination method or with some barrier between successful pollination and the production of the mature acorn. Although the pollination method, particularly in relation to the detection of a 'receptive' stigma, was somewhat haphazard, it is felt that a more likely explanation lies in the natural abortion of maturing seed. Under natural conditions, rarely

more than three acorns are ever produced on any one peduncle - this is particularly true of Q. petraea, where the short peduncle brings the acorns into close proximity to the stem and petioles, so that there is physically no space available to mature more than three acorns. In Q. robur the same is true, since the flowers are clustered at the end of the peduncle; but here also the physical strength of the peduncle must be called into question. During pollination, many more than three flowers were found on each peduncle, and it seems likely that either space or strength of the peduncle or both have contributed in some way to bring about the natural abortion of some maturing seeds. Frequently, it is possible to see immature seeds on peduncles at the end of summer, apparently 'crowded-out' by the mature seeds.

Hybridisation between the two species was singularly unsuccessful, particularly when compared with intraspecific crossings - the level of success was approximately 1% when compared to such crossings. Over the three years, 22 hybrid acorns were produced out of a total of 3934 flowers pollinated, a success rate of 0.56%. Using Q. robur as the female parent proved more successful in all three years, and was significantly different from that using Q. petraea as the female parent during 1970 but not during 1969 and 1971. Such low levels of success are similar to those obtained by Dengler (1941) and Pyatnitski (1939, 1954 and 1960).

Attempts were made to germinate some of the 22 acorns formed during the three years. Two acorns from 1969 were dissected in order to determine whether or not the embryo was fully formed. The acorns generally were smaller than those of either pure species, but as far as could be determined, the embryo was fully developed and appeared normal. The acorns were sown in John Innes Compost No. 2, one per 3" diameter black polythene pot. No mature seedlings have been produced from these acorns,

but some degree of success was obtained - this is summarised in Table 8.2.

Of the 22 acorns, five were infected by the fungus Sclerotinia pseudotuberosa which converts the whole of the acorn into a black sclerotium; three failed to show any signs whatsoever of germination; six produced a radicle but no further development took place; two produced an extensive root system, but no plumule emerged; two acorns produced an extensive root system, but the seedlings died shortly after the plumule emerged; two acorns produced weak seedlings, with fully emerged plumules, but these too died after 8 and 14 weeks. The high percentage of acorns infected by the fungus Sclerotinia pseudotuberosa (nearly 25%) is very much higher than non-hybrid acorns. The acorns used for the experiments described in Chapter 4 were also infected with the fungus, but to a lesser degree (4%) suggesting that hybrid acorns may be more susceptible to attack by the fungus than non-hybrid acorns. It is noticeable from Table 8.2 that the acorns appear to stop development at specific points - they either fail to germinate at all, or die after production of a radicle, or die after an extensive root system was produced, or after a plumule emerged or during leaf expansion. Such barriers to further development might well reflect times at which different sets of genes come into action although this is pure conjecture.

Acorn Characters

The production of a large number of acorns of known parentage provided an excellent opportunity to study acorn characters. The characters used to distinguish acorns are listed in Chapter 5. Briefly, three characters are useful - the presence of olive green longitudinal stripes on the outside of the mature, fresh acorn, present in Q. robur

TABLE 8.2 THE FATE OF THE 22 HYBRID ACORNS

1969

Acorn	1	Used for dissection of embryo
"	2	Used for dissection of embryo
"	3	Acorn failed to show any signs of germination
"	4	Acorn failed to show any signs of germination
"	5	Acorn produced radicle, ½" long, but failed to show any further development
"	6	Acorn produced radicle 1½" long, but failed to show any further development
"	7	Acorn produced tap root, with side root branches, but no plumule emerged
"	8	A weak seedling produced that died after 14 weeks

1970

Acorn	9	Acorn infested with fungus identified as Sclerotinia pseudotuberosa
"	10	Acorn infested with fungus identified as Sclerotinia pseudotuberosa
"	11	Acorn failed to show any signs of germination

1971

Acorn	12	Acorn infested with fungus identified as Sclerotinia pseudotuberosa
"	13	Acorn infested with fungus identified as Sclerotinia pseudotuberosa
"	14	Acorn infested with fungus identified as Sclerotinia pseudotuberosa
"	15	Acorn produced radicle, 1" long, but failed to show any further development
"	16	Acorn produced radicle, 1" long, but failed to show any further development
"	17	Acorn produced radicle, 1" long, but failed to show any further development
"	18	Acorn produced radicle, 1"long, but failed to show any further development
"	19	Acorn produced extensive root system, but no plumule emerged
"	20	Acorn produced extensive root system, but died shortly after plumule emerged
"	21	Acorn produced extensive root system, but died shortly after plumule emerged
"	22	A weak seedling produced that died after 8 weeks

The radicles of acorns 6 and 17 were used for the analysis of the karyotype.

but absent in Q. petraea; the acorn colour, pale fawn in Q. robur but a dark brown in Q. petraea, and acorn size and shape, the Q. robur acorn being longer and thinner than that of Q. petraea.

These three characters were measured on a random sample of the acorns produced from the artificial crosses. It was found possible to score the longitudinal stripe as strongly present, weakly present and absent, and acorn colour similarly as pale fawn, dark brown and intermediate. Acorn shape was determined as the ratio acorn length : diameter of the acorn at the widest part, the measurements being made with an industrial micrometer. Acorn colour and stripe characteristic of Q. robur was scored as 0, when characteristic of Q. petraea as 2 and intermediate states as 1. The results are presented in Table 8.3.

Both acorn colour and stripe differentiate the species very clearly, there being little variation in either of these two characteristics from the basic type. This contrasts with population samples observed by Wigston (1971). He noted the colour and stripe of naturally produced acorns in six woodlands. In the Q. robur populations, generally only 50% of the acorns were of the characteristic type, approximately 10% were of the other species, and the remaining 40% were intermediate. The same distribution was found in the Q. petraea population studied. Jones (1959) regards both colour and stripe as useful characters in distinguishing the species and the results presented here would agree closely with such an assertion, but it must be remembered that these were from artificially produced acorns whilst Wigston's results were based on population samples. The hybrid acorns were generally intermediate for colour and shape, but showed a wide range of variation (see Table 8.3).

Acorn shape in the study by Wigston (1971) showed very anomalous behaviour - in the Q. petraea woodland studied, the shape index ranged

	Acorn Shape	Acorn Colour	Acorn Stripe	Sample Size
Selfed *Q. petraea*	1.49 ± 0.19	1.94 ± 0.17	1.94 ± 0.08	76
Intraspecific *Q. petraea*	1.36 ± 0.26	1.96 ± 0.13	1.86 ± 0.10	144
Selfed *Q. robur*	1.71 ± 0.12	0.44 ± 0.24	0.39 ± 0.43	67
Intraspecific *Q. robur*	1.68 ± 0.14	0.36 ± 0.19	0.28 ± 0.26	200
Q. robur x *Q. petraea*	1.57 ± 0.25	0.95 ± 0.43	0.74 ± 0.71	22

TABLE 8.3 VARIATION IN ACORN CHARACTERS FROM ACORNS DERIVED FROM ARTIFICIAL CROSSES

from 1.00 to 1.40 whilst in the three Q. robur woodlands studied, the ranges were 1.3-1.7, 1.0-1.3 and 1.0-1.4, i.e. two of the Q. robur woodlands producing identical shape ranges to the Q. petraea woodland acorns. Again, these are natural acorns, but more importantly, since shape is a function of growth, both actual and differential, it is likely to be markedly influenced by external parameters particularly environmental factors. As noted earlier, Jones (1959) believes that long, warm summers favour the growth of acorns. Forbes (1914) also records that acorns produced in the southern part of England are much larger than those produced in Scotland and Ireland.

The results for acorn shape are presented in Table 8.3. These show very large differences between the species (see Analysis of Variance table below) but little differences between selfed and intraspecific crosses within each species. The differences in shape - the rounder acorns of Q. petraea and the longer, thinner acorns of Q. robur are consistent with published accounts of acorn shape, except Wigston (1971), and since they are from controlled crossings can probably be regarded as 'typical' of the species.

The shape of the hybrid acorns falls midway between Q. robur and Q. petraea, but again is very variable and overlaps the ranges shown by each species to a considerable degree. It should also be noted that the acorns were smaller overall than either species.

Difference in acorn shape between Q. petraea acorns (both from selfed and intraspecific crossings) and Q. robur acorns (both from selfed and intraspecific crossings)

Item	Sums of Squares	Degrees of Freedom	Variance	Variance Ratio	Probability
Between species	10.52	1	10.520	302.3	$<0.001^{***}$
Within species	16.88	485	0.035		
Total	27.40	486			

Karyotype Analysis

The acorn material obtained from the experimental crosses provided material for the study of the karyotype of the species, since these were of known parentage - acorns collected normally whilst attached to trees can only be determined as far as the maternal parent is concerned. Three acorns from each type of cross, excluding the interspecific crosses, were grown in John Innes No. 2 potting compost in 3" diameter plastic pots. Germination was rapid (five weeks) and produced small side branches off the main tap root when the latter was about 2" long. The tips of these side branches were removed and fixed in acetic alcohol (3 parts absolute alcohol : 1 part glacial acetic acid), and stored until required in stoppered vials at 5°C. When required, the roots were hydrolysed in N hydrochloric acid for 7 minutes at 60°C, and then transferred to Feulgen stain for 60 minutes in the dark. Squashes of the small area of the root tip that had stained pink were made in acetic-orcein. The combined use of both Feulgen and acetic-orcein was found to give a stronger, more durable staining reaction which showed up the cell surface and cytoplasm. The squashes were examined at x1000.

The acorns from the interspecific crosses were required to raise hybrid progeny and consequently possible destructive sampling was to be avoided. As the acorns failed in the main to germinate (see previous section) such precautions were unnecessary. Two hybrid acorns which produced radicles were used for staining and squashing. Both acorns were from the Q. robur female x Q. petraea male cross.

In the analysis of the karyotype (at mitotic metaphase) it is possible to observe five different characteristics:

1. The difference in absolute size of the chromosomes
2. Differences in the position of the centromere
3. Differences in relative chromosome size
4. Differences in the basic number of chromosomes
5. Differences in the number and position of satellites

If suitable mitotic prophase stages are available, it is also possible to observe differences in the degree and distribution of heterochromatic regions (Stebbins, 1971). The chromosomes of Quercus are rather small - the largest measured in this study being 2.5 u. (Strickberger (1968) notes that most metaphase chromosomes fall into the range 3.5 u to 10 u with fungi and birds being exceptional in producing chromosomes of 0.25 u.) Such a small size, possibly accompanied by the cytological inexpertise of the author, made observations of the six characteristics mentioned above rather difficult. It was, however, possible to prepare a count of the chromosomes, note their relative sizes and measure the chromosomes. Some observations were possible on the position of the centromere and the occurrence of satellites, but only on relatively few chromosomes. Differences in the degree and distribution of heterochromatic regions was not possible due to the absence of suitable nuclei. In all, the material yielded the following 'good' cells for observation:

1. Selfed Q. robur - 5 root tips, 18 cells
2. Selfed Q. petraea - 4 root tips, 13 cells
3. Intraspecific Q. robur crosses - 5 root tips, 22 cells
4. Intraspecific Q. petraea crosses - 6 root tips, 17 cells
5. Interspecific crosses (Q. robur female x Q. petraea male) - 2 radicles, 11 cells

Basic Chromosome Number:

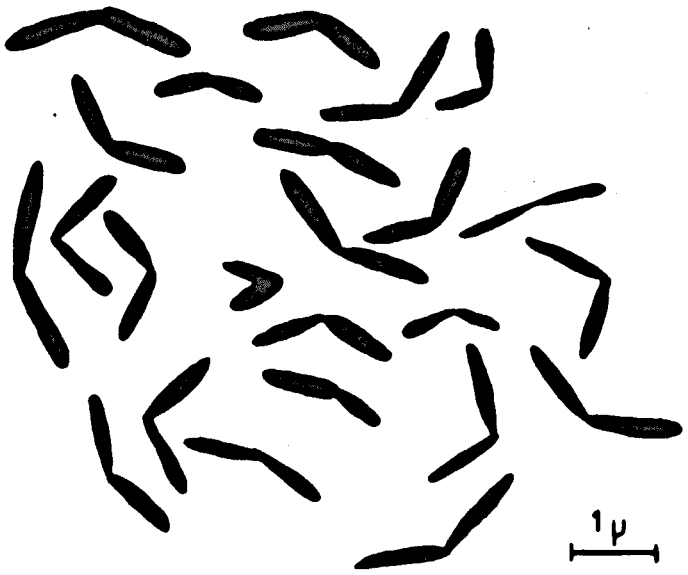
The number of chromosomes recorded in the majority of cases was 2n = 24. This agrees with the findings of Jaretzky (1930) for Q. petraea and Høeg (1929) and Poucques (1949) for Q. petraea and Q. robur, but disagrees with the first report of chromosome number in Quercus given by Cosens (1912) as 2n = 8. Wetzel (1929) reported 2n = 22 for Q. petraea. Sax (1930) records n = 12 for a range of mainly North American oak species and this would appear to be the basic number for the genus, although Tutajuk and Turčaninova (1968)record 2n = 28 in apical meristems of buds of Q. castaneaefolia. All other reports of somatic number in species of the genus give 2n = 24, eg. Grimpu (1929), Friesner (1930). There were,

however, some exceptional numbers found during the present investigation. Two cells of the selfed Q. robur preparations showed a somatic number 2n = 22, but these counts were almost certainly spurious and probably due to poor squashing out. Two cells of the interspecific crosses gave counts of 2n = 22 and 2n = 25. Both counts were in well prepared nuclei, and in the case of the 2n = 22 nucleus, there was no evidence of any chromosomes overlapping, thus giving an erroneous count. In the case of the 2n = 25 nucleus, there was no evidence of breakage in any of the chromosomes or division of sister chromatids although both are possibilities. Høeg (1929) recorded normal behaviour of pollen grains in suspected hybrids, but noted some irregularities particularly 'detached chromosomes and empty grains', and the observations noted here might reflect similar irregularities.

The Karyotype:

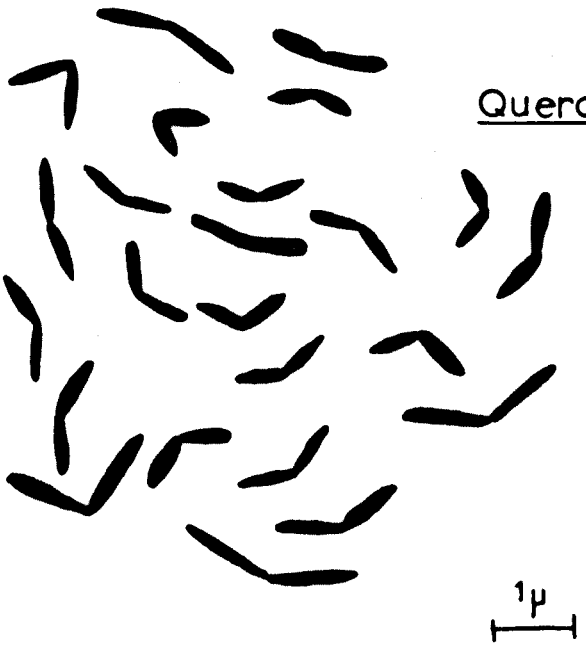
No differences (other than the exceptional two counts of 2n = 22 noted above) were observed between the karyotype of the selfed individuals and intraspecific crosses, and for the purposes of the discussion below, they will be treated as identical.

The chromosomes of Q. robur were generally smaller (mean length - 1.42 μ) than those of Q. petraea (mean length - 1.84 μ). Diagrams8.1 and 8.2 show typical squashes of the two species. Jaretzky (1930) had noted that the chromosomes of Q. petraea were very similar in size, whilst four of the reduced number of twelve chromosomes in Q. robur were smaller than the others, and one was of intermediate length. Table 8.4 lists the sizes of the chromosomes found in the two nuclei depicted in Diagrams 8.1 and 8.2. In the Q. robur nucleus, there are certainly eight or nine chromosomes which are smaller than the rest (below 1.3 μ) and this has been confirmed in the other nuclei studied. However, the nucleus of Q. petraea also shows four chromosomes of rather



Quercus petraea

DIAGRAM 8.1



Quercus robur

DIAGRAM 8.2

DIAGRAMS 8.1 and 8.2 The karyotype of Q. petraea and Q. robur respectively observed at mitotic metaphase in root tip squashes.

	Q. petraea	Q. robur	Theoretical Hybrid	Origin	Mean of 11 Hybrid Nuclei
	1.25	1.00	1.00	R	1.10
	1.25	1.00	1.10	R	1.10
	1.25	1.10	1.15	R	1.20
	1.25	1.10	1.25	P	1.20
	1.60	1.10	1.25	P	1.20
	1.75	1.20	1.30	R	1.25
	1.75	1.30	1.35	R	1.40
	1.75	1.30	1.40	R	1.40
	1.75	1.30	1.40	R	1.50
	1.75	1.40	1.40	R	1.50
	1.75	1.40	1.40	R	1.50
	1.75	1.40	1.50	R	1.60
	1.85	1.40	1.68	P	1.70
	2.00	1.40	1.75	P	1.75
	2.00	1.40	1.75	P	1.80
	2.00	1.40	1.75	P	1.80
	2.00	1.40	1.90	R	1.95
	2.00	1.40	1.92	P	2.00
	2.00	1.50	2.00	P	2.00
	2.25	1.50	2.00	P	2.20
	2.25	1.80	2.12	P	2.20
	2.25	2.00	2.18	R	2.20
	2.25	2.10	2.25	P	2.20
	2.50	2.25	2.38	P	2.35
Mean Chromosome Sizes	1.84	1.42	1.63		1.68

TABLE 8.4 CHROMOSOME SIZES IN Q. petraea, Q. robur AND HYBRID ROOT TIP NUCLEI

small size (about 1.25 μ) and these were not noted by Jaretzky. The occurrence of rather small chromosomes appears to be a characteristic shown by several species within the genus - in the rather poor drawings of Jaretzky (1930), it is possible to recognise two types of chromosome, small and large in Quercus glandulifera, Q. pontica, Q. macranthera and Q. robur but not in Quercus petraea, Q. Delechampii, Q. Libani nor Q. nigra. Fourage (1939) notes that the chromosomes of both Q. petraea and Q. robur are identical in size and shape and is obviously at variance with the results presented here and by Jaretzky (1930).

The karyotype of the hybrid nuclei proved interesting. In order to assess whether the individual species have contributed equally in the production of the hybrid, it was necessary to have some expectation of the hybrid karyotype assuming equal representation. Since only absolute and relative sizes were available to characterise the chromosomes (see below), the chromosomes from each somatic cell were paired as regards size to produce the twelve possible sizes for each species (the haploid condition). These together represent the theoretical karyotype of the hybrid assuming that both species contributed equally and that the chromosome complement has remained intact. This theoretical karyotype is presented in Table 8.4.

The karyotypes of three of the hybrid nuclei have been drawn in Diagram 8.3. As noted above, there was a small variation in chromosome number, but generally, 2n = 24. The distribution of chromosome sizes in the two pure species was such that in the theoretical hybrid nucleus, ten of the twelve smallest chromosomes would have come from the Q. robur component, and ten of the twelve largest chromosomes would have come from the Q. petraea component. The karyotypes of the eleven nuclei studied conform reasonably well to the theoretical type, both in terms of the mean chromosome size expected and in the distribution of the

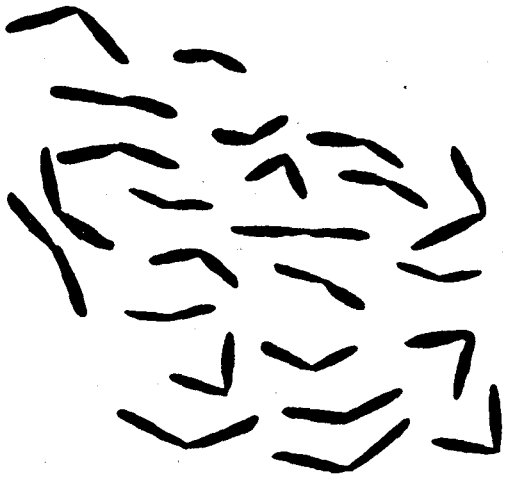


DIAGRAM 8.3

Quercus robur ♀
X Q. petraea ♂

1μ

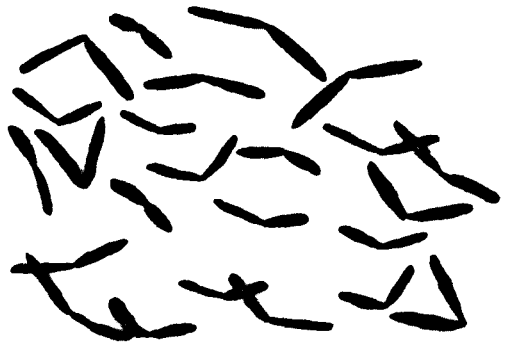


DIAGRAM 8.3 The karyotype of three nuclei of root tip cells observed at mitotic metaphase from the cross Q. robur ♀ x Q. petraea ♂

individual chromosomes (see Table 8.4 and Diagram 8.3). The hybrid nuclei certainly have more very small chromosomes than would be expected for a Q. petraea nucleus and more larger chromosomes than would be expected for a nucleus of Q. robur. For comparative purposes, Table 8.4 lists the mean values of the smallest through to the largest chromosome sizes found in the eleven hybrid nuclei. Good agreement can be seen between this list and the theoretical karyotype and it is therefore concluded that the species have contributed equally to the karyotype of the hybrid.

In well prepared cytological material, it should be possible to record other details of chromosome morphology particularly the position of the centromere and the number and position of the heterochromatic regions. The chromosomes of Quercus are too small to allow these features to be recognised with certainty particularly by the non-specialist. Only general observations can, therefore, be made concerning these aspects of the cytology of Q. robur and Q. petraea. Most chromosomes appeared to be metacentric or sub-metacentric. In Q. petraea four chromosomes at mitotic metaphase appeared to be telocentric, whilst in Q. robur, two chromosomes appear telocentric. Satellite portions were not observed on any chromosome studied and it is concluded that these are absent in the species.

Discussion

The low success rates for artificially created hybrids noted in this chapter and by other authors (eg. Pyatnitski, 1939; Dengler, 1941) prompt the question whether or not such a low success rate can create and maintain the high level of hybridity described by Cousens (1962, 1963, 1965), Wigston (1971) and the present investigation. The level of interspecific success rates would appear to be approximately 1%, and such a low level would persuade authors such as Jones (1959) to believe

that large numbers of intermediates recorded in natural populations are not of hybrid origin but are in Jones' words "mostly based on imperfect understanding of the specific characters". Cousens (1963) in discussing the results of Dengler (1941) and Pyatnitski (1939, 1941 and 1947) believes that since oaks show so much morphological variation within each species, any crossing plan should attempt to include all morphological types - those at the very extremes as well as those showing elements of the other species. Although an admirable suggestion, lack of manpower has prevented such an inevitably large crossing plan to be undertaken in the present investigation. Cousens (1963) also notes the importance of attempted backcrosses, an important point, since once established in a population, an F_1 hybrid is able to produce a whole range of backcross progeny and consequently, once the barriers to production of an F_1 hybrid are defeated, the rate of spread of alien genes depends largely on the success of backcrosses. As Cousens (1963) argues, the interspecific fertility may be relatively unimportant if the hybrids are fully fertile with either parent.

No information on this point is forthcoming from the researches presented here, since the exercise was specifically to determine the successfulness of interspecific crosses using trees of known morphological purity and not to consider intermediates. The pollen viability results discussed in the previous chapter would suggest that since apparent backcross derivatives have a high pollen viability, the production of F_3's from F_2 backcross derivatives crossed with the pure species would be relatively successful compared to the initial production of F_1's. There is, however, no direct evidence on this point.

Stebbins (1950) also raises an important issue - oaks are generally long-lived individuals and consequently although the success rate of

production of hybrids is fairly low, the long period over which the hybrids are produced means that in absolute terms, the number of hybrid acorns may be quite high. For example, Jones (1959) records that 120-140 year old standard trees produced on average 50,000 acorns per year, and if only 0.1% of these are of hybrid origin, this represents 50 acorns per tree per year for several centuries. Under natural conditions, an oak species must receive large quantities of foreign pollen (Stebbins et al., 1947) and consequently the possibilities of hybrids are always present.

Aspects of this argument are investigated further in Chapter 11.

Both Klotzsch (1854) and Dengler (1941) reported heterosis in the F_1 hybrids, whilst in the present investigation, it has proved impossible to raise mature seedlings. However, as mentioned above, F_1 hybrids can be produced from individuals at the extremes or individuals that are morphologically closer, and since no notes are available as to the morphological type of the individuals used in earlier studies, it is difficult to compare results. The work of Klotzsch is regarded with suspicion by Dengler (1941) but the work of Dengler (1941) was carried out using modern tree breeding methods. If, as seems possible, the hybrid is more vigorous than either parents, this might overcome the objections proposed by Jones (1959) to the view of widespread hybridisation in British oak species. A more vigorous plant is obviously more likely to flower earlier and conceivably produce more flowers which might well be the advantage required by the hybrid to make it and its backcross derivatives successful. It might well prove profitable in future to examine the growth performances of the progeny of intermediate trees for signs of heterosis although it should be remembered that, if the progeny are the result of 'open-pollinations', only the maternal parent will be known to be intermediate.

The karyotype analysis lends good support to the separation of the two morphological types into specific categories. As noted in Chapter 1, several authors at the close of the last century designated the Q. petraea types to varietal status, eg. Bentham and Hooker (1886), and the confusion of the status of the many subspecific and intermediate forms has allowed this view to persist. Schwarz (1970) in preparing the account of the genus for Flora Europaea separates the two species and clears much of the difficulties involved in the taxonomy of the species. The differences in karyotype supports this division. However, the differences in the karyotype might well lead to problematical pairing between the different haploid sets in the F_1 hybrid. As seen in Table 8.4, in the theoretical karyotype, ten of the largest twelve chromosomes are from Q. petraea and ten of the smallest twelve chromosomes are from Q. robur. This would lead to very difficult pairing during meiosis, and possible queries concerning the ability of the F_1 hybrid to function as a reproductive entity.

SECTION FIVE

COMPARATIVE PHYSIOLOGY

CHAPTER NINE

LEAF MORPHOLOGY AND TRANSPIRATIONAL RESISTANCES

Introduction

Leaf morphology within the genus Quercus is strikingly variable, from the round non-dissected leaves of Q. copeyensis, the lanceolate Q. ilex, the toothed Q. fusiformis, the very shallowly lobed Q. prinus through a gradation of lobed forms from Q. petraea and Q. alba to the deeper lobed Q. robur and the exceptionally deep lobes of Q. kelloggii. Leaf size varies from the small leaves of Q. coccifera (1.5-4 cm long) to the large leaves of Q. rubra (12-20 cm long). Leaf hairiness varies from the completely glabrous leaves of Q. rubra to the hairy leaves of Q. petraea to the densely pubescent leaves of Q. pubescens.

These differences may well result from the evolutionary pressures producing leaf types suited to particular environments. Tucker (1974) has described the leaf form of several species pairs belonging to different subgenera of the genus Quercus which are remarkably similar in gross leaf morphology. These species pairs came from similar habitats and in several cases, eg. Q. turbinella and Q. dunnii they coexist together. Benson et al. (1967) have described an interesting situation in a hybrid swarm of Q. Douglasii x Q. turbinella subsp. californica in California. Although each population of the hybrid swarm was heterogeneous, each was markedly restricted and this restriction appeared to be correlated with exposure to the sun, i.e. trees on north east facing slopes resembled, but not completely Q. Douglasii whilst those on south west facing slopes resembled Q. turbinella subsp. californica. Slopes facing other directions had populations correspondingly intermediate between the two species. The oaks of the subgenus Sclerophyllodrys, Q. coccifera, Q. ilex and Q. rotundifolia

are all evergreen, and have their centre of distribution and almost their complete distribution throughout the Mediterranean. They also have a basic leaf structure in common - their leaves are ovate-oblong to lanceolate, small, very thick in texture and glabrous. Indeed these species have leaves typical of the sclerophylls commonly found in the Mediterranean region. Both these examples and the situations described by Tucker (1974) suggest a close correlation and possible adaptation of leaf structure to the environment within the genus Quercus. The work of Nescjarovič and Smirnova (1969) have shown in Q. robur seedlings that those seedlings from acorns collected in eastern, southern and south western provinces had a more xeromorphic character than seedlings grown from acorns from northern and north eastern areas of Russia. It would appear, therefore, that leaf structure of Q. robur might show adaptations to gross differences of environmental conditions.

Lewis (1972) has discussed the possible physiological significance of the variations exhibited in leaf structure, particularly the effect of leaf structure on the diffusive resistances to water vapour and demonstrated that variation in leaf size and shape and leaf thickness can have a very important effect on the rates of transfer of heat energy, carbon dioxide and water vapour from the leaf to the environment. Q. robur and Q. petraea are generally thought to have different geographical and edaphic 'preferences' in Britain, although the situation is very confused with planting, and as shown in Chapters 2-4 there may also be differences in their response to the environment - Q. robur having leaves behaving as 'sun' leaves, Q. petraea having leaves behaving as 'shade' leaves. The leaves of the two species might, therefore, show physiological behaviour typical of 'sun' and 'shade' leaves.

This chapter reports an investigation into the diffusive resistances to water vapour loss from oak leaves under a variety of conditions

representative of sun and shade in order to demonstrate how the leaves of the two species react under these conditions.

Method

The method of investigating diffusive resistances to water vapour loss from leaves follows the 'cut-shoot' method described by Eckardt (1960). The experimental theory and derivation of the resistances from the experimental results are discussed in Appendix 7.

The measurements were completed under laboratory conditions - it had originally been intended to perform the experiments inside a growth chamber under controlled conditions, but the constant switching of micro-switches in the control gear and vibration made the use of a balance very difficult. An artificial environment was, therefore, created by suspending a 750 watt quartz halogen lamp from the ceiling and at a height of 100 cm above a bench top. Under the lamp a wire frame was constructed so that layers of muslin could be interposed between the lamp and the bench top at a height of 75 cm, thus permitting levels of light intensity to be altered. Under this frame a second frame was constructed 10 cm high which consisted of fine wire strung at 1cm intervals across a dexion frame, so that a leaf lying on this frame was suspended in mid-air, 10 cm above the bench top. To one side of this frame a small fan was sited to provide a 'wind' across the wire. This fan was kept constant throughout the whole series of experiments at 0.5 mt/sec, not particularly to simulate natural conditions but to prevent side draughts in the laboratory influencing the experiment unduly. Without the fan air movements across the frame were random and non-predictable; with the fan air movements were constant. It was found possible to create a range of different conditions by altering the number of muslin layers - not only was light intensity affected but also air temperature and relative humidity. For the purposes of the

experiments conducted here, three 'conditions' were used - 'sun', 'medium' and 'shade'. The differences between these are summarised in Table 9.1.

For each experiment the following procedure was adopted: the experimental plant was well watered, placed in a polythene bag, sealed and kept in a dark cupboard for four hours. At the end of this period the plant was removed from the cupboard and placed, still in the polythene bag, under a bright light source for approximately 30 minutes. Such a pre-treatment optimised the saturation of the leaves and ensured that the stomata were fully open. A single leaf was detached from the plant, immediately weighed on a top-pan balance to the nearest mg and placed on the experimental frame in the path of the 'wind'. The leaf was reweighed at two minute intervals, care being taken to cause little disturbance to the leaf during the weighing process. Leaf temperature was measured throughout the whole experiment using a copper/constantan thermocouple linked into a Comark Electronic Thermometer Type 1624. The output from this instrument was fed into a two-pen Servoscribe Chart Recorder to obtain a constant trace of leaf temperature. The second pen of the Servoscribe recorded air temperature similarly from a second thermocouple held at the same level but to one side of the leaf. The leaf thermocouple was held in a modified micromanipulator so that it could be lowered onto the leaf surface without unduly interfering with the leaf. Several times during the course of each experiment the relative humidity was measured using a clockwork psychrometer.

Figure 9.1 records the typical decline observed in leaf weight under these conditions. When phase CD of the graph had been reached, weighings were taken every five minutes for about twenty-five minutes.

At the end of the experiment a replica of the leaf was cut from Whatman's No. 1 filter paper, and this was weighed so as to calculate leaf area. It was then soaked in distilled water, the excess blotted off, the

	Air temperature	Relative Humidity	Light intensity	No. muslin layers
"Sun"	$28.80^{\circ}C \pm 0.74^{\circ}C$	$37.5\% \pm 4.0\%$	0.443 cals/cm^2/min	0
"Medium"	$22.86^{\circ}C \pm 0.77^{\circ}C$	$46.5\% \pm 4.2\%$	0.122 cals/cm^2/min	3
"Shade"	$16.30^{\circ}C \pm 0.78^{\circ}C$	$56.0\% \pm 4.7\%$	0.042 cals/cm^2/min	8

N.B. Light intensity (measured using a dome solarimeter) fluctuated slightly during the course of the experiments due to a window in the laboratory, but the variation was small.

TABLE 9.1 AVERAGE CONDITIONS FOR THE THREE EXPERIMENTAL TREATMENTS

FIGURE 9.1

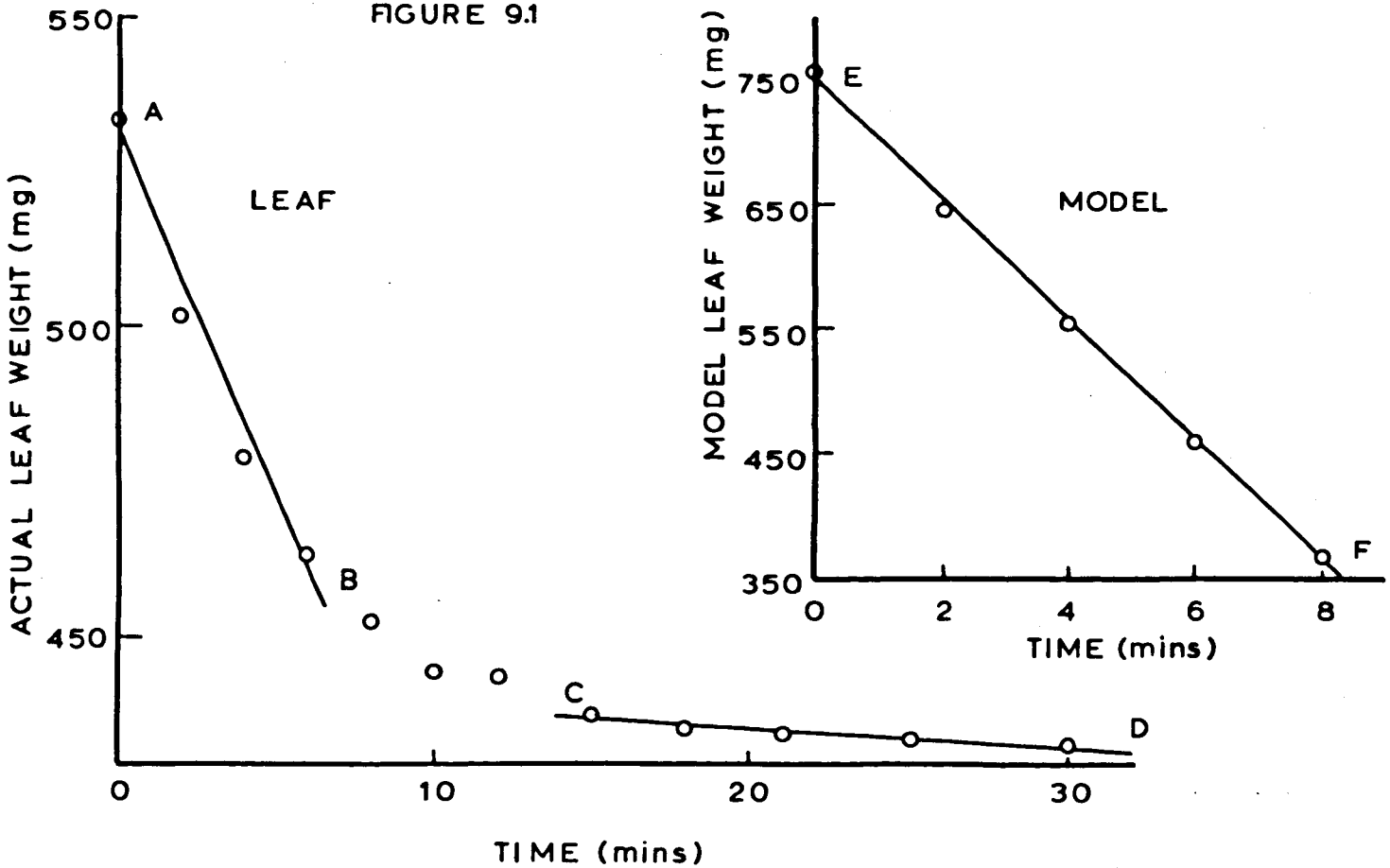


FIGURE 9.1 Decline in weight of a detached oak leaf and its corresponding filter paper model

model leaf weighed, and placed under the same conditions as experienced by the original leaf. This too was reweighed at two minute intervals, and its temperature and air temperature constantly monitored. Relative humidity was also measured during this period.

Seven different groups of leaves were used for experiments in this investigation. The seedlings grown under different light intensities described in Chapter 4 provided five groups, i.e. 100%, 75%, 50%, 25% and greenhouse seedlings, and adult branches of sun and shade leaves collected from woodlands and brought back and kept for short periods in water in a greenhouse provided the other two groups. All groups were represented by the two species, and for all groups the two species were examined under the three different environmental conditions of sun, medium and shade, using 20 leaves for each treatment. When changing conditions from 'sun' to 'medium' to 'shade', the experimental set-up took approximately 2-4 hours to equilibrate to the new conditions. Relative humidity and air temperature were constantly monitored throughout this period to determine when equilibrium had been reached.

Results

For each leaf and its corresponding model, graphs were drawn of weight decline against time (see Figure 9.1). Regression lines were fitted to the different phases of the graph AB, CD and EF and these used to calculate the different components of leaf resistance as described in Appendix 7. The following measurements have been subjected to a three-way analysis of variance in which the main effects are species, leaf types and treatments: total cuticular resistance, R_C, total boundary layer resistance, R_B, and stomatal resistance, R_S. These analyses are presented together with a list of means and standard deviations for these measurements and a least significant difference of means calculated from the

analyses of variance in Appendix 8.

Discussion

Generally, the resistances to water vapour loss were extremely high, and in the range expected of a thick, leathery leaf.

Boundary layer resistance (R_B) - Figure 9.2

Over all comparable treatments, the leaves of Q. petraea showed a higher boundary layer resistance. Leaf dissection is known to influence the R_B characteristics of leaves, such that narrowly dissected leaves have lower values (eg. Lewis, 1972). Since Q. robur has the more dissected leaf of the two species, it would be expected to have the lower R_B. The results also showed large differences between sun and shade leaves of the same species, the less dissected shade leaves having higher values. The rather poor dissection of the growth chamber seedlings was reflected in the higher values of R_B even for the seedlings from the greenhouse. The magnitude of R_B is determined by two important factors, wind speed and leaf geometry such that:

$$R_B = K \left(\frac{W}{V}\right)^{\frac{1}{2}}$$

Where K = constant
W = effective downwind width (m)
V = wind velocity (m/sec)

and it would appear in this case that since wind speed was constant for the range of experiments the differences were due to differences in leaf geometry. Slatyer (1967) has shown, for example, that a cotton leaf 10 cm wide would have an R_B at a wind speed of 10 cm/sec of 3 sec/cm whilst a grass leaf, 1 cm wide at the same wind speed would have an R_B of 1.0 sec/cm. Throughout the three treatments there were very few significant differences between comparable results suggesting that the different conditions of the treatments, i.e. air temperature, humidity and light intensity did not unduly affect R_B. Leaf hairiness may affect R_B by increasing its value,

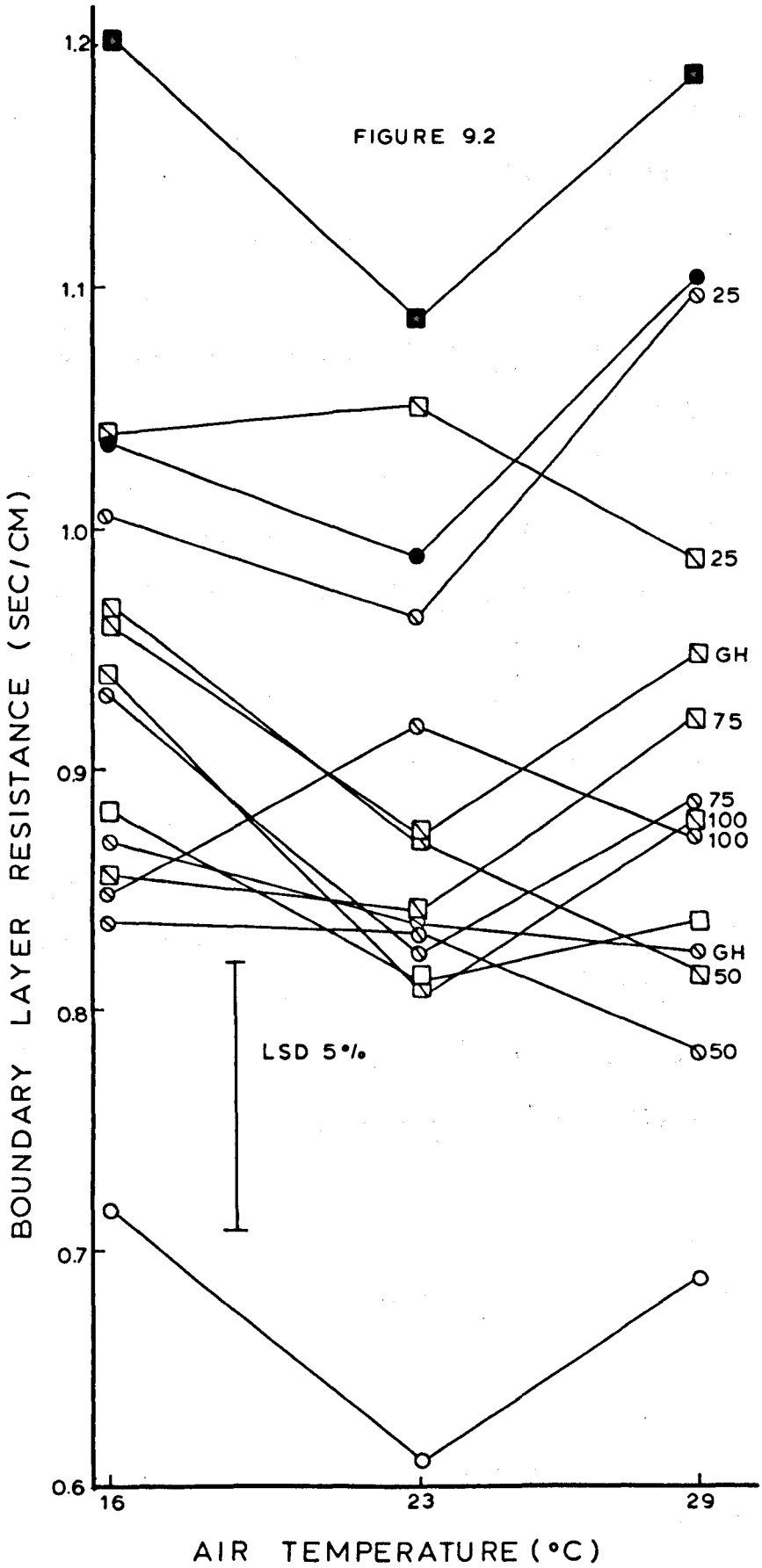


FIGURE 9.2 Boundary layer resistance to water loss from adult leaves (sun and shade), and seedling leaves (GH, 100, 75, 50, 25) at different environmental conditions for Q. robur and Q. petraea

LSD 5% = least significant difference of means at 5% level

○ Q. robur sun leaves
● Q. robur shade leaves
□ Q. petraea sun leaves
■ Q. petraea shade leaves
⊘ Q. robur seedling leaves
▨ Q. petraea seedling leaves

See Appendix 8 for standard deviations

but the differences recorded here, Q. petraea having the larger R_B, must be due to shape differences since the model leaves do not possess hairs. The presence of hairs on the actual Q. petraea leaf would serve only to increase the differences between the species. Very hairy leaves of Verbascum thapsus have been shown to lose more water and heat than hairless leaves of the same species (Wuenscher, 1970) and indeed as Gates (1962) points out heat loss from a leaf by convection is usually very high and this is limited by the boundary layer resistance. Vogel (1968, 1970) has investigated the loss of heat by convective cooling from model leaves and determined that loss from model sun leaves of Q. alba was greater under all conditions than loss from model shade leaves of the same species. Consequently, although R_B has a very small influence on water loss from the leaf in comparison to R_C and R_S, it is of paramount importance in regulating heat loss by convection.

Cuticular resistance (R_C) - Figure 9.3

The values for cuticular resistance would place both Q. petraea and Q. robur in the range of xerophytic plants (Holmgren et al. 1965). Q. robur sun leaves show higher values for R_C, greater than those of either Q. robur shade leaves or Q. petraea sun leaves. Generally, the shade leaves had lower R_C values than corresponding sun leaves and Q. robur leaves had higher R_C values than corresponding Q. petraea leaves. The differences between sun and shade leaves were also reflected in the results for the seedling leaves which showed a decreasing R_C as the light intensity under which the seedlings had been growing decreased. Seedling leaves too had generally lower values than adult leaves for R_C even when grown under greenhouse conditions. Variation in cuticular resistance is almost certainly related to cuticular structure, and possibly cuticular thickness, although as Cowan and Milthorpe (1968) point out, little information exists on this point. During the investigation of leaf

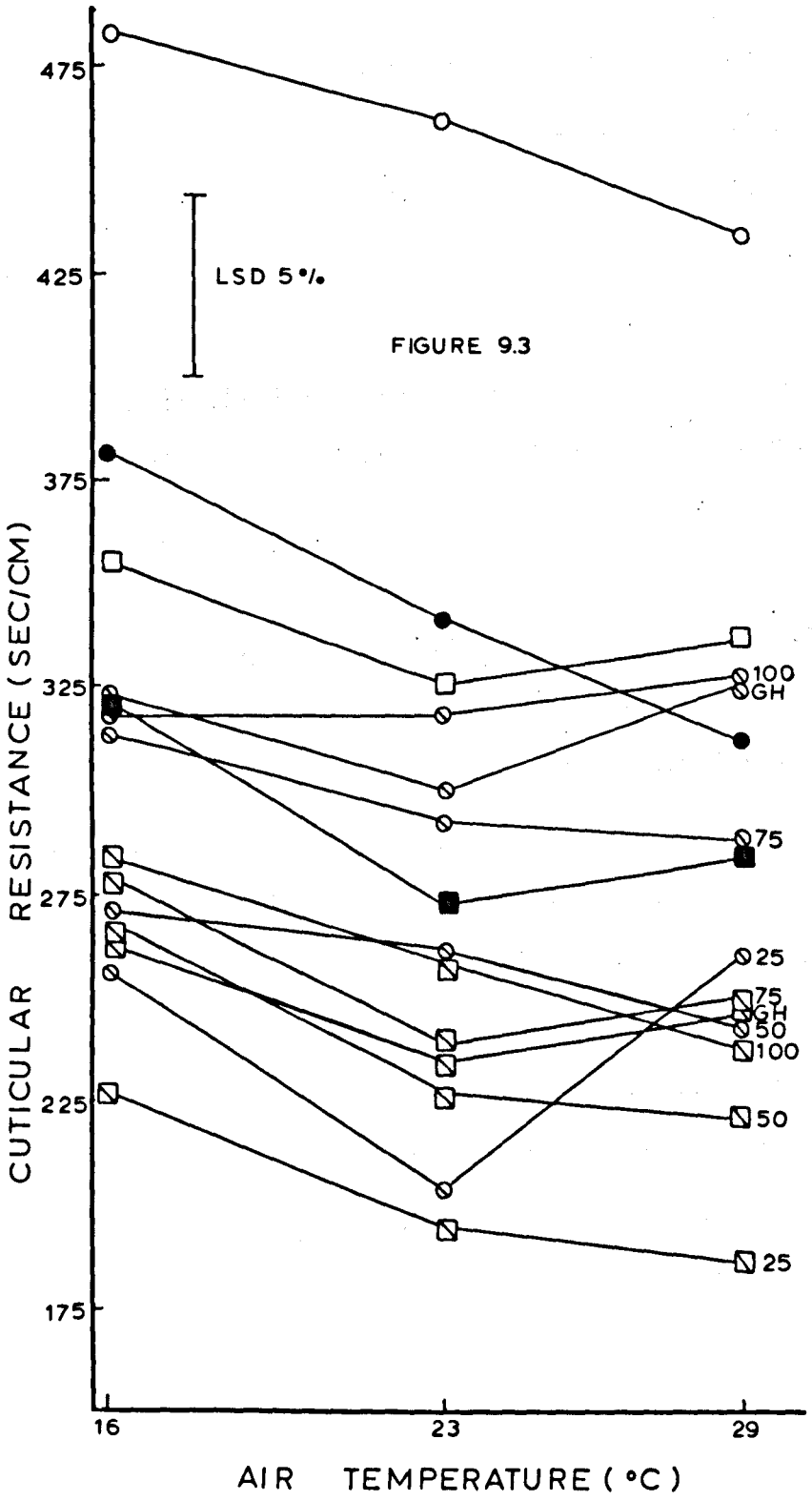


FIGURE 9.3 Cuticular resistance to water loss from adult leaves (sun and shade), and seedling leaves (GH, 100, 75, 50, 25) at different environmental conditions for Q. robur and Q. petraea

LSD 5% = least significant difference of means at 5% level

○ Q. robur sun leaves ● Q. robur shade leaves

□ Q. petraea sun leaves ■ Q. petraea shade leaves

⊘ Q. robur seedling leaves

⊠ Q. petraea seedling leaves

See Appendix 8 for standard deviations

anatomy in Chapter 2, no measurements were made of cuticular thicknesses, although some later work has suggested that there may be thickness differences between the cuticles of the two species by up to 20%, Q. robur having the thicker cuticle. The coriaceous Q. ilex was found by Larcher (1960) to have a higher cuticular resistance than Q. pubescens, a species comparable with Q. petraea, suggesting that the cuticular resistance differences between these species parallel those between Q. robur and Q. petraea.

Cuticular resistance was also found to vary significantly with changing treatments, the R_C being lowest under the 'sun' conditions. Holmgren et al.(1965) have shown a similar result for Lamium galeobdolon, Betula verrucosa and Acer platanoides which ranged from 59-87 sec/cm (Lamium), from 105-287 sec/cm (Betula), and from 286-556 sec/cm (Acer) over a five degree centigrade range 22°C to 17°C. The changes in Quercus are somewhat smaller for a species with resistances comparable to those of Acer. From medium conditions at an air temperature of about 23°C to shade conditions with an air temperature of about 16°C, R_C increased by amounts ranging from 0% to 25.8%, but an interesting species difference was that the average increase over this temperature range was 8.6% for Q. robur and 15.0% for Q. petraea suggesting that the possible shade Q. petraea had a cuticle more influenced by light and temperature. Since cuticles have a high component of cuticle waxes, high leaf temperatures are likely to affect the functioning of the cuticle in regulating water loss, and the results noted above would suggest that the cuticular structure of Q. petraea leaves are much more affected than Q. robur under high temperature conditions.

Stomatal resistance (R_S) - Figure 9.4

Values of stomatal resistance for both species are comparable with those recorded for other tree species (eg. Holmgren et al. 1965), and in

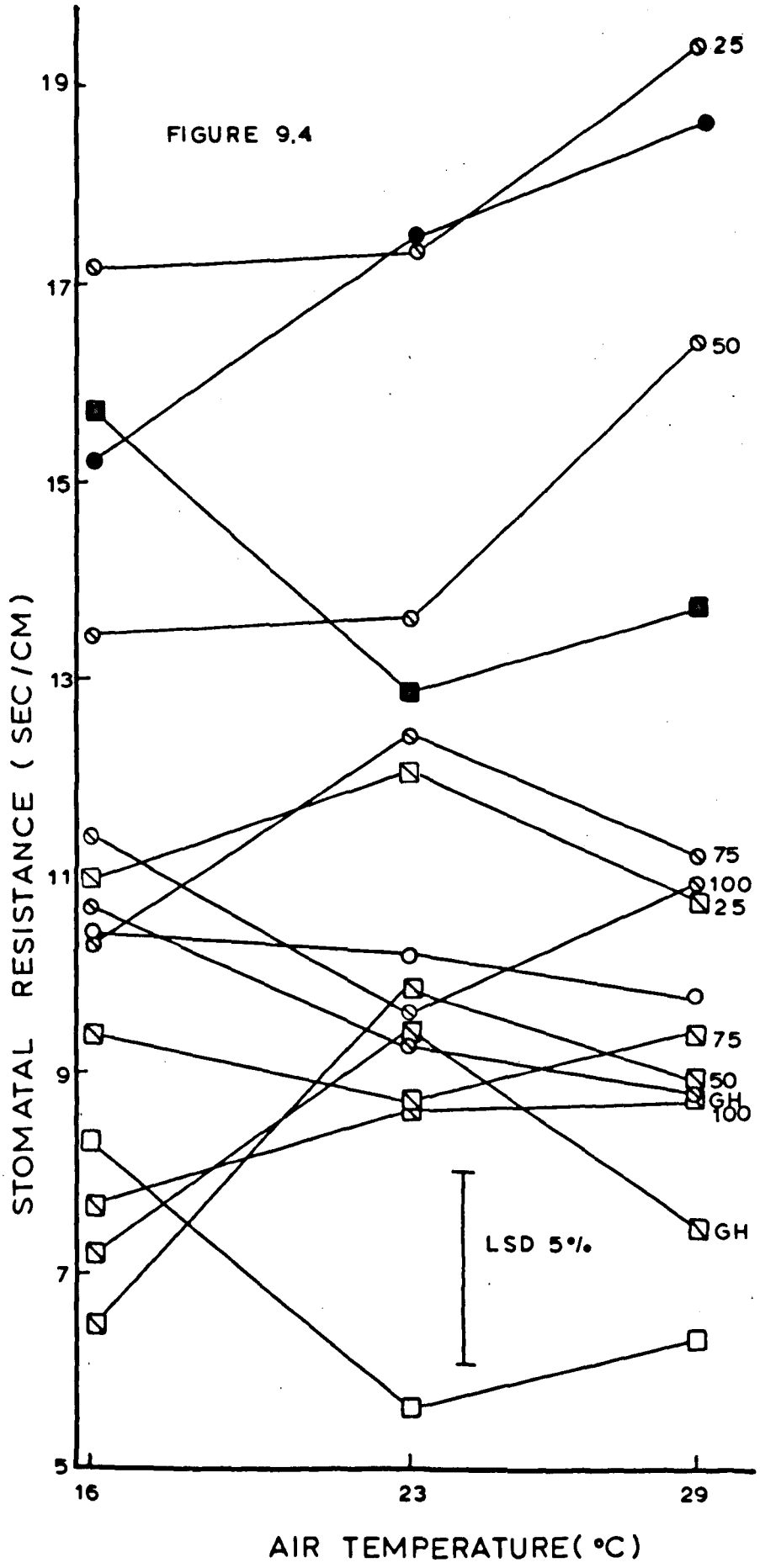


FIGURE 9.4 Stomatal resistance to water loss from adult leaves (sun and shade), and seedling leaves (GH, 100, 75, 50, 25) at different environmental conditions for Q. robur and Q. petraea
LSD 5% = least significant difference of means at 5% level

- ○ Q. robur sun leaves
- ● Q. robur shade leaves
- □ Q. petraea sun leaves
- ■ Q. petraea shade leaves
- ⊘ Q. robur seedling leaves
- ▨ Q. petraea seedling leaves

See Appendix 8 for standard deviations

excess of values for more herbaceous plants, eg. Helianthus annuus 0.45 sec/cm. The stomatal resistance of Q. robur was generally higher than that of Q. petraea but the shade leaves of both species were also higher for R_S than corresponding sun leaves, a somewhat perplexing result. Lewis (1972) noted that populations of Geranium sanguineum produced leaves with lower R_S values under Alvar and Steppe conditions (i.e. exposed conditions) than populations from woodland conditions. The measurements calculated here are 'open-stomata resistances', which are different from the resistance of the stomata when closed. It must be supposed that the resistances of closed stomata are infinite, and this would seem a not unreasonable assumption since Slatyer and Jarvis (1966) failed to detect N_2O movement through a cotton leaf when the stomata were closed.

Stomatal resistance is inversely proportional to the size and frequency of the pores (Cowan and Milthorpe, 1968), and consequently for pores of equal size, stomatal resistance is inversely proportional to frequency. In Chapter 2 it was shown that the leaves in sun conditions had a higher density of stomata than comparable shade leaves, and also that Q. robur leaves had a higher density than Q. petraea, which would lead to a higher resistance in shade and Q. petraea leaves. The results presented here confirm the larger resistance shown by shade leaves, but the stomatal resistance of Q. robur is greater than that of Q. petraea. It should be noted, however, that comparable frequencies can only be used if the pore size of the stomata, and other anatomical features of the stomata of the two species are identical. No information exists on this point, but it would seem a reasonable explanation for the results obtained that pore sizes and other stomatal parameters are different between the species. (It is hoped to study the anatomical features of the stomata of these two species shortly using scanning electron microscope techniques.)

One further point concerning stomatal resistance is the concavity of leaves of Q. petraea observed in certain Welsh populations. Leaves of these populations are concave in shape, not flat, so that the under-surface of the leaf with the stomata comes to lie inside the concavity, just as the leaves of Ammophila arenaria roll in dry conditions to enclose the stomata. These leaves, however, appear to be permanently concave. Since the stomatal resistance of the Q. petraea leaves is small (compared to Q. robur), this would appear to be a modification to gross leaf morphology to increase the boundary layer resistance and possibly influence stomatal resistance. This curvature of leaves has never been observed in Q. robur by the present author.

Rijtema (1965) has shown that under conditions of changing light intensity, stomatal resistance increases with decreasing light intensity. A similar response was observed in adult oak leaves, with the exception of Q. robur shade leaves, but not in seedling leaves.

The results presented here would support the contention that Q. robur has generally sun leaves and Q. petraea shade leaves. When the stomata are closed, the cuticle is the largest barrier to water loss, the resistance being up to 500 x that of the boundary layer, and therefore under stress conditions when the stomata are closed Q. robur will have the higher resistance. This is a somewhat paradoxical situation, since Q. robur is generally found on the wetter, moisture retaining soils where the tree would possibly not experience water stress conditions, but it also occurs more in the east of Britain where rainfall is lower and this might be an important factor. With the stomata open, the leaves show little resistance to water loss as can be seen by comparing loss at this time with loss from a freely evaporating model leaf - some leaves losing up to 25-30% of the water lost by the corresponding freely evaporating model.

Consequently, factors likely to be important in influencing water stress, eg. geographical position, soil type, drainage, slope, temperature, wind speed, humidity, etc. may also be important in determining the species to be found in a given area. The distribution of the hybrid is also likely to be influenced by such factors, including spatial considerations of the hybrid inside populations. The outer parts of a woodland are under a more exposed environmental regime where wind speed is greater, light penetration greater due to a sparser foliage and humidity lower due to the effects of wind, light and temperature, and therefore there will be gradients of environmental parameters from the woodland periphery to its centre. The hybrid, if it is intermediate for leaf resistance values, may also be adapted to a 'hybrid habitat' or indeed to specific parts of the woodlands of the two parental species.

CHAPTER TEN

COMPARATIVE GROWTH ANALYSIS STUDIES

Introduction

Growth analysis is a convenient method of assessing the performance of a plant under a variety of environmental regimes. Callaghan and Lewis (1971) have used the techniques of growth analysis to study the performance of the tetraploid Phleum alpinum L. under a series of contrasting habitat types on South Georgia. The effect of shading on Impatiens parviflora has also been investigated by Evans and Hughes (1961) and Hughes and Evans (1961) using growth analysis as an estimate of performance. Within tree species several parallel studies exist. For example, Loach (1970) investigated the growth parameters of the seedlings of five tree species grown under a range of light intensities from 3-100% daylight. Gordon (1969) used similar methods in an investigation of the effect of light intensity on yellow birch (Betula alleghaniensis) seedlings.

Some growth analysis work has been carried out on the two British oak species. Ovington and Macrae (1960) used seedling dry weight as an estimate of growth of Q. petraea seedlings grown under a variety of conditions differing in soil type and cover. A parallel experiment using different soil types and different levels of cover but with Q. robur seedlings has recently been reported by Karpisonova (1971).

The importance of growth analysis studies lie in their ability to assess the performance of the species/population/ecotype etc. under a range of conditions and therefore estimate to what degree the organism is able to adapt. Section 2 considered adaptation taking place at the morphological and anatomical level, but growth analysis, since it is concerned with the efficiency of photosynthesis and the partitioning

of photosynthetic products to the different plant organs is a measure of physiological adaptation. Jarvis (1964) completed an important study on the adaptability of Q. petraea seedlings to different levels of light intensity. His researches covered growth experiments under different degrees of shading, the measurement of photosynthesis under laboratory conditions and field experiments to determine the importance of light in seedling growth and survival. His experiments did not, however, seek to compare the performance or survival of Q. petraea and Q. robur. Only the work of Plaisance (1955) attempted a comparative growth study of the two oak species. He was able to show that in one year old seedlings, root length, stem diameter and total plant weight were all a maximum for Q. robur under 100% light intensity, but for Q. petraea these parameters reached a maximum at light intensities below 100%. Such a difference again reflects the possibility that Q. robur is a 'sun' plant and Q. petraea is a more 'shade' tolerant species.

This chapter reports the adaptations of seedlings of Q. petraea and Q. robur to different light intensities using the techniques of growth analysis.

Method

Growth analysis for a tree species can be calculated over one growing season or over a shorter period when photosynthetic activity might be considered maximal. Since the object of this study was a comparative investigation, it was felt that the latter would provide more useful information. The methods follow very closely therefore the methodology used by Jarvis (1964) when studying similar short-term growth analysis of Q. petraea seedlings, the major difference being that the experiment performed by Jarvis was conducted under field

conditions, with the seedlings sown directly into the soil; in this experiment, the seedlings were under controlled growth chamber conditions.

The light intensities used for the final growth chamber experiment reported in Chapter 4 were utilised for this experiment, the range of intensity being from 2-21% daylight (i.e. 10-100% growth chamber light). The conditions of the growth chamber were 15°C, 95% relative humidity and a 16 hour light/8 hour dark day.

Thirty acorns of each species were sown under each light intensity, one per 4½" diameter black polythene pot filled with John Innes No. 2 compost. Following Jarvis (1963), the acorns were of a more or less uniform weight and had been collected during Autumn 1970 from Uffmoor Wood and the Wyre Forest. The acorns were sown on 10th January 1971 after being stored for three months in damp Sphagnum sp. moss at 5°C. Some acorns failed to germinate, and consequently in order to keep sample sizes equal and therefore not complicate the analysis of results, the number of seedlings of each species under each light intensity was reduced to twenty. Jarvis (1964) sampled his first harvest of seedlings after six weeks, when all initial leaf expansion had been completed, but leaf expansion under growth chamber conditions was slower and the first harvest was not therefore taken until the seedlings were eight weeks old. On each seedling, the following parameters were recorded:

Cotyledon dry weight - mg (including the acorn shell) C_1

Stem dry weight - mg (excluding leaf petioles) S_1

Root dry weight - mg - since the seedlings were grown in compost, cleaning the roots of soil particles proved difficult, but the long main tap root with few side branches helped as there was little fibrous root growth R_1

Total leaf dry weight - mg (including leaf petioles) L_1

Total leaf area - cm^2 - estimated by printing the leaf outlines onto ammonia-developed Diazo paper as described in Chapter 2 A_1

Total number of leaves per seedling N_1

Ten seedlings of each species under each light intensity were sampled at this time, the ten remaining seedlings of each species/treatment were kept under the growth chamber conditions. These were harvested three weeks later, and the above parameters were measured on this second batch of seedlings. These have been designated C_2, S_2, R_2, L_2, A_2 and N_2 corresponding to the characters above.

Results

a) Derivation of secondary growth parameters

The following indices of growth performance were derived from the raw data:

Root/stem weight ratio	R/S	no units
Leaf area ratio (LAR)	A/W	cm^2/g
Specific leaf area (SLA)	A/L	cm^2/g
Mean leaf area (MLA)	A/N	cm^2/leaf
Total dry weight (W)	C+S+R+L	mg

These parameters were calculated for each of the two seedling harvests. The following parameters were estimated for the growth period, at the end of the experiment:

Dry weight increment: $W_2 - W_1$ mg

Leaf area increment: $A_2 - A_1$ cm^2

Net assimilation rate E: $\frac{2 \times (W_2 - W_1)}{(A_2 + A_1)(t_2 - t_1)}$ after Coombe (1960)

where t_1 = time of first harvest

t_2 = time of second harvest

Relative growth rate G: mean A/W x E
after Blackman and Wilson (1951).
where mean $A/W = (A_1 + A_2)/(W_1 + W_2)$ (Jarvis, 1964)

b) Analysis of results

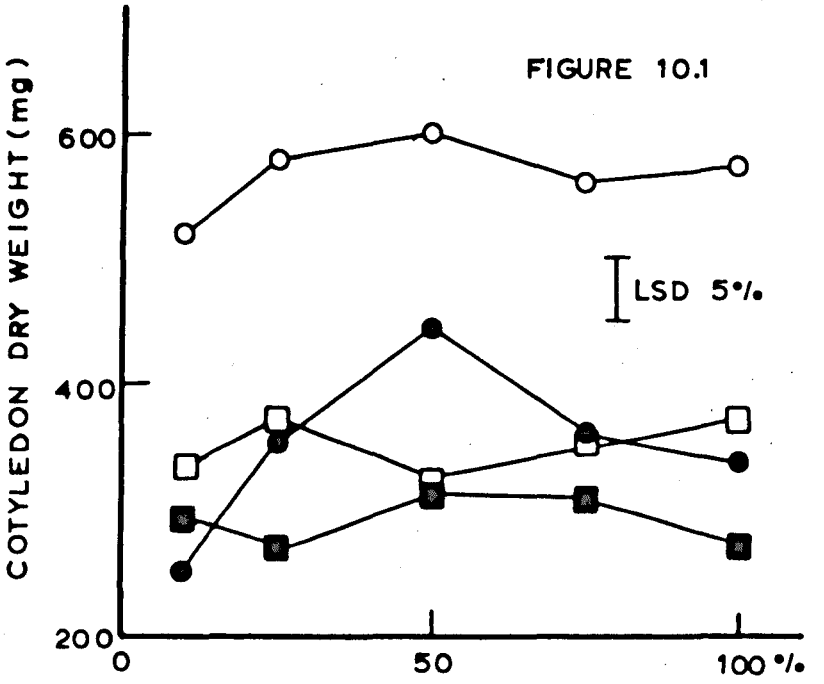
The analysis of the results and tabulated means and standard deviations are presented in Appendix 9. For those parameters which were measured at both the first and second harvest, i.e. all the above parameters excluding dry weight increment, leaf area increment, E and G, a three-way analysis of variance is presented in which the main effects are species, light intensities and harvests. Those parameters estimated only at the final harvest have been subjected to a two-way analysis of variance in which the main effects are species and light intensities. A least significant difference of means has been calculated from both the three and two-way analyses of variance and these are presented along with each analysis.

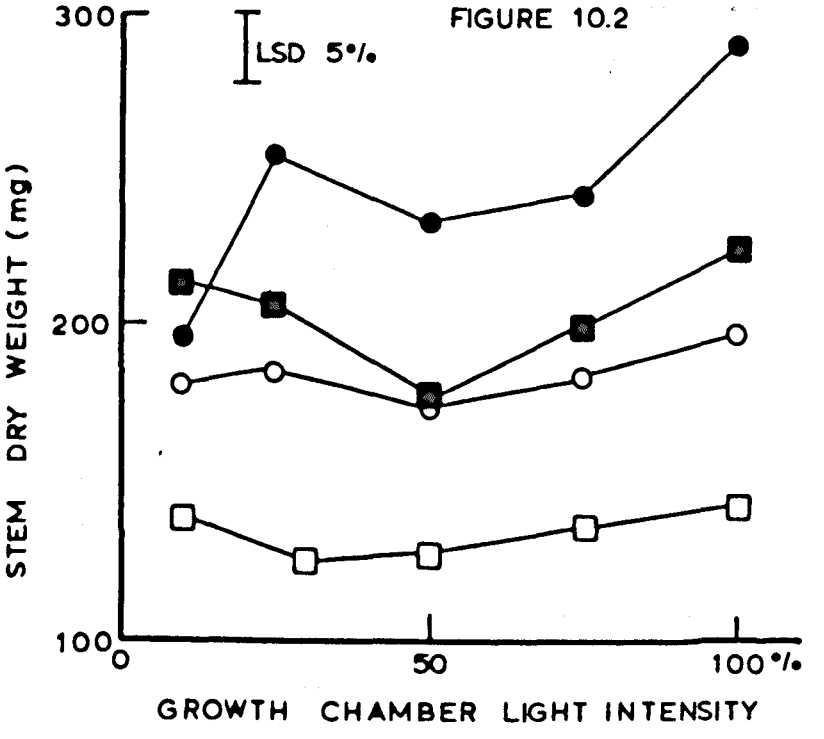
The response of the indices of growth performance is graphed in Figures 10.1 to 10.15.

Discussion

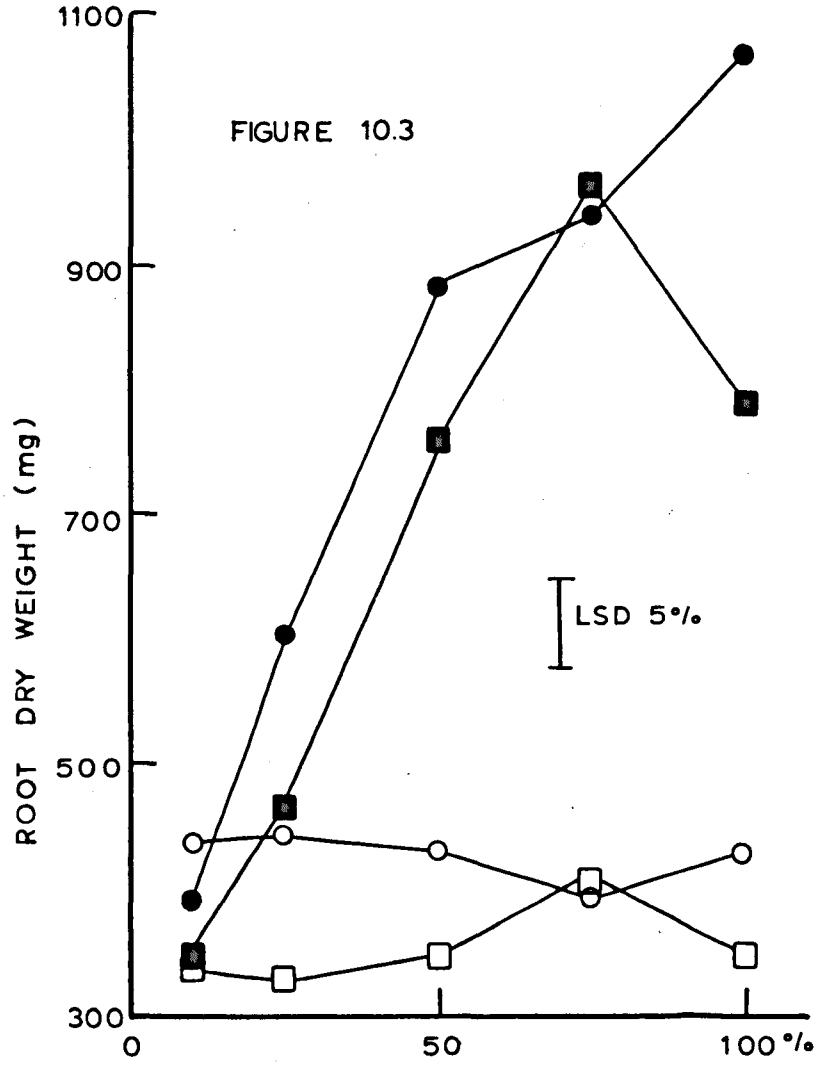
Since many of the individual components of growth, i.e. the dry weights of individual plant parts, show interesting and instructive differences between species and light intensities, the discussion will include reference to these individual components of growth.

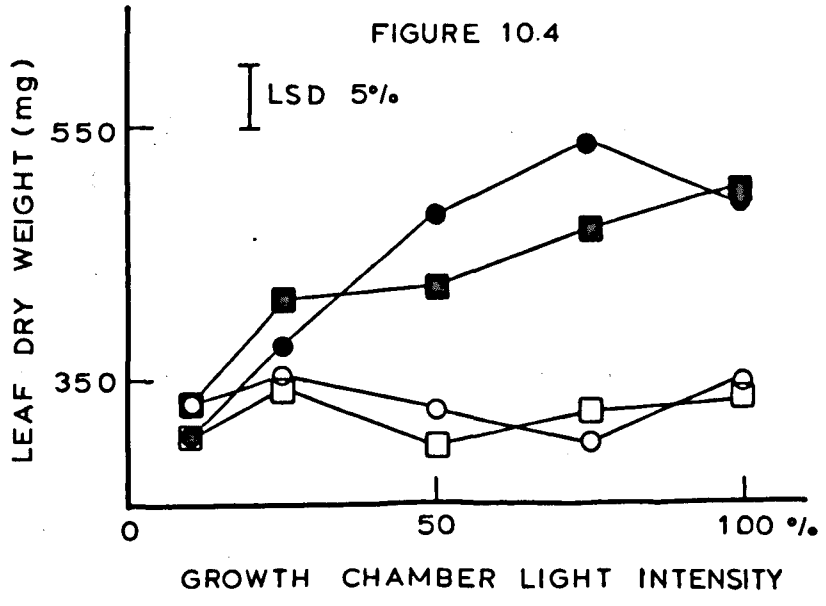
Total plant weight (Figure 10.5) increased through the three weeks of the experiment, most seedlings under the 100% light increasing in weight by approximately 45%. Under the lower light intensities, however, increases were not so great and indeed the 10% Q. robur seedlings had a negative dry weight increment at the end of the experiment (Figure 10.12). A large percentage of the increase in dry





FIGURES 10.1 and 10.2 Growth analysis of oak seedlings under different light intensities; Cotyledon weight and Stem weight respectively
(○ Q. robur Harvest 1; ● Q. robur Harvest 2
□ Q. petraea Harvest 1; ■ Q. petraea Harvest 2)
LSD 5% = least significant difference of means at the 5% level
See Appendix 9 for standard deviations





FIGURES 10.3 and 10.4 Growth analysis of oak seedlings under different light intensities; Root weight and Leaf weight respectively
(○ Q. robur Harvest 1; ● Q. robur Harvest 2
□ Q. petraea Harvest 1; ■ Q. petraea Harvest 2)
LSD 5% = least significant difference of means at 5% level. See Appendix 9 for standard deviations.

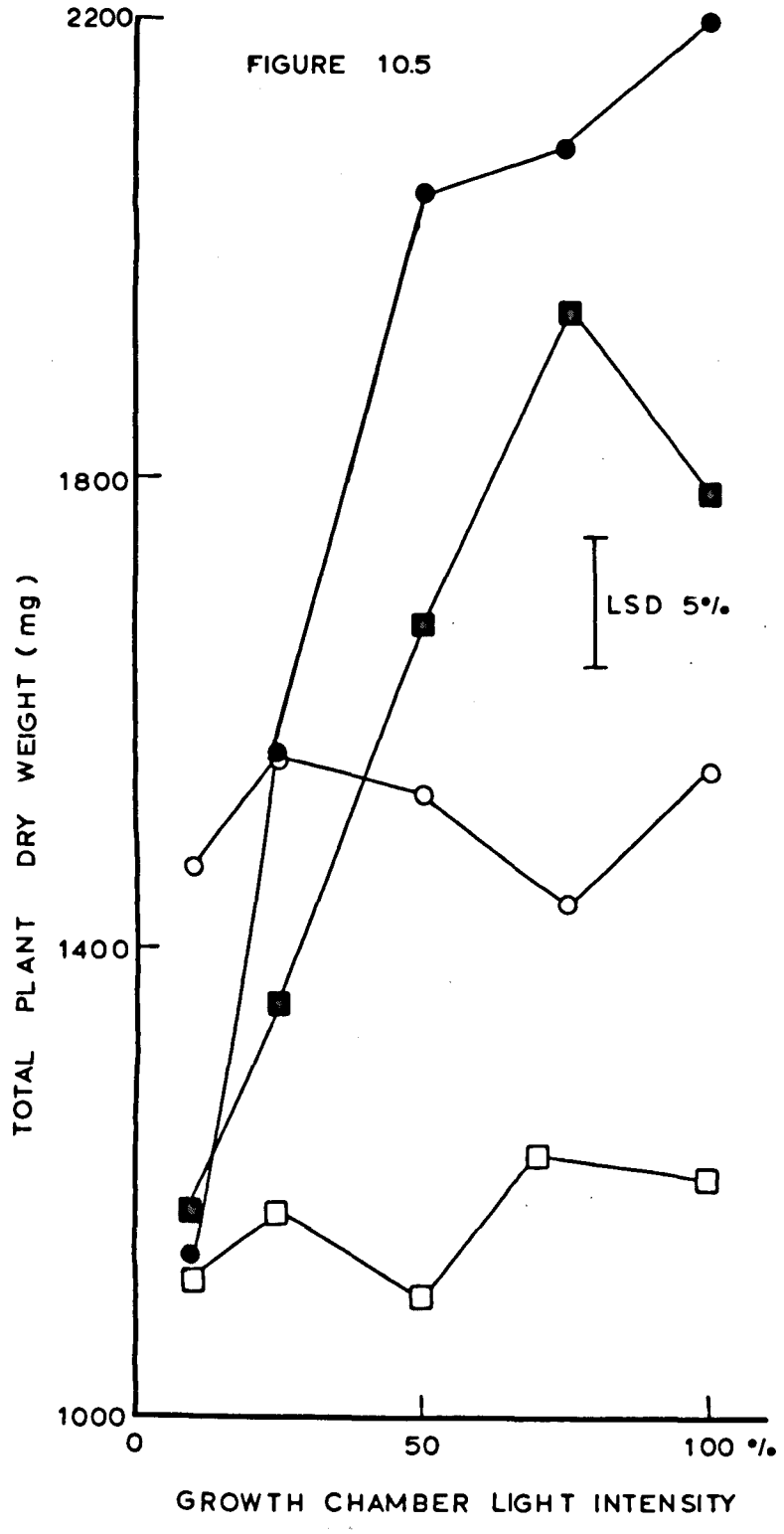


FIGURE 10.5 Growth analysis of oak seedlings under different light intensities; Total plant weight
(○ Q. robur Harvest 1; ● Q. robur Harvest 2
□ Q. petraea Harvest 1; ■ Q. petraea Harvest 2)
LSD 5% = least significant difference of means at 5% level.
See Appendix 9 for standard deviations.

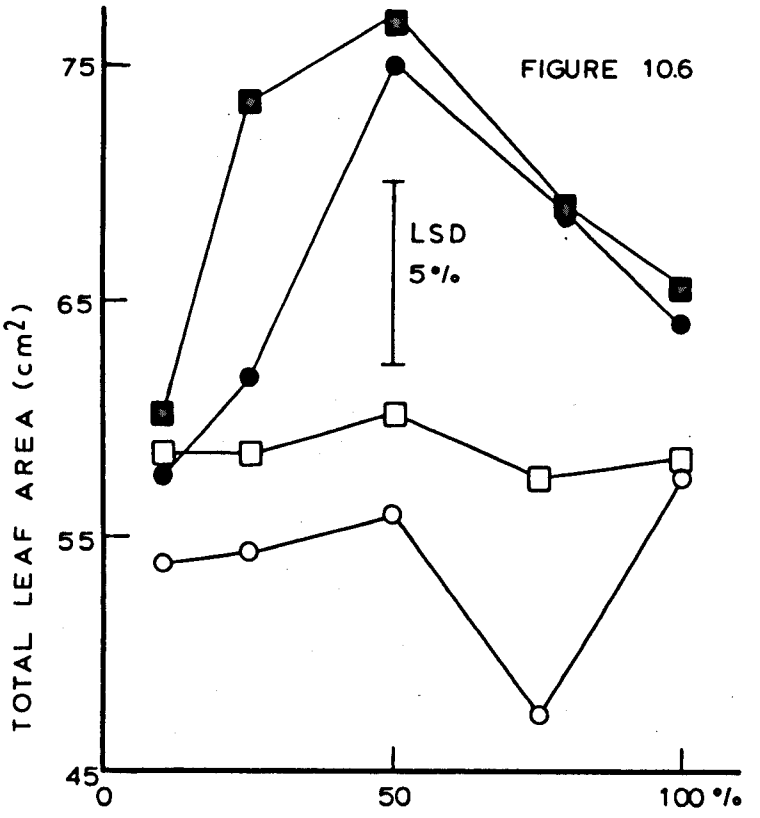
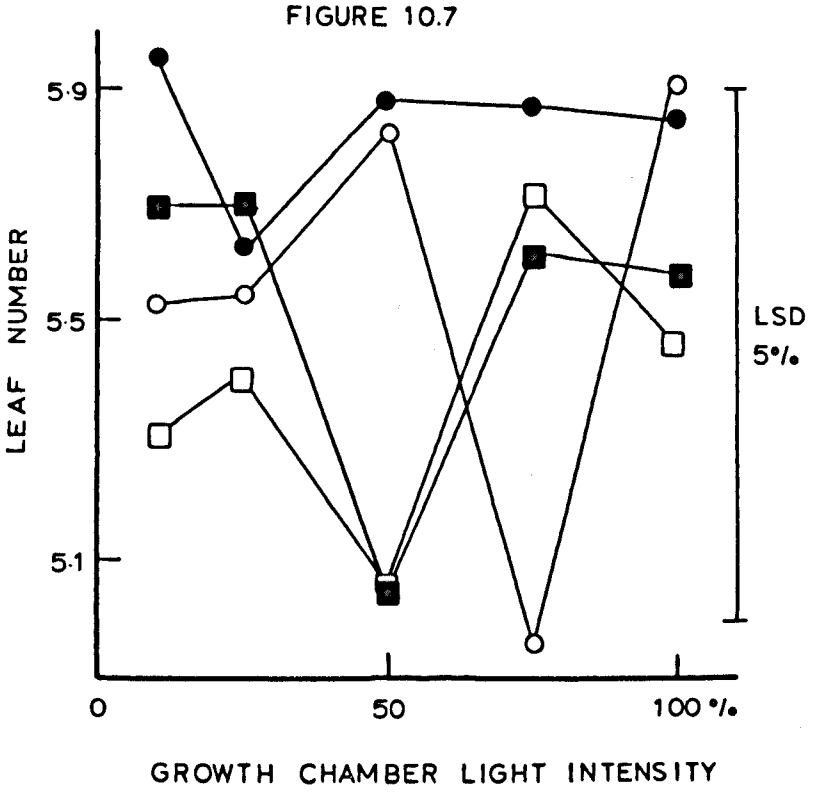
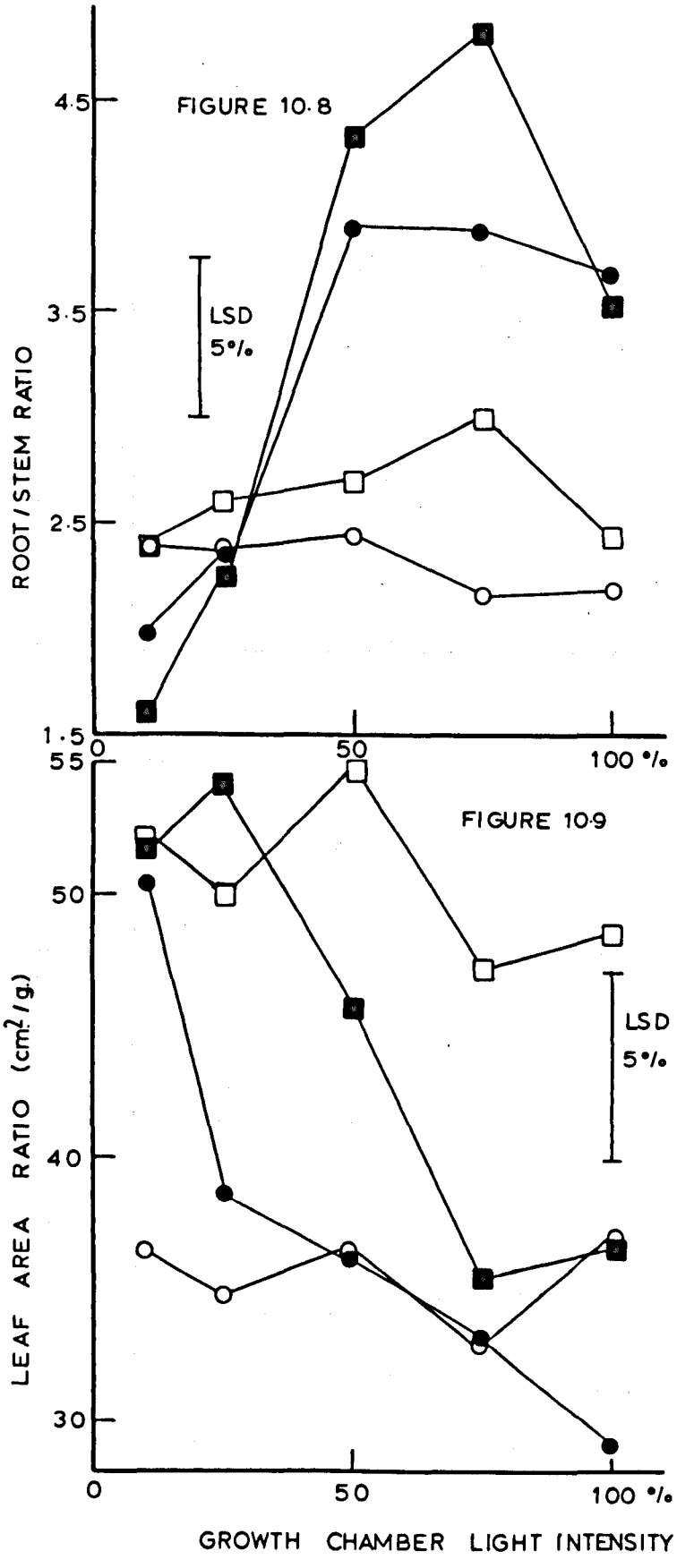


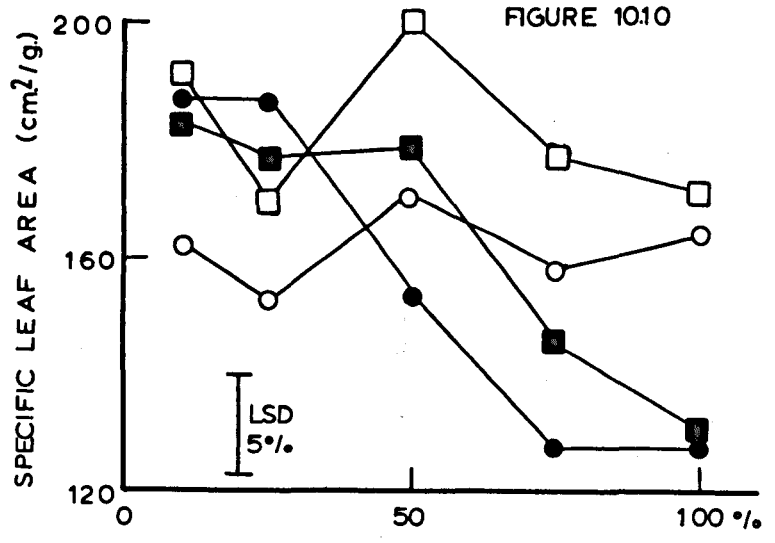
FIGURE 10.7

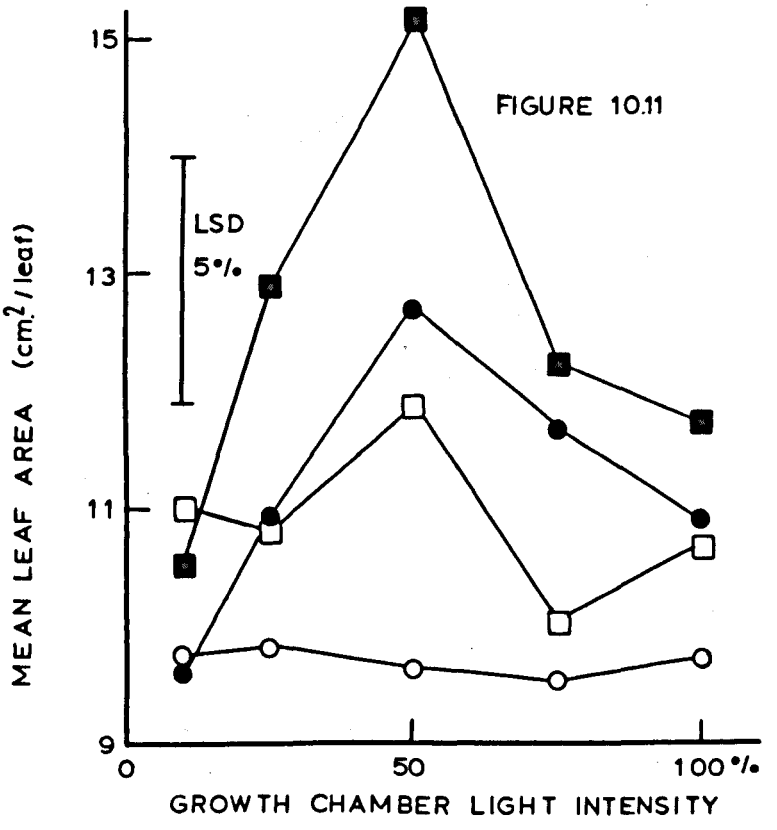


FIGURES 10.6 and 10.7 Growth analysis of oak seedlings under different light intensities; Total leaf area and total number of leaves per plant respectively
(○ Q. robur Harvest 1; ● Q. robur Harvest 2
□ Q. petraea Harvest 1; ■ Q. petraea Harvest 2)
LSD 5% = least significant difference of means at 5% level. See Appendix 9 for standard deviations.

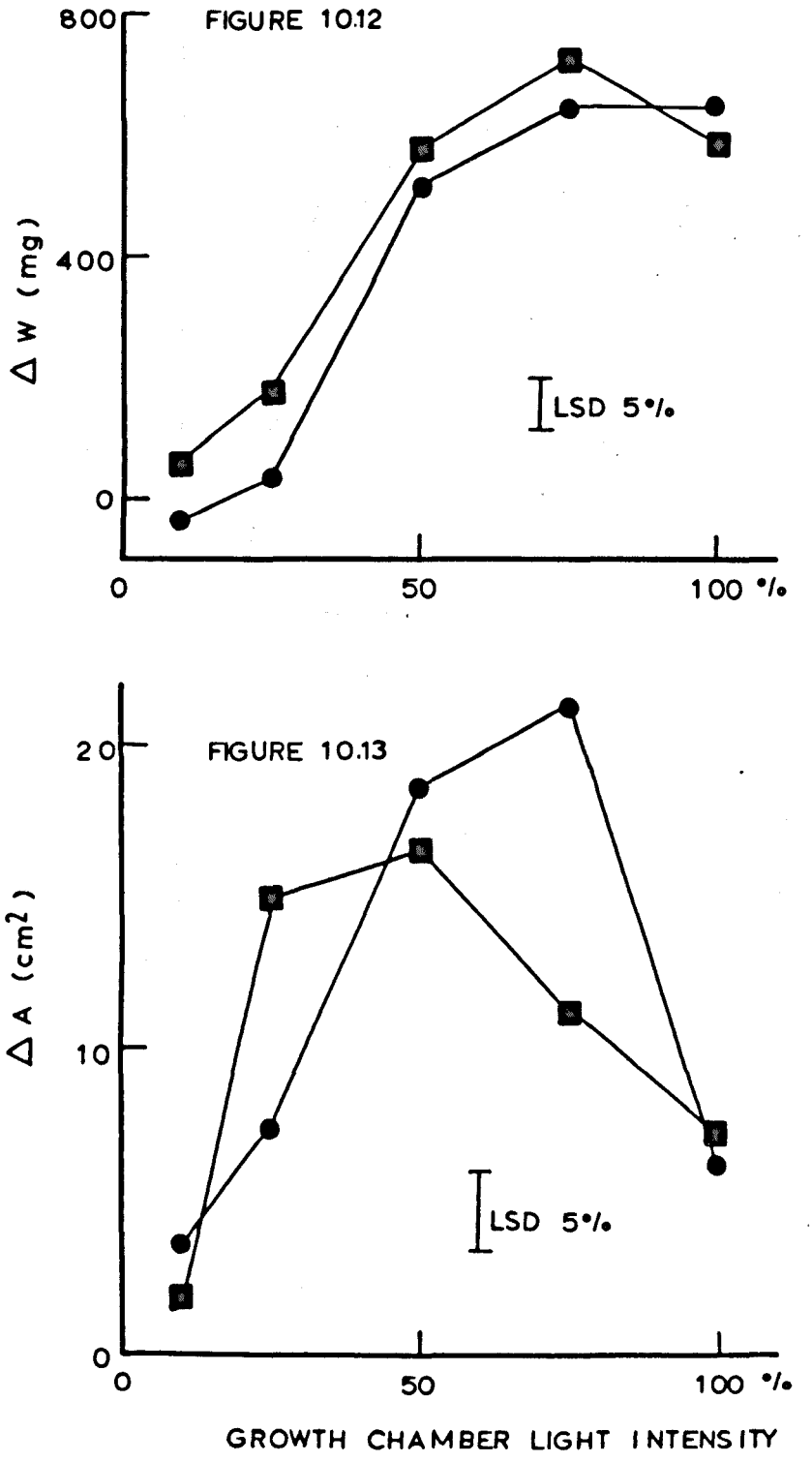


FIGURES 10.8 and 10.9 Growth analysis of oak seedlings under different light intensities; Root/Stem weight ratio and Leaf area ratio respectively
(○ Q. robur Harvest 1; ● Q. robur Harvest 2
□ Q. petraea Harvest 1; ■ Q. petraea Harvest 2)
LSD 5% = least significant difference of means at 5% level. See Appendix 9 for standard deviations.

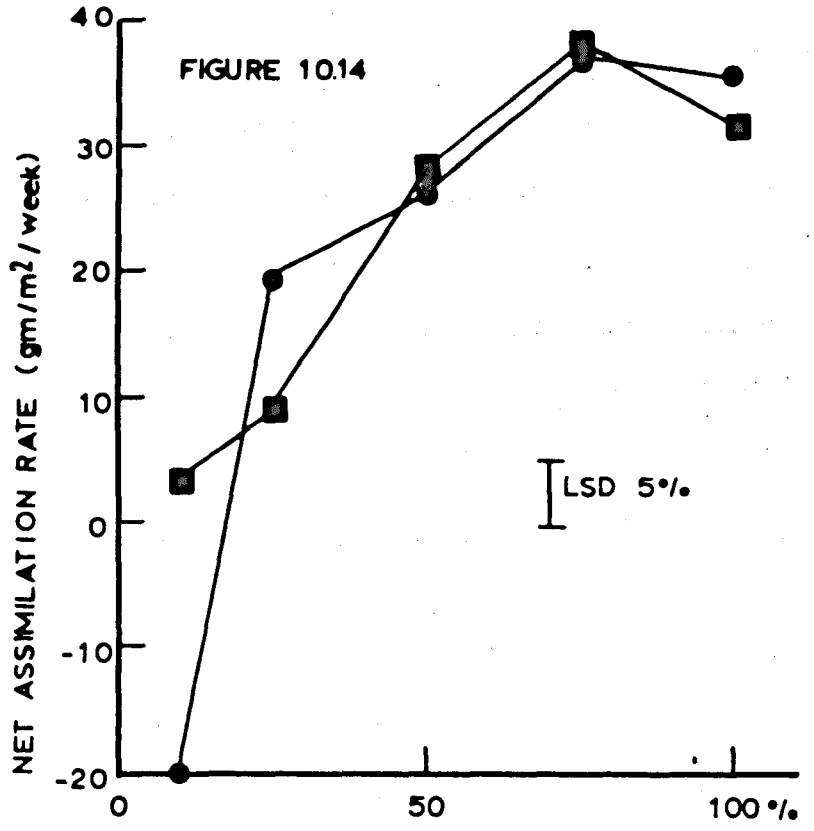


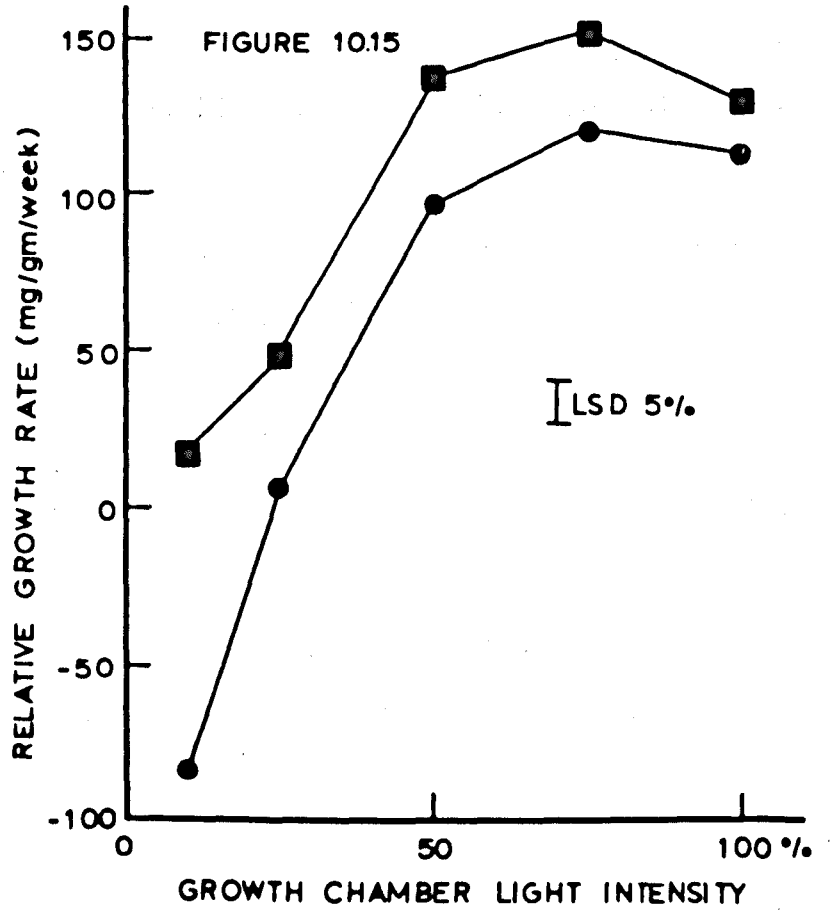


FIGURES 10.10 and 10.11 Growth analysis of oak seedlings under different light intensities; Specific leaf area and Mean leaf area respectively
(○ Q. robur Harvest 1; ● Q. robur Harvest 2
□ Q. petraea Harvest 1; ■ Q. petraea Harvest 2)
LSD 5% = least significant difference of means at 5% level. See Appendix 9 for standard deviations.



FIGURES 10.12 and 10.13 Growth analysis of oak seedlings under different light intensities; Change in plant weight and change in leaf area over the two harvests respectively.
(● Q. robur; ■ Q. petraea)
LSD 5% = least significant difference of means at the 5% level
See Appendix 9 for standard deviations





FIGURES 10.14 and 10.15 Growth analysis of oak seedlings under different light intensities; Net Assimilation Rate and Relative Growth Rate respectively
(● Q. robur; ■ Q. petraea)
LSD 5% = least significant difference of means at the 5% level
See Appendix 9 for standard deviations

weight over the growth period at the higher light intensities was due to exceptional increases in root dry weight (Figure 10.3), which increased on average 84.6% over the three-week period. Increases in stem and leaf dry weights were more modest, stems increasing by 41.5% on average, and leaves by 33.0% (Figures 10.2 and 10.4 respectively). At the lower light intensities, root increment was virtually zero or negative (in the case of Q. robur). Q. petraea appeared to cope better with the very low light intensities - at 10% light intensity, stem weight increased by 54%, root weight by 1% and leaf weight by 8%, whilst at the same light intensity for Q. robur, stem weight increased by 8%, root weight decreased by 11% and leaf weight decreased by 7.5%. The seedlings of Q. robur appeared to be making more use of the reserves stored in the cotyledons to supplement photosynthates since at the lower light intensities, cotyledon weight declined by 53% (521 mg to 247 mg) whilst the cotyledons of Q. petraea declined during the same period by only 12.5% (336 mg to 294 mg). At the higher light intensities too, Q. robur utilised more of the dry weight of the cotyledons than Q. petraea - Q. robur 41%, Q. petraea 27% (Figure 10.1).

The seedlings of Q. robur produced slightly more leaves on average than Q. petraea (Figure 10.7) but this was not significant ($F = 2.63$, $P = 0.20\text{-}0.05$). Total leaf area (Figure 10.6) did, however, show significant differences between the species ($F = 11.15$, $P = 0.01\text{-}0.001$). These differences are reflected in the mean leaf area, i.e. A/N (Figure 10.11). Both species showed the highest mean leaf area at 50% light intensity, except the first Q. robur harvest, where it occurred at 25% light intensity. These results compare well with those of Jarvis (1964) who found that the maximum mean leaf area occurred at 34% light intensity. However, ambiguity in Jarvis' paper make direct comparisons difficult. His experiment was set up in an

enclosure at Padley Wood, near Sheffield, and in his experiment, he used shaded screens to produce the different light intensities. These he recorded as relative light intensities, the frames without screening being 100%, those with screening down to 10%, but these he describes as being 100%, 54%, 10%, etc. of daylight. However, since the screens were set up under the forest canopy they could not represent light intensities relative to open conditions (eg. Chapter 2) since the frames with no screening represented 100% light. The 100% light intensity used probably represented, therefore, the average light intensity on the forest floor, which has been estimated at between 17% and 24% (Fairbairn, 1954). Consequently, the highest light intensity would represent approximately the highest used in this investigation and the lowest also the same as the present investigation. Although the highest mean leaf areas were recorded at 50% light, the largest increase in leaf area, i.e. $A_2 - A_1$ (Figure 10.13) for Q. robur was at 75% and for Q. petraea at 50%.

Specific leaf area (A/L) increased with decreasing light intensity in both species (Figure 10.10) although the increase appeared more noticeable in the Q. robur leaves. An increase in specific leaf area suggests either an increase in leaf area or lighter and therefore possibly thinner leaves, a response noted in Chapter 4 for seedling leaves under low light intensities. Leaf area ratio (A/W), since it relates leaf area to total plant weight is a measure of the leafiness of the plant, and this too increased with decreasing light intensity, i.e. at the lower light intensities the seedlings were producing more leaf area per unit weight of plant, possibly to make more efficient use of the low light intensity available (Figure 10.9). One species difference here, however, was the initial difference between the leaf area ratios, that of Q. petraea being much higher

initially at all light intensities than that of Q. robur. During the three weeks of the experiment, however, LAR of the Q. petraea high light intensity seedlings fell due to a large increase in total plant weight, whilst at the same light intensity, Q. robur seedlings remained static, but increased greatly at the lower light intensities, due mainly to total plant weight loss at the low light conditions. Therefore, although Q. petraea is able to function and adapt to high light intensities, it would appear that the seedlings of Q. robur cannot function well and not adapt to low light conditions, a conclusion that can also be derived from the data of Plaisance (1955).

Net assimilation rate (E) of both species under high light intensities was high, and of the same order of magnitude (Q. robur 35.5 g/m^2/week, Q. petraea 31.5 g/m^2/week), but at lower light intensities, differences appeared (Figure 10.14). At 10% light intensity, although it had fallen in both species, Q. petraea still had a positive E, 3.4 g/m^2/week, whilst Q. robur had a negative E of -19.9 g/m^2/week. Again this suggests differences between the species in their ability to cope with shade conditions. This is also reflected in total plant relative growth rate (Figure 10.15) where Q. robur at 10% light intensity had a negative relative growth rate, and a very low relative growth rate at 25% light, in comparison with Q. petraea which remained positive and high throughout all light intensities. These differences are also apparent if the total plant relative growth rate is partitioned between cotyledon, root and shoot growth rates (see Table 10.1). At the low light intensity, Q. petraea maintained a high level of shoot production (approximately 50% that of the 100% light intensity), but the level of root production fell off very rapidly. Both root and shoot relative growth rates were negative at the lowest light intensity for Q. robur, but the roots again were the organ that appeared to be sacrificed. At these low

Relative growth rate (mg/g/week)		Relative Light Intensity (%)				
		100	75	50	25	10
Cotyledons	Q. robur	- 41.3	- 38.1	- 29.1	- 47.5	- 70.1
	Q. petraea	- 22.3	- 9.3	- 2.9	- 27.5	- 12.2
Shoots	Q. robur	43.2	56.6	40.3	20.1	- 2.5
	Q. petraea	54.9	44.3	41.7	39.4	28.5
Roots	Q. robur	113.2	103.2	85.2	34.6	- 12.3
	Q. petraea	99.8	116.8	98.7	36.4	1.2
Ratio						
Root RGR	Q. robur	2.62	1.82	2.11	1.73	4.85
Shoot RGR	Q. petraea	1.82	2.63	2.36	0.92	0.04

TABLE 10.1 INDIVIDUAL COMPONENTS OF THE RELATIVE GROWTH RATES OF OAK SEEDLINGS

light intensities, Q. robur was also utilising the reserves from the cotyledons much faster than Q. petraea, and this appeared to be true throughout the whole range of light intensities, suggesting that not only does Q. robur require cotyledonous reserves at normal seedling light intensities, but also that even though the Q. robur acorn is initially larger, it would become depleted before that of Q. petraea. This conclusion has obvious implications in the survival of oak seedlings in nature, particularly when seedlings rely on reserves in the cotyledons for regeneration after grazing damage. Q. petraea would possibly survive such treatment better.

Relative growth rates of shade tolerant and shade intolerant species have been noted by Grime (1965). Quercus rubra is the only oak species recorded and is described as a shade tolerant species, with relative growth rates in the range 0.61 to 2.87 mg/g/hr. Other shade tolerant species range from -0.09 mg/g/hr for Pachysandra spp. to 2.93 mg/g/hr for Tsuga canadensis, with an average of 1.22 mg/g/hr. Shade intolerant species range from 1.95 mg/g/hr for Ailanthus altissima to 17.12 mg/g/hr for Betula populifolia with an average of 6.13 mg/g/hr. When expressed as mg/g/hr, the relative growth rates of Q. robur and Q. petraea would appear to suggest that both species belong to the shade tolerant grouping (total plant relative growth rate at 100% light: Q. robur - 0.68 mg/g/hr; Q. petraea - 0.78 mg/g/hr. However, the grouping of species was carried out using relative growth rates determined under sunny conditions; under shade conditions, Q. robur and Q. petraea show very divergent behaviour. It should also be remembered that the results presented here represent seedling relative growth rates, whilst the values noted above for other species were for adult plants.

Several studies exist in which the effect of shading on relative

growth rate or photosynthetic activity has been investigated. Logan (1970) found that the photosynthetic apparatus of yellow birch (Betula alleghaniensis)adapts poorly to shade conditions. Using sun and shade grown seedlings, he was able to show that both sun and shade leaves of the yellow birch had the same rates of apparent photosynthesis, although in sun conditions the sun leaves were higher. Gordon (1969) using the same species produced very similar results, the shade seedlings being taller with larger leaf area, but the sun seedlings accumulated more dry weight. Interestingly, the dry weight distribution showed that there was a greater percentage of dry weight in the leaves and stems of shaded seedlings, but in unshaded seedlings, the greater percentage of dry weight was in the roots. Similar results have been described here for the Q. robur and Q. petraea seedlings - the root/stem ratio of Q. petraea being lower than that of Q. robur at the low light intensity, i.e. at the low light intensity, the supposed shade Q. petraea puts more dry weight into stem than the supposed sun species Q. robur. Loach (1970) investigated a series of seedlings of tree species of different shade tolerances in a range of light intensities. The results presented here parallel those of Loach. Net assimilation rates of shade tolerant species remained high down to 44% daylight but then declined as shade increased. Intolerant species, however, fell sharply from 100% daylight and one species, Populus tremuloides, had a negative net assimilation rate at 3% daylight. Q. robur in the present study also showed a negative net assimilation rate at the very low light intensities.

Two pieces of work, Karpisonova (1971) and Ovington and Macrae (1960), have investigated the relationship between soil type and shade on the growth of oak seedlings. Karpisonova (1971) described experiments with Quercus robur grown for three years in combinations

of full sunlight or shade and rich or poor soils. He found that light intensity was a much more important factor than soil fertility in determining growth of the seedlings. In Q. petraea, using again a range of light intensities and soil types, light intensity was again shown to be the more important factor in controlling seedling growth (Ovington and Macrae, 1960).

The closest work to the present investigation is that of Jarvis (1964). The results for Q. petraea described by Jarvis (1964) for a comparable experiment to that performed here agree broadly with the present results, the major differences being ones of magnitude rather than behaviour. Dry weight increments were somewhat larger in the present experiment and so too was leaf area increment and these differences are reflected in higher relative growth rates and net assimilation rates at the higher light intensities, although not at the lower light intensities.

Jarvis (1964) also recorded relative growth rate over a much longer period - one growing season. The response of the seedlings to increasing shade was generally the same as for the short term experiment, i.e. the seedlings showed increases of leaf area, specific leaf area, leaf area ratio, and decreases in net assimilation rate, relative growth rate, root weight and root/stem ratio with increasing shade conditions. Jarvis (1964) also recorded photosynthesis, and the chlorophyll content of the seedling leaves. He was able to show that sun grown plants had a slightly lower maximum rate of photosynthesis and a less steep initial slope (when plotting net photosynthesis against light intensity) which Jarvis attributed to a lower capacity on behalf of the sun grown seedlings of the photochemical processes and therefore a less efficient utilisation of weak light. The chlorophyll content of the shade leaves was found to be almost twice that of sun leaves

when measured on a mg/g dry weight basis for one year old plants grown under sun and shade conditions for fifteen weeks. This lower chlorophyll content of sun leaves was thought to be an obvious sign of the intolerance of bright light by the photosynthetic mechanism of the sun plants leading to a lower overall photosynthetic capacity. It is interesting to note that Polster (1963) has reported that the shade leaves on the north side of Q. robur trees were more efficient in photosynthesis under field conditions than sun leaves on the south side.

Compensation points for Q. petraea have been variously estimated at 2.1% and 7.9% relative light intensity (Ovington and Macrae , 1960) and 2.0% and 5.9% relative light intensity (Jarvis, 1964). Following Jarvis (1964), the relative light intensity at the compensation point has been estimated from the regression of dry weight increment ($W_2 - W_1$) on log light intensity, and this reveals a compensation point at 9.4% light intensity for Q. robur and 3.4% relative light intensity for Q. petraea, i.e. the compensation point occurs at a lower relative light intensity for Q. petraea than that for Q. robur, emphasising the ability of Q. petraea to manage better at lower light intensities.

(Attempts were made during this investigation to follow the course of photosynthesis in seedling leaves held in a perspex chamber whose air supply was monitored using an infra-red gas analyser in a photosynthetic and transpiration measuring apparatus built by the present author after the design of Bierhuizen and Slatyer (1964). Although some measurements were completed, problems of overheating in the leaf chamber prevented a full-scale investigation of the photosynthetic behaviour of Quercus leaves. However, two general conclusions from the small number of runs completed were:

a) Photosynthetic rate is at a maximum between 21°C and 30°C, but falls off rapidly below or above these limits. Consequently the

seedlings in the growth chamber at an air temperature of 15°C were probably not at maximum photosynthetic capacity, suggesting that the species differences might have been larger if the temperature had been higher.

b) Photosynthetic rate remains at maximal values down to light intensities equivalent to the 75% growth chamber light intensity (about 16% daylight) and then falls off rapidly with decreasing light intensity. Since oak seedlings were grown at much lower light intensities, they could not have been operating at maximal levels.)

The morphology and anatomy of adult leaves of the two British oak species showed differences consistent with the theory that Q. robur is a 'sun' species and Q. petraea is a 'shade' species. The investigation presented here into the growth analysis of seedlings leads to the same conclusion. There are important implications here not only for the physiological behaviour of the hybrid, but also of the hybrid seedling. Hybrid vigour is a well-known phenomenonin plant species (Strickberger, 1968) and if the hybrid seedling were to have the physiological ability to adapt to low light intensities like the seedlings of Q. petraea it might be at a distinct advantage in a Q. robur forest surrounded by Q. robur seedlings which might be less well able to take account of the prevailing conditions. Acorn food reserves would also appear to be an important consideration.

SECTION SIX

SIMULATION MODELS

CHAPTER ELEVEN

SIMULATION MODELS: A NAIVE APPROACH

Introduction

Although models of many genetical systems exist, and although many are under investigation, the production of a genetical model of introgression has not proceeded very far. Two possible reasons for this might be the lack of interest shown by biologists in introgression compared with other genetical situations, and coupled with this the failure of some biologists to accept introgression as a valid phenomenon, and secondly, as Wigston (1971) points out, the genetic mechanisms involved in introgression are of far greater complexity than any so far used in model systems. Anderson (1949) has attempted to explain the genetical basis for introgression, particularly in finite populations, but this still remains a largely uninvestigated problem.

In the production of simulation models of genetical systems, the following procedures attempt to link the model and field observations:

1. Patterns of variation are observed and described.
2. From the observed variation, a possible genetical basis is derived which it is thought may account for the variational patterns.
3. A model is produced which simulates the matings between individuals.
4. The patterns of variation in the model population and the observed natural variation are compared.
5. If the two patterns of variation agree, then the genetical model can be accepted as a reasonable explanation.

Gleaves (1973) has, for example, shown that gene flow by means of airborne pollen can be described by an inverse power law model, the model adequately describing the observed situation. Crosby (1960) has used a similar approach to the one detailed above in investigating heterostyly

in Primula vulgaris. Skellam (1952) has modelled the increase in size of an oak population using an extension of the random walk problem, to determine whether normal dispersal mechanisms would account for the movement of oak across Britain after the last Ice Age. He concluded that factors such as dispersal by rooks must have been of prime importance in extending the range of the oak, but several of his basic assumptions concerning oak biology are suspect. For example, he believed that oaks do not fruit until they are 60 or 70 years old - seedlings used in this present work have fruited in 5 years; Jones (1959) gives figures of between 15 and 49 years under natural conditions. The work of Skellam does provide, however, the only example of modelled oakwood changes.

The work presented in this chapter represents continuing research, and the models and results discussed here are only the first faltering steps in attempting to model introgression in oaks. No real genetical basis for introgression is proposed, the aim being to identify the important variables operative in the system; to attempt to provide some order of their magnitude and lastly to produce a model which will at least mimic the structure of an oak population, and modify that structure to produce the different population types observed in nature. As such, it is considered to represent a naive attempt; future models should help to provide a more realistic, genetical basis for introgression.

The Models

Two models will be considered, and be referred to as the COMPLEX and SIMPLE models for reasons which should become apparent later. Chronologically, the COMPLEX model was developed first, and since it is based on a more realistic assessment of an oak forest, it will be given priority. The COMPLEX model:

The basis of the model is the population. In later versions of this

model, the population was considered to consist of an 'orchard' like arrangement, with trees in straight lines, spreading in two dimensions. For the purposes of discussion, the population can just as easily be thought of as a line of trees. Computationally, there is little difference; the line of trees being held in a one-dimensional array, the orchard like configuration in a two-dimensional matrix. The only difference in the behaviour of the models is that for a line of trees, each tree is flanked on either side by other trees (apart from those at the end of the line), whilst a tree in a matrix is flanked on all sides by other trees, again apart from the edge individuals. Each tree can be considered to have two characteristics, age (TAGE) and taxonomic affinity (TTYPE). TTYPE can register a tree belonging to one species, or another, or any position in between - it is in essence a form of hybrid index. Each tree may be considered to possess also a population of seedlings, i.e. under natural conditions, there will be a number of seedlings underneath the oak canopy, and some of these may ultimately replace the mature trees. Since each tree will have several seedlings at its base, those considered for a line of trees would be stored in a two-dimensional matrix, those for a two-dimensional matrix of trees in a three-dimensional matrix. Again each seedling can have two characters, age (SAGE) and taxonomic affinity (STYPE).

Change in such a population will be brought about by the removal of individuals, and their replacement by other individuals. The COMPLEX model assumes a stable situation insofar as the population size of the trees and their seedlings is constant. Consequently, in such a population there is only replacement of individuals not the creation of new spaces for new individuals, i.e. the population is not expanding.

Death of individuals is thought to be due to one of three factors - old age (trees certainly 'age', and under given circumstances there is probably some age limit for trees), random processes (lightning strike

and other extrinsic factors), and natural selection (i.e. processes connected with the 'fitness' of the individual). In a simplified system, these can be considered to represent separate tolls on the population, although almost certainly in reality they are closely linked. Both old age and random death are easy to simulate - the former requires monitoring of TAGE and SAGE, the latter requires generation of random coordinates for the matrices and removing chosen individuals. The rate of change in the population can thus be governed in two ways by altering the maximum age permitted and by changing the rate of random death. Change in the seedling population may be similarly considered.

Selection is best considered as a two-stage process, the initial selection against particular genetical combinations at the formation of the acorn stage, and a secondary selection process operating in the choice of seedlings to fill gaps in the forest canopy caused by removal of adult trees. In a finite, closed population, such adult replacement by seedlings will involve selection from those seedlings beneath, or almost beneath the removed adult tree. This recruitment may be considered to be a random process, or a deterministic process - the determining factors being either age (the oldest seedling being recruited) or genetic constitution (perhaps selection for hybrids as they may show heterosis or selection against hybrids as they might not be well-fitted in the habitat of a parental type)or more probably a complex interaction of many factors. The rather inadequate but simpler solution considered here is that selection against specific genetic combinations occurs at the acorn formation stage, recruitment is considered to be a random process, with a minimum age required for the recruited individual.

Removal of seedlings, therefore, occurs for three reasons: random death, recruitment to the adult stage, and finally death due to old age. The latter is difficult to envisage - in nature, random death must occur

at an exceptionally high rate (since recruitment rate will be very low) in order to prevent a complete understorey of large seedlings (i.e. bushes) forming. Death by old age in the model situation probably redresses the balance caused by an inefficient random death process. An assumption must be made at this point that the seedling population must be considered to be of finite size, and that just as seedlings are recruited to adult status so acorns are recruited to seedling status. There must, therefore, be provision within the model for the reproductive process. It is considered that a tree will only reproduce if gaps exist in the seedling population below it. This is to save computer time rather than a statement of a natural situation.

Reproduction in the population involves a complicated procedure. Basically, acorns may be derived from selfing, crossing with the nearest trees, or crossing with alien pollen from outside the population, considered usually to be from the other species. In the simplest case, crossing can be considered to occur only with adjacent trees (for the 'nearest' trees) although more complex situations may be derived by considering concentric zones of crossing moving out from an individual, so that the majority of the crossing takes place with closest individuals, but is permitted at a lower level with individuals further away. Other variables such as wind direction may be easily built into the model at this stage. In the case of the line of trees, acorns can be formed, therefore, from trees on either side, from selfing and from alien crossing. (Special situations may arise at the edge of a forest, where no trees exist on one side, but where alien crossing may be considered greater.) A number of acorns to be produced say N_A is derived as follows:

$$A = N_A * NNI * FF_i$$

$$B = N_A * SELF * FF_j$$

$$C = N_A * OUT * FF_k$$

where NNI, SELF and OUT = the proportion of crossing expected between the tree and others:

NNI = proportion of crossing between the tree and its neighbours
SELF= proportion of crossing between the tree and itself
OUT = proportion of crossing between the tree and aliens

i.e. NNI + SELF + OUT = 1 (in above equations A+B+C will yield N_A acorns if $FF_i = FF_j = FF_k = 1$)

NNI can be and usually is partitioned into NNI_a, NNI_b, NNI_c etc. where

NNI_a = proportion of crossing between the tree and its immediate neighbour
NNI_b = proportion of crossing between the tree and its next immediate neighbour
NNI_c = proportion of crossing between the tree and its next immediate neighbour

and so on, the limitation being that:

$NNI_a > NNI_b > NNI_c$ ..... etc.

and

$NNI_a + NNI_b + NNI_c$ ..... = NNI

Colwell (1951) has used P^{32} labelled pine pollen grains to study the dispersal of pollen from a point source, and determined that 90% of the pollen may be collected within 26 ft. of the source, which would suggest that: $NNI_a \gg NNI_b \gg NNI_c$ ..... etc.

FF_i, FF_j and FF_k are modifiers, which measure the success of a possible cross and adjust the final result accordingly. These are in fact selection parameters, and operate in the following manner.

The success of a cross is determined by how remote (in taxonomic distance) are the two parents. For example, the success when two members of the same species cross is likely to be close to 100%. Selfing (on the evidence presented in Chapter 8) is likely to be much less, and crossing with an alien species is likely to be very low indeed. The taxonomic distance between two individuals is measured as the difference between their TTYPE (i.e. hybrid index), thus the success of a cross between two individuals depends on their mean TTYPE:

$$X_{ij} = \text{INTEGER}\left(\left(\frac{TTYPE_i + TTYPE_j}{2}\right) + 0.5\right)$$

X_{ij} is an integer, and can be used to find the X_{ij}th position in an array FF which holds success rates for such a cross. For example, suppose a tree of TTYPE = 1 crosses with a tree of TTYPE = 10 then X = 6. By consulting array FF at position 6, the success of such a cross may be determined.

From above, if $FF_i = FF_j = FF_k = 1$ then N_A acorns would be produced in total from the three crosses. Since FF_i, FF_j and FF_k will not equal 1, but will generally be smaller than 1 (approaching 1 for within species crosses, approaching 0 for between species crosses), the total number of acorns produced will be lower than the total permitted.

The type of acorns produced from a particular cross will obviously be:

$$STYPE_{ij} = \text{INTEGER}\left(\left(\frac{TTYPE_i + TTYPE_j}{2}\right) + 0.5\right)$$

Consequently A acorns of type $STYPE_A$ will be produced, and B acorns of type $STYPE_B$ etc. These can be stored in an array, and chosen at random to fill in the gaps in the seedling population.

At the end of every annual cycle, all seedlings and trees increase in age by one year.

To summarise the processes of the COMPLEX model:

1. A scan of adult trees for tree age; if any are above the maximum permitted they are eliminated.

2. The generation of a random number. This determines if a tree will die randomly in the population during that year. This may be modified as follows:

   Generate a random number between say 0 and 100. If that number is greater than a set number, then random tree death will occur. Modification of the set number will determine how frequently trees will die randomly.

   Elimination of trees randomly involves generation of a random co-ordinate and elimination of that particular tree.

3. The eliminated trees are replaced by seedlings directly beneath - the oldest seedling being chosen provided that it is of minimum age. Should all seedlings under one eliminated tree be under the minimum age required, the gap in the canopy is left unfilled until the following annual cycle when a seedling might have reached the required age.
   It is possible, therefore, for a permanent 'hole' to appear in a population due to a tree and all its seedlings dying.

4. Elimination of seedlings reaching the maximum permitted age.

5. Elimination of seedlings randomly by the generation of random co-ordinates.

6. A check is carried out at this stage to determine if any seedlings have been eliminated. If not, then the simulation passes on to No. 8.

7. If seedlings have been eliminated, then they are replaced by acorns produced during the reproductive process. A number of acorns are produced and these selected randomly to join the seedling population.

8. All extant trees and seedlings are increased in age by one year.

The COMPLEX model worked extremely well, with one major drawback. Small populations of 10-20 trees could be easily coped with and took little computer time, but populations of a more realistic size (100 trees, 10 seedlings/tree, producing a maximum of 1000 acorns each during reproduction) took several minutes to compute only a few years' cycling. Although many runs have been completed using small populations, an alternative faster model was developed, the SIMPLE model, for larger populations.

The SIMPLE model:

The basic difference between this model and the previous one is that operations in the COMPLEX model are completed on the individual as the basic unit, whilst in the SIMPLE model, the population becomes the unit and all operations are computed on the mean individual. The same processes occur, however, the only major change being the restriction of characteristics of the population to TYPE only, AGE being neglected. The sequence of events of this simulation is as follows:

1. Two parameters characterise the population T1, the number of trees in the population and T2, the <u>total</u> taxonomic character for the population,

   i.e. $T2 = TTYPE_1 + TTYPE_2 + TTYPE_3 \ldots\ldots TTYPE_{T1}$

2. At every annual cycle, a number of individual trees are removed thus decreasing T1. This number has two components:

   F1 = a fixed number, i.e. a constant which represents trees being eliminated due to their reaching the maximum age.

   V1 = a variable number derived from a random number generator which represents the random event in tree death.

   Therefore $T1_2 = T1_1 - F1 - V1$

   where $T1_2$ = new population size

   $T1_1$ = old population size

   T2 becomes similarly reduced.

3. Eliminated trees (i.e. F1 + V1) are replaced by seedlings of the mean seedling population type:

   i.e. S1 = total number of seedlings
   S2 = total seedling type

   Therefore M_S = S2/S1 where M_S = mean seedling type

   $T2 = T2 + (M_S * (F1+V1))$

   F1 + V1 seedlings are recruited, of mean M_S type, to the adult population, restoring T1 to its previous level - again this considers a population of finite fixed dimensions.

4. The process of seedling elimination is now carried out, eliminating again a fixed number and a variable number of seedlings representing fixed and random effects, F2 and V2 respectively. The seedling population is deprived, therefore, of F1 + V1 + F2 + V2 seedlings during every annual cycle.

5. Replacement during the reproductive phase is carried out in the same form as that described previously for the COMPLEX model, except using mean values, and only considering two types of cross:

   Crossing between individuals of the same population (included here are selfings);

   Crossing between individuals in the population and alien individuals normally considered to be of a separate species.

   During replacement, F1 + V1 + F2 + V2 need to be replaced, then:

$Y1 = 10,000 * W1 * FF_{MT}$

and

$Y2 = 10,000 * O1 * FF_{AL}$

where W1 = level of populational inbreeding
O1 = level of populational outbreeding
FF_{MT} = success rate of a cross between trees of the same population
FF_{AL} = success rate of a cross between trees of the population, and the alien species
10,000 = any convenient number
Y1 = number of acorns formed from populational inbreeding
Y2 = number of acorns formed from populational outbreeding

$Z1 = (Y1/(Y1 + Y2)) * M_T * (F1 + F2 + V1 + V2)$

and

$Z2 = (Y2/(Y1 + Y2)) * ((M_T + 20)/2) * (F1 + F2 + V1 + V2)$

where $Y1/(Y1 + Y2)$ = proportion of the total number of acorns formed that are from populational inbreeding
M_T = mean tree TYPE of the population, and therefore the TYPE of the populational inbred acorns
F1 + F2 + V1 + V2 = number of replacement acorns required
Z1 = total TYPE of the replacement acorns derived from inbreeding

Similarly with Z2 which equals the total type of the replacement acorns derived from outbreeding.

$(M_T + 20)/2$ = mean TYPE of an alien cross with the population, the alien being considered as of TYPE = 20

Hence for replacement:

S2 = S2 + Z1 + Z2, and S1 = S1 + F1 + F2 + V1 + V2

This model is obviously not open to the same manipulative possibilities of the previous model. It is not possible for example to investigate the possible effect of wind direction on pollen flow, 'edge' effects and very importantly, the spread of alien genes once infiltrated into a population. This last point may be crucial, since introgression is supposedly the infiltration and assimilation of genetic material from one species to another, and once alien genetic material is in a population, it might be argued that this will tend to spread throughout the immediate vicinity of the original F_1 hybrid. Such effects cannot be studied using the SIMPLE model.

The models have been programmed in both BASIC and ALGOL (1900 and

4100) but since the models are in a process of flux, details of the programs have not been included here.

Typical Values

One of the main problems concerning simulation of introgression in an oak population is the lack of basic population description information such as population size, maximum age of trees, numbers of seedlings, etc. Certain starting values are required to define the population, and its structure, in order to start the model; some of these and their possible derivations are discussed below:

Population size - a very variable parameter. During sampling of populations, groups of trees as small as 18 trees were noted, which seemed to constitute isolated populations. It is argued that from the point of view of a model, population size is required to be realistic, but not accurate. In consequence, for the COMPLEX model, test runs used a size of 10 individuals, actual runs used 100 individuals, although these were very lengthy computer runs. The SIMPLE model used 1000 trees.

Seedling number - again a variable parameter, and almost certainly variable with time since trees have good and bad years for acorn production. Grazing intensity is also likely to influence the numbers, and this too may vary. Generally, five seedlings were considered to each tree, this being based on field observations.

Taxonomic structure of the population - the results of Chapter 5 would suggest that no tree is ever completely 'pure' in a hybrid index sense (since no tree scored zero in the 0-340 range), but for simplicity the taxonomic structure of the populations was always assumed to be extreme, i.e. all individuals being given the value 1 unless runs were being completed in which the effect of an alien tree actually in the population was being considered. Similarly, the seedlings were also adjudged to

possess the same taxonomic ranking as their parent tree.

Rate of change in the population - from the point of view of trying to estimate the period of time required for change to take place in a population (one of the important reasons for modelling in the first instance), the rate of death in the population is of paramount importance. No figures exist in the literature on this point. The approach used here was to sample natural populations for trunk diameters at breast height. This enabled these individuals to be divided into trunk diameter classes, which it was argued would represent age classes (a survivorship curve). The size of the largest class would represent those very old trees in a population that would be eliminated ('die') during the next cycle. The rate of random death could be considered similarly to be the sum of the differences between one diameter class and the next. This approach has two objections - firstly the arbitrariness of the diameter class, and secondly a related problem that although such an approach allows diameter classes to be produced, there is still no indication of the actual period of time involved, i.e. the change is described, but the rate of change is still elusive. This was resolved by obtaining from the Forestry Commission yield tables, and trunk diameters, so that an estimate of the likely age of the largest trunks could be made, and then dividing the distance between the largest and smallest trunk diameters accordingly. An added objection to this approach, however, is that it does assume stable growth conditions. The rate of change of the seedling population proved much more problematical, stem height and diameter measurements gave reasonable distributions, but there appeared to be no estimate of error - for example, seedlings can be heavily grazed, which would lead to an old seedling being both short and with a small stem diameter. In the absence of an alternative, it was decided to use stem diameters, constructing diameter class distributions and estimating from these random

death and possible seedling death due to old age. Good and Good (1972) have studied the population dynamics of tree seedlings including several North American oak species using similar size class distribution histograms and relating size classes ultimately to age classes. They also provided figures of 'turnover' rates of populations which have proved useful for values of some starting parameters.

From such basic survivorship curves, it is possible to consider either populations changing faster or slower than the base rate.
The success of different types of cross - again no information exists on this point except the scattered results of Dengler (1941) etc. and the results presented here. The pollen viability of individuals of different morphologies (Figures 7.5-7.9) it was thought may prove useful since here is expressed the fertility of at least the male part of the reproductive process. It was argued that if two extremes were crossed, then the success of that cross is likely to be very low - and possibly comparable with the fertility of the pollen at the mid-point between the extremes, i.e. the pollen viability curve represents the shape of the curve required, but probably not with the correct values. These it was assumed might be close to 1 for a cross of two very close individuals, and almost zero for two very dissimilar individuals. This curve was, therefore, redrawn with the lowest value at 0.02 (i.e. a success rate of 2%) and the highest value at 0.90 (i.e. a success rate of 90%). Initially this curve was divided into 20 equal parts, and the mean success rate determined for each part of the curve. This could now be used in the following way:

Consider a cross - TREE 1 x TREE 20

The success of this cross could be determined from consulting the mean success rate at the 11 part of the curve - FF_{11}. This value could then be multiplied by the maximum number of acorns expected, eg. if the

maximum number of acorns formed were 100, and the success rate were 0.02 for FF_{11} then there would be 2 acorns formed out of a possible 100.

Although there is no theoretical basis for the above, it would seem to be a close approximation to reality, and an attempt to describe a situation not considered in the literature. The effect of using different FF ranges is discussed below.

Levels of populational outbreeding and inbreeding - although typical values were useful initially, determining possible levels was one of the reasons for producing the models, and consequently typical values for these parameters remain for experimentation.

Some properties of the models

Since the work presented in this chapter represents work in progress, it is not proposed to discuss details of all results so far obtained, but to consider some of the properties of the models and consider how they might prove useful.

1. The COMPLEX model

Table 11.1 and Figure 11.1 illustrate one of the first test runs of the COMPLEX model, using ten trees. The starting values for the model were:

Population size: 10 No. of seedlings/tree: 5
TYPE of trees and seedlings: 1
Extreme TYPEs: 1 and 10, 10 representing the alien species
Breeding levels: Selfing 10%, Nearest Neighbour 20%
Populational outbreeding 30%

(a deliberately high value of outbreeding was chosen in order to bring about observable changes over the 250 year time span of the simulation)

Table 11.1 and Figure 11.1 show three levels of turnover rate in the population:

Fast rate: death rate - 1% per year (both random and old age)
Medium rate: death rate - 0.7% per year
Slow rate: death rate - 0.38% per year

For the seedlings the corresponding values are 26.5%, 17.5% and 9.5%.

											Adult death rate
0	Years	1	1	1	1	1	1	1	1	1	
50	"	1	1	6	1	1	1	1	1	1	
100	"	1	1	6	1	1	1	1	1	1	0.38%/Year (Slow rate of turnover)
150	"	1	1	6	1	1	6	1	1	1	
200	"	1	1	6	1	1	6	1	1	1	
250	"	1	4	8	1	1	4	1	1	1	
0	Years	1	1	1	1	1	1	1	1	1	
50	"	1	1	1	1	1	1	1	1	1	
100	"	1	1	1	1	1	1	1	1	6	0.7%/Year (Medium rate of turnover)
150	"	1	1	1	1	1	1	1	5	5	
200	"	1	1	1	1	1	8	3	4	8	
250	"	1	1	1	6	1	8	7	4	8	
0	Years	1	1	1	1	1	1	1	1	1	
50	"	1	1	6	6	4	1	1	6	1	
100	"	1	5	9	9	4	3	8	8	6	1.0%/Year (Fast rate of turnover)
150	"	8	7	9	10	7	6	8	8	6	
200	"	8	7	9	9	9	9	7	9	9	
250	"	8	7	10	9	10	9	7	9	9	

TABLE 11.1 RESULTS FROM THREE RUNS OF THE COMPLEX MODEL USING DIFFERENT TURNOVER RATES, BUT OTHERWISE THE SAME POPULATIONAL PARAMETERS. TEN TREES ARE REPRESENTED IN LINEAR CONFIGURATION.

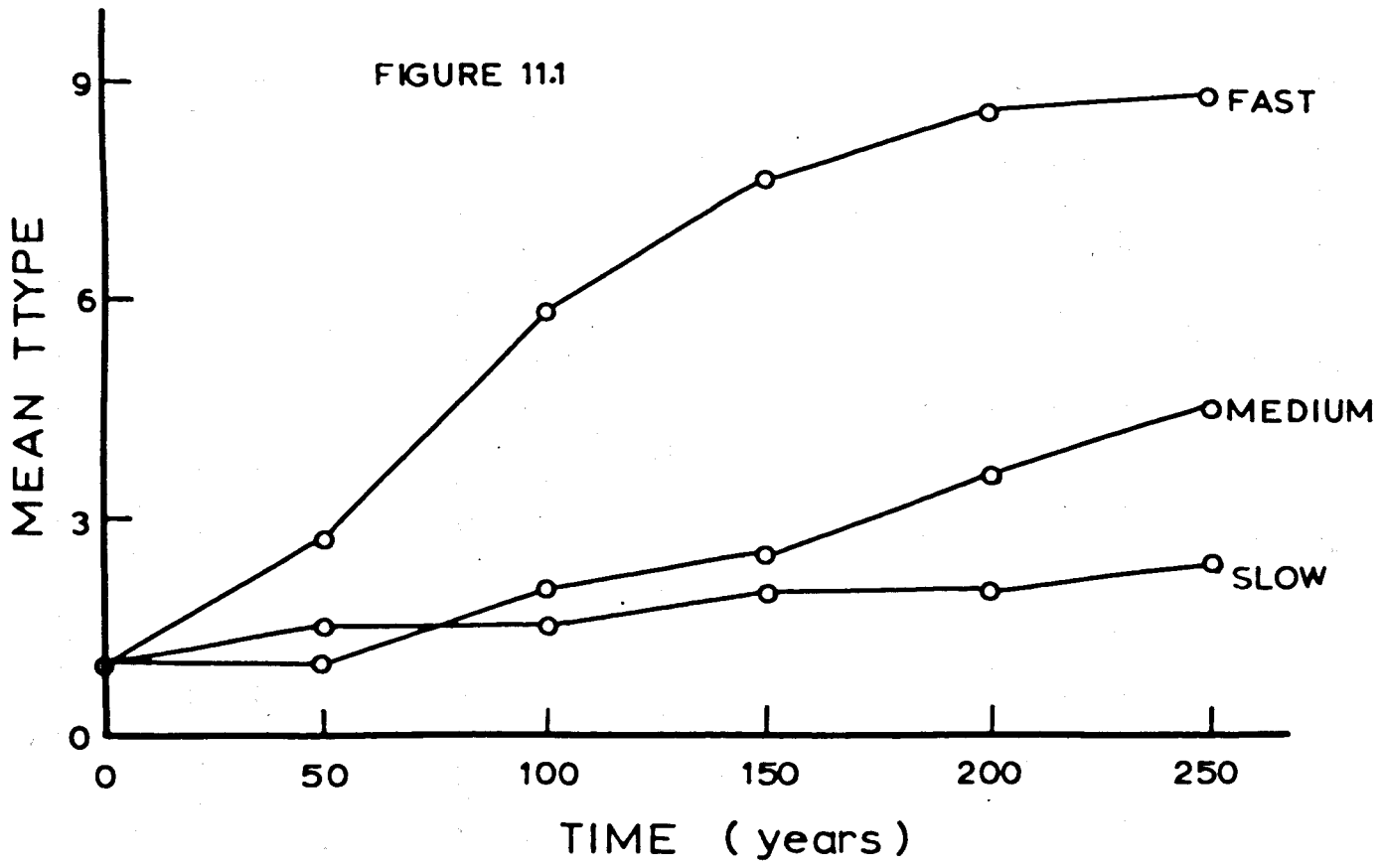


FIGURE 11.1 Change in the morphological index (TTYPE) with time of three model oak populations each under a different turnover rate (using the COMPLEX model)

This simple example illustrates many of the facets of this model.

The first, and most important, is the difference in the death rates, and the changes which occur as a result of changes in death rate. As can be seen from Figure 11.1, after 250 years, the higher death rate and in consequence the faster turnover population has reached a mean taxonomic level of 8.80, whilst the medium and low rates have reached only 4.50 and 2.30 respectively. Therefore rate of turnover of the population may be just as important a factor in determining the level of hybridisation observed in a population as the level of populational outbreeding itself.

It is also possible to detect in this example the spread of alien genetic material once it appears in the population. In the medium population (Table 11.1) F_1 hybrids are formed at the very end of the line of trees. As time progresses, the alien genes spread to the left so that by the 250th year, the five trees on the left of the population are to some degree hybrid. At this time also a third F_1 hybrid has been formed further over to the left. In the fast rate population, hybrids occur initially towards the middle of the population, and alien genes begin to spread both ways to the left and right. An F_1 hybrid formed in the low rate population remains isolated for some time, and only later in the simulation does it cross and begin to spread.

Lastly, the random element can be observed. It would be expected that since the rate of turnover is greater in the medium and high rate populations, they would produce hybrids before the low rate population. In fact, the low rate population produced its first F_1 hybrid in the 46th year, the medium rate population in the 57th year, and the high rate population in the 20th year. The random element can therefore be an important feature of oak populations - if an adult tree dies, the composition of the seedlings underneath at the time of death is critical in determining the future course of the population.

This example also illustrates another point which in one sense might argue that the model is a poor model of introgression. From Figure 11.1 it may be seen that during the progression of time, the mean taxonomic index, i.e. TYPE of the population increases, so that eventually in the high rate population, it is 8.80 close to the extreme 10 representing in this case the alien species. Introgression, as it is described, does not behave in this fashion, it being specifically described as the gradual infiltration and assimilation of germ plasm from one species into another. Indeed, all quoted examples recognise introgression to be a situation where the majority of the population under investigation are of an extreme type together with a proportion of F_1 and backcross F_2, F_3 etc. individuals. It would appear obvious that an F_1 is more likely to be successful crossing with the alien species than is the pure type of the population in which the F_1 finds itself. Similarly, F_2, F_3 backcrosses are more likely to be successful in crossing with the alien than is the pure type. It naturally follows that once hybrids are established in a population, even though they backcross with the population parental type, future hybridisation with the alien species must be enhanced, and the consequent result of that would be a gradual shift of the mean population type until eventually the population had completely reverted to the alien species.

Examples of this phenomenonin the literature are somewhat rare, due possibly to the long period over which observations would have to be made. (The rate of change initially may be very slow and difficult to detect - see Figure 11.1.) One excellent example is that of Helianthus described by Stebbins and Daly (1961). A hybrid population of Helianthus, (H. annuus x H. bolanderi) in California became divided into two distinct groups of plants by the invasion of a grass species. These groups were about 120 m apart but each behaved completely independently

of the other. One group maintained a high proportion of H. bolanderi type plants, whilst the second changed from being at first intermediate in character to H. bolanderi type individuals, and then back again, finally having a large majority of H. annuus type plants. These changes took place in a matter of a few years, suggesting that changes of this type may be extremely rapid. In sympatric introgression, there would appear to be no reason why the character of a population should not change completely from one species through to an alien species. Rate of turnover of individuals and the intensity of selection against hybrids would appear to be the only restrictions to this happening. Selection against hybrids produced as a result of backcrossing the F_1 to the alien species, a possibility since it must be envisaged that the population is experiencing a continual alien pollen 'rain', is likely to be very intense since in few situations are hybrids of this type described. Ecological selection is likely to be operative against such F_1 x alien hybrids since they will be found in the population of the maternal parent, and therefore having to survive in what is probably an ecologically hostile environment from the point of view of an F_2 hybrid of this type.

2. The SIMPLE model:

This behaves similarly to the COMPLEX model under comparable situations. Figure 11.2 illustrates one particular result. The starting values for this run were as follows:

Adult population size: 1000 Running time: 5000 years
Seedling population size: 5000
TYPE of seedlings and adults: 1
Extreme TYPE values: 1 and 20, i.e. 20 represents the alien species
Populational inbreeding: 0.80 Populational outbreeding: 0.002-0.20

Three different death rates are used:

0.15%/year - slow rate of turnover
0.30%/year - medium rate of turnover
0.45%/year - fast rate of turnover

Much lower death rates can be used in this model, since it can run for more realistic periods of time without taking up excessive computer time.

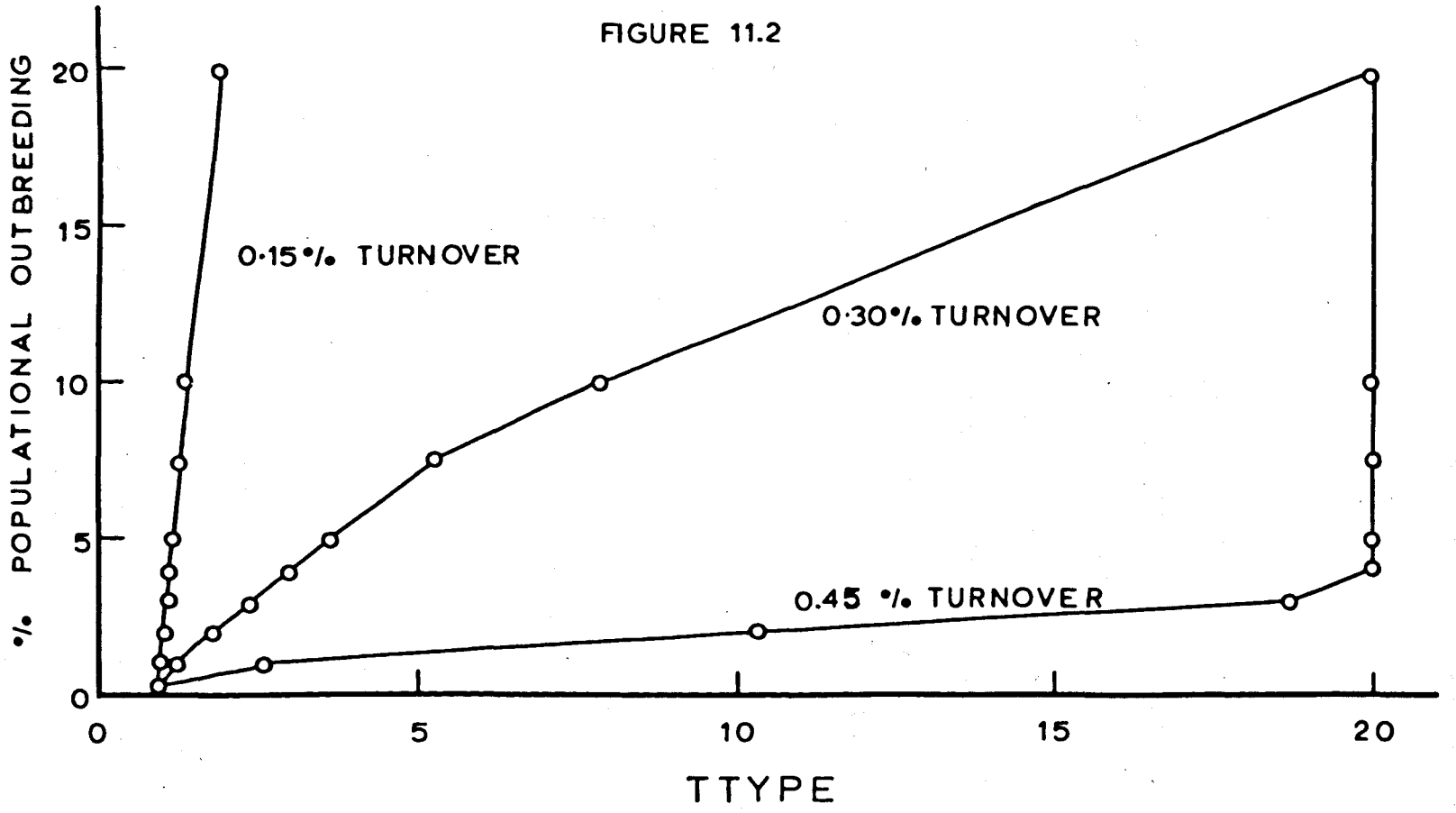


FIGURE 11.2 Change in the morphological index (TTYPE) with differing levels of populational outbreeding for three oak populations each under a different turnover rate (using the SIMPLE model)

Figure 11.2 shows the very divergent behaviour of the three runs using the different death rates. In the figure, each point along the lines represents the morphological TYPE or index reached by the population with the corresponding level of populational outbreeding after 5000 years. At the highest death rates, the population achieved the status of the alien species after 5000 years, with all but the lower outbreeding rates. Even an outbreeding rate of 0.02 or 2% would achieve the mid-point between the species after 5000 years. The low death rate by contrast remains comparatively unchanged, even at a high outbreeding rate. Such a population over geological time, therefore, has approximately stabilised, in that over short periods of time, change in the population would be undetectable. The medium death rate represents the mid-point between the two extremes of low and high death rate. The low death rate population has stabilised in a more or less pure state, but there must be a family of curves between the low and medium death rates some of which may be more or less stable with a proportion of hybridity. These are likely to lie close to the low death rate curve.

As before, it should be noted that intense selection must operate against hybrid types to prevent complete reversal of the population to the alien species.

One set of variables not yet mentioned are the 'fitness factors', the correction parameters applied to different types of cross, to compensate for the taxonomic difference between the crossing individuals. Figure 11.3 shows the results of four simulation runs using the SIMPLE model, and different values for the fitness factors and different death rates. Table 11.2 lists the two ranges of fitness factors used, Range 2, having very low values for hybrids, so that any intraspecific cross will be markedly unsuccessful in comparison to the same cross using Range 1 fitness factors.

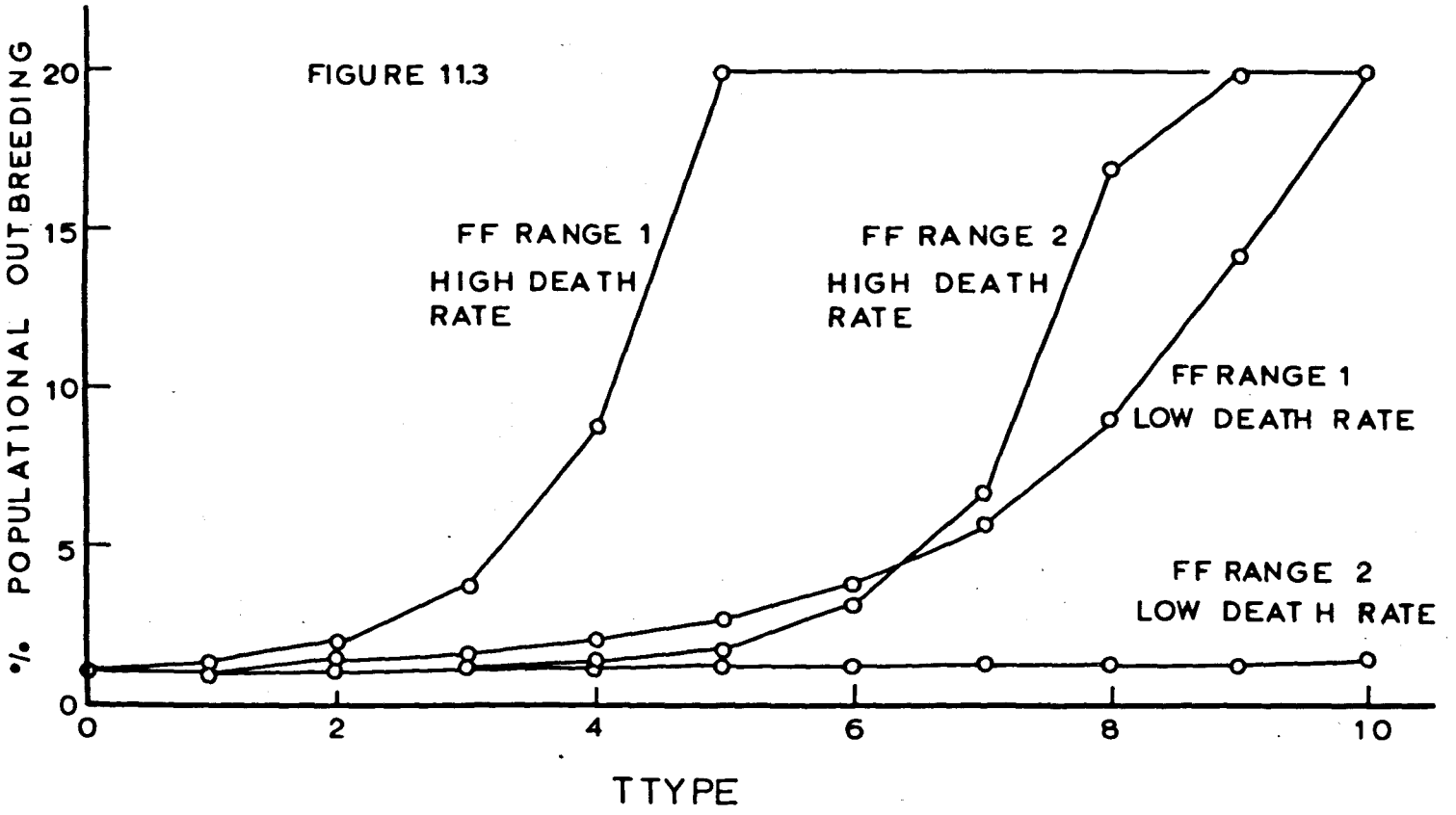


FIGURE 11.3 Change in the morphological index (TTYPE) with differing levels of populational outbreeding for four oak populations experiencing combinations of different turnover rates and fitness factors (using the SIMPLE model)

Taxonomic position of the cross *	Range of Fitness Factors	
	1	2
1	0.883	0.900
2	0.883	0.450
3	0.883	0.300
4	0.874	0.200
5	0.846	0.150
6	0.783	0.100
7	0.664	0.060
8	0.497	0.040
9	0.410	0.020
10	0.378	0.010
11	0.378	0.010
12	0.410	0.020
13	0.497	0.040
14	0.664	0.060
15	0.783	0.100
16	0.846	0.150
17	0.874	0.200
18	0.883	0.300
19	0.883	0.450
20	0.883	0.900

* Eg. - a cross between an individual of TYPE 20 and an individual of TYPE 10 would be corrected for the Fitness Factor 0.783 (i.e. (10 + 20)/2 = 15 = a fitness factor of 0.783) or 0.100 depending on the range of factors employed.

TABLE 11.2 TWO DIFFERENT RANGES OF FITNESS FACTORS USED IN THE COMPLEX AND SIMPLE MODELS - RESULTS FOR THESE ARE ILLUSTRATED IN FIGURE 11.3

The population parameters are as described in the previous example, except that in this instance the populations were allowed to run for 10,000 years.

At very low death rates, and very low fitnesses for intraspecific crosses (i.e. Range 2), the population remained almost unchanged. Alteration of the range, however, caused a large increase in the proportion of hybrids in the population. Using higher death rates, the same differences between the ranges apply, but it takes a shorter period of time to reach the same end point. Consequently, variation in the success rates of crosses can be easily and successfully mimicked in the models.

A last example of the use of the models is illustrated in Figure 11.4. This figure is based on the following reasoning: sampling oak populations during the population investigation yielded 50 trees, and approximately 2 of these or 4% would need to be hybrids before the population would be recognised as having some degree of hybridity. If therefore the morphological TYPE scale ran from 0 to 10, then a population with a mean TYPE score of 0.40 if sampled as a real population would be detected as containing hybrids. In simulations of populations, therefore, the time taken to reach a mean TYPE score of 0.40 can be recorded, and this period would represent the length of time required for hybridisation to have been progressing under the conditions of the population before it could be detected by the sampling methods used in this thesis.

Figure 11.4 represents the results of such a series of computer runs with different levels of outbreeding, the time axis representing the length of time required before hybridisation would be detected. For example, at a 2% level of outbreeding, hybridisation would not be detectable for 250 years, at 5% for 100 years, and so on. The curve can also be interpreted in the opposite fashion, i.e. if a population has a

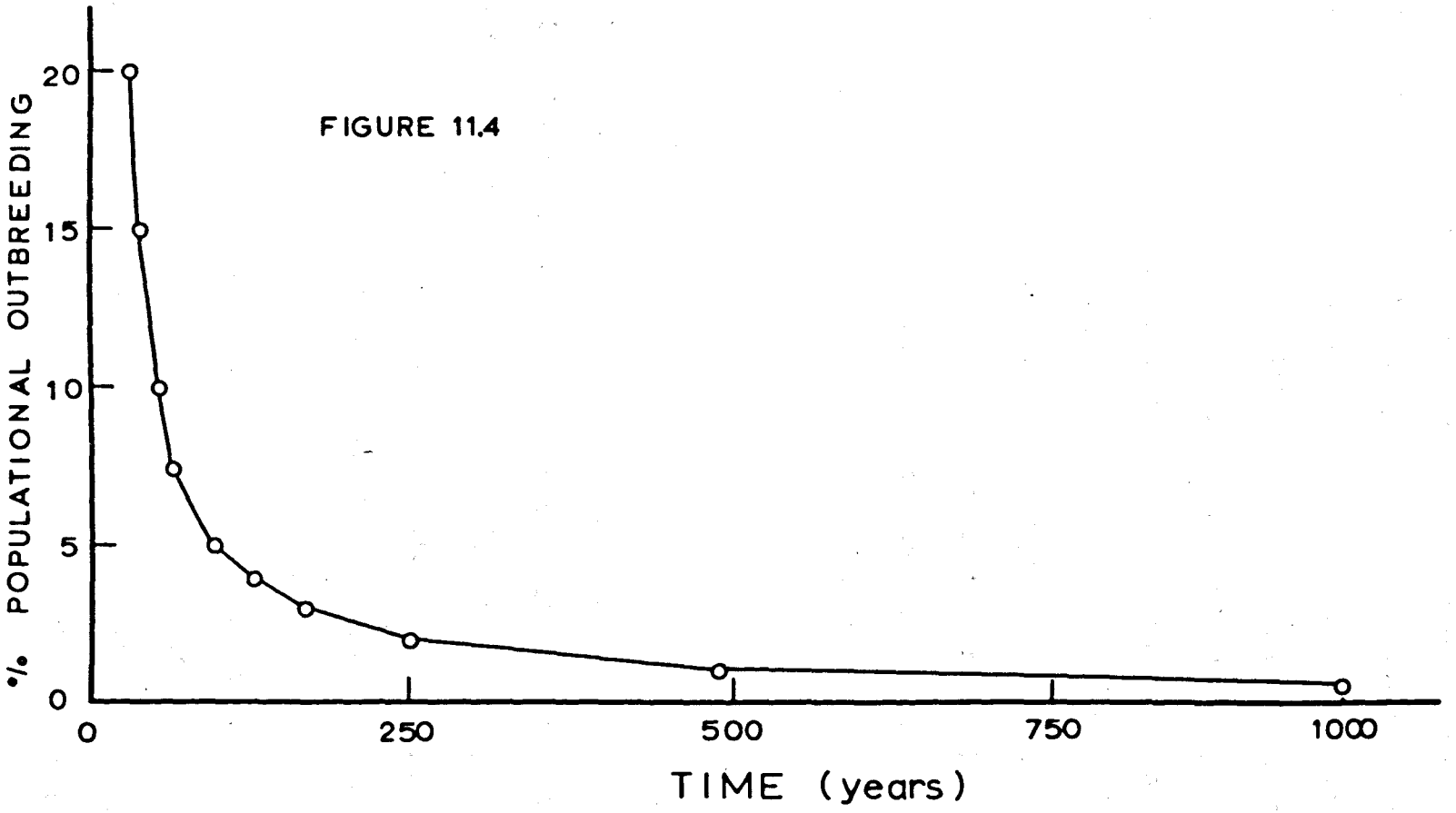


FIGURE 11.4 Graph showing the time taken for hybridisation to become apparent in an oak population for differing levels of populational outbreeding (using the SIMPLE model)

detectable level of hybrids and is say 200 years old, the level of outbreeding required to have brought about such a situation would be 2.7% outbreeding. Of course, such interpretations would depend on two things. Firstly an assumption that conditions had remained stable over the 200 years and secondly that if the interpretation was an attempt at describing actual forests, then the original forest parameters must have been accurately determined. This latter approach to simulation models, i.e. trying to estimate levels of outbreeding or periods of time to bring about hybridisation is a continuing aspect of this research, and is being pursued by attempting to obtain more accurate information for the population parameters, since this is imperative if estimates of outcrossing, etc. are to be made realistically and accurately.

It is hoped that such an approach might help allay some of the criticisms against introgressiin in woody species namely the low success rate in crossings between the species and also the long generation time. The use of Leslie matrices to model complex populations (see Williamson, 1972) might be used to model an oak woodland, with the classes being different size or age classes. This might prove a useful intermediate model to the COMPLEX and SIMPLE models described here; the COMPLEX model being replaced by a faster operational model, the SIMPLE model which only considers the dynamics of the whole population being replaced by a model where the dynamics of a group of classes could be studied. If adequate models can be built and operated using real population data, then this should prove an important contribution to our knowledge of variation in oak woodlands. The work presented in thischapter represents only a naive initial attempt; many improvements are needed before the simulation model can adequately describe and mimic a natural population.

SECTION SEVEN

DISCUSSION

CHAPTER TWELVE

A CONCLUDING DISCUSSION

The species concept and the genus Quercus L.

Formal delimitation of specific taxa is still a matter for debate (Briggs and Walters, 1969) but it is possible to recognise two main species concepts:

a) The Taxonomic Concept based on orthodox taxonomy in which individuals having common attributes may be separated from other groups by morphological discontinuities and

b) The Biological Concept based on genetics and cytogenetics in which either interbreeding between members of a species is stressed or reproductive isolation from other groups is considered important.

Both concepts applied to the genus Quercus are unsatisfactory. In orthodox terms, oak species are delimited from each other by rather trivial characters of a mainly quantitative nature. Thus Q. robur is separated from Q. petraea by depth of lobe, length of petiole, number of lobes, length of peduncle, etc., single diagnostic characters being absent not only for this species pair but for all oak species. Indeed, leaf and fruit characters are remarkably uniform throughout the genus; American, European and Asiatic oaks produce leaves and acorns of exceptional similarity. Floral characters are even more conservative - a detailed search for floral differences between Q. robur and Q. petraea made during the course of this thesis failed to detect any observable difference. Davis and Heywood (1963) record a similar situation in Euphorbia, where insignificant flowers appear relatively uniform throughout the group. They comment:

"Would Euphorbia be treated as a single genus if its flowers were as conspicuous as a lily's? The very narrow species concept often employed in Paeonia and Pulsatilla is no doubt a result of their having showy flowers; like pretty girls they get a lot of attention."

Such morphological differences as do exist in Quercus become obscured by a wide expression of distinguishing characters brought out by phenotypic plastic responses. These may be so great as to completely obscure the existence of two taxa - Garilov (1969) has described a comparable example where an ecotypic form of Q. petraea is indistinguishable from Q. polycarpa at low altitudes whilst another ecotype is indistinguishable from Q. dalechampii at higher altitudes. Although some differences probably exist between these ecotypes and other species, orthodox taxonomy operating on overall morphological similarity, would be found to be severely wanting in this instance. Jones (1968) has argued for a broader concept of oak species so as to take account of a wider range of variation than might otherwise be acceptable, but even this would not help in the situations described by Garilov (1969).

The oak taxonomist must inevitably turn to the Biological Species Concept in the majority of cases where morphological criteria are insufficient, but here again difficulties arise. Since species would be conceived as interbreeding entities reproductively isolated from other such entities, the widespread hybridisation observable within the genus would cause problems. Assuming that in the majority of cases, reported hybrids between oak species are actual hybrids and not unusual forms of one species or the other, then the level of hybridisation between component species of the genus represents a very high level. This is, of course, a bad assumption since only in a relatively few instances have reports of oak hybrids back-up genetical information to substantiate the claim, but nevertheless if only half of all reported hybrids are actual hybrids, the proportions are still large. It is in fact somewhat difficult to generalise about the isolating mechanisms operating to maintain specific integrity within the genus. On the American continent, eco-geographical isolating barriers would appear to be widespread, since in many instances,

allopatric species freely hybridise in areas of contact. Muller (1952) has described many situations where this would appear to be the case. Heslop-Harrison (1953) has summarised the position in North American oak species. He regards many species as interfertile, but argues that although growing sympatrically, interfertile species do not intergrade due to rapid elimination of hybrid progeny. In Europe, closely related species tend to be more sympatric, perhaps with different ecological preferences, but these are usually insufficiently strong to allow complete allopatricity to develop. Seasonal, temporal or mechanical isolating mechanisms would not seem to be important, since flowering times overlap greatly, and floral structure is remarkably uniform. The work of this thesis suggests that post-zygotic mechanisms do exist, and that these may arise at different stages in the growth of the seedling, but pre-fertilisation, post-pollination mechanisms are probably as important. Consequently, within the genus, isolating mechanisms that exist are comparatively easily broken and therefore any biological species concept for oaks developed along the lines of reproductively isolated, inbreeding groups of individuals would appear suspect. Davis and Heywood (1963) note that the 'feeble internal sterility barriers' make the genus 'a bête noire for all but the devoted monographer'!

A hyper-space approach might prove useful in these cases, where every dimension of the hyper-space represented different distinguishing or partially distinguishing characteristics. Thus an eco-geographical dimension would give only partial separation of Q. robur from Q. petraea, whilst a 'crossability' dimension would give almost complete separation. Previous work on British oaks has concentrated on the morphological aspects of species differences; some of the results of this thesis would add new dimensions to the hyperspace differentiation of Q. robur and Q. petraea. The karyotype analysis of the species and their artificially produced hybrids have shown conclusively that differences at the morpho-

logical level are reflected in differences at the chromosomal level, the chromosome sets being sufficiently different as to permit better identification of individuals than with morphological characters alone. The existence of a 'hybrid' karyotype in the hybrid cells serves only to emphasise the differences between the species in this respect. The results of the attempted artificial crossings similarly enhance the differences. Although high levels of hybridisation have been reported, attempts at producing artificial hybrids remain singularly unsuccessful, so that the reproductive isolation of the two morphological groups is almost complete and rarely permits hybrids to be formed. Even morphological characters adequately differentiate the taxa provided that a multivariate approach is considered. The wide range of phenotypic plastic responses reported in earlier chapters, although reducing specific differences, is insufficient in some important characters to mask species differences. Physiological differences might similarly provide a dimension of possible separation. Only by a multivariate approach of this type can adequate recognition of the taxa Q. robur and Q. petraea be performed. Indeed only by careful inspection of morphological characters can sufficient clarity of difference be observed to detect possible hybrids, and then only through a multivariate approach can the work prove particularly useful.

Consideration of the species concept as applied to Quercus illustrates some interesting differences between the genus in America and in Europe. As argued earlier, in America, isolating mechanisms appear to exist only at the eco-geographical level, whilst in Europe, sympatric species are separated by post-pollination isolating mechanisms. It must be argued that, in America, the invasion by Quercus might have been particularly recent, the only isolating mechanisms being produced at the eco-geographical level. In Europe, more stringent mechanisms exist to

separate the species, and since many European oak species now live sympatrically, the indications are that in Europe, eco-geographical separation has allowed other reproductive isolating mechanisms to be developed in the past under allopatric conditions and these mechanisms have been maintained now as the species live sympatrically. It is difficult to envisage post-pollination isolating mechanisms being developed under sympatric situations, unless these are the result of polyploidy, and since polyploids have not been recorded for the genus, this would seem an unlikely situation. The impact of man on the more 'natural' areas of Europe has undoubtedly been influential in bringing about sympatric situations between European oaks, but the importance of man has rarely been called into question in explaining hybrid oak situations in America. Solbrig (1970) has argued that natural phenomena such as glaciations, mountain building, erosion, etc. in America have created disturbed habitats in the past. If such areas were available for colonisation, then introgression between oaks may have been proceeding for a relatively long period of time, the introgressed populations existing in the naturally disturbed habitats. Data from Asiatic oak species on the occurrence of hybrids, ecological preferences, etc. might prove very useful in detecting the history and evolution of the genus, but as yet little work has been completed on the Asiatic species. Without such critical work, Europe would seem to be the best candidate for the original differentiation and spread of the genus, with the American oaks recently, and still rapidly, evolving.

Introgression between *Q. robur* and *Q. petraea* - the evidence

For all the extensive and intensive work performed in the field of introgression, no criteria exist for its recognition. Anderson (1949) records only the loose association of characters as being the proof

required for accepting the presence of introgression, but this is clearly insufficient particularly in situations where the parental species themselves are so very variable. In these situations, it is perhaps of importance to clarify the position as to presence or otherwise of hybrids before considering whether such hybrids have by backcrossing produced an introgressed situation. Gottlieb (1972) has recorded the criteria neecssary for the establishment of hybrid origin:

1. Morphological intermediacy.
2. An additive profile for biochemical characters that are present in each parent, but not in both of them.
3. Unusual amounts of interpopulational morphological variability thought to result from segregation of populational differences.
4. Distribution of the 'hybrids' in the zone of geographical overlap of the parents.
5. Occurrence of the 'hybrid' in more recent geological formations than the parents.
6. Occurrence of the 'hybrid' in ecologically intermediate habitats and showing intermediacy for physiological characters.
7. Existence of at least partial fertility in F_1 hybrids between the parents to permit the possibility of the production of segregant genotypes.
8. Experimental synthesis of individuals resembling the hybrid taxon in segregants of hybrids between the parents.

No single criterion with the possible exception of the last can prove conclusively that hybrids exist. For example, morphological intermediacy might reflect ancestral rather than hybrid status; unusual amounts of inter-populational morphological variability might reflect differences in the breeding system, environmental heterogeneity, founder effects, etc. Only a composite answer in which several criteria are considered can provide an adequate answer if synthesis of actual hybrids is lacking. The work of this thesis would serve to provide information on most of these criteria. Individual trees exist which are certainly intermediate for a large number of characters. No biochemical differences between

the species have been reported, and therefore recognition of additive profiles in suspected hybrids is inappropriate. (Work completed with Mr. S. J. Wainwright of Leeds University on trying to detect acid phosphatase differences between the species proved negative.) Unusual amounts of populational variability exist in British oak populations, and in many instances these are consistent with hybridisation, but the possible backcrossing in these populations with subsequent removal of certain segregate types would therefore not normally result in the production of a complete range of segregate types, these having been selected against at the seedling stage. Although no fossil evidence exists, there is good evidence that British oak species are to some extent ecologically separated and that hybrids do occur in hybrid habitats paralleling the more obvious situations in America. Geographical overlap is complete, and in Russia, where Q. petraea is absent but Q. robur extends, no trees resembling hybrids between Q. robur and Q. petraea have been described. Partial fertility of the F_1 hybrid is only important where F_2 and backcross progeny are considered to occur and thus is of paramount importance to the proof of introgression. Fertility as such can be considered as a variety of facets of the reproductive process - it may be male fertility, female fertility, etc., and its measurement must always be relative. The results from the assessment of pollen viabilities in a variety of different oak populations indicate that morphologically intermediate types have low pollen viability, thus satisfying that criterion. Synthesis of the hybrid has not, however, been possible, at least as far as producing a fully matured viable individual is concerned, but the partial success, coupled with the karyotype analysis of the hybrid seedlings formed, goes some way to satisfying that criterion. On balance, therefore, the evidence supports the view that hybrids exist between Q. robur and Q. petraea.

Having established the existence of hybrids, it remains to establish that backcross derivatives also exist. Only circumstantial evidence is available. Trees of morphologically intermediate type exist in oak populations, but the range of plasticity must cast doubt on any firm interpretation of the status of these trees using morphological criteria alone. The circumstantial evidence comes from the pollen viability studies. It has been recognised in several instances that F_2 backcross derivatives may be expected to possess restored or partially restored fertility. A correlation, therefore, between fertility and morphological type as recorded in Figures 7.5-7.9 over the whole range of morphological types is good, but circumstantial evidence for backcross derivatives. Anderson (1949, 1953) has emphasised the morphological aspects of backcross derivatives, and the analysis of the population data from oak populations adequately covers this aspect of introgression in oaks, the use of PCA possibly providing a more objective approach than the PSD used by Cousens (1963, 1965), Wigston (1971) and other workers in this field.

Criticisms of introgression exist, however, and these may be considered under two headings: 1) criticisms against introgression *per se* and 2) criticisms against introgression specifically as it applies to *Q. robur* and *Q. petraea*.

Heiser (1973) has summarised general arguments against introgression, and the review of these below owes much to his researches (see Heiser, 1949, 1973). Seven major arguments have been levelled at introgression as alternative explanations to what have been regarded as examples of introgression:-

1. The presence of characters of two species in individuals does not necessarily indicate hybridity, the individuals may, for example, represent remnants of ancestral populations out of which the two species differentiated. This 'genetic-pool hypothesis' has been extensively criticised

by Anderson (1953) who argued that unintrogressed species might be found in older areas, whilst introgressed species are more likely to appear in newly invaded areas. In Q. robur and Q. petraea, hybrids occur throughout their sympatric ranges, but the situation in Scotland, at the westerly limit of the species, represents the most variable area described. It is unlikely that Scotland is the ancestral home of these species. Anderson (1953) has also pointed out the loose association of characters found in introgressed types not the wholesale presence of characters of one species in individuals of another as suggested by this alternative.

2. Apparent introgressed situations may be brought about by mutational effects. An excellent example of a related phenomenon occurs in Q. robur. Generally the species is regarded as completely glabrous, and observation of a wide range of material confirms this view. Very occasionally stellate hairs are found on the undersurface of the leaf particularly in the axils between the main vein and lateral veins, and these in the past have been ascribed to the hybrid origin of that individual (eg. Cousens, 1963). Where spangle galls of Cynipid wasps are produced on the undersurface of otherwise glabrous Q. robur leaves, the outer surface of the galls are covered with stellate hairs. The genetic apparatus of Q. robur must contain coding for stellate hairs, but this remains latent until the cancerous growth of the gall is produced. A similar situation has been noted by the author on glabrous Sycamore leaves which produce hairs around similar insect infestations. Although these instances are not thought to represent examples of mutation they do emphasise the difficulty in interpreting such characters as evidence of hybridisation.

3. Autogamy has been suggested as a possible mechanism for the production of introgression-like effects, but Quercus is an exclusively out-breeding genus and not subject to such arguments.

4. Similarly, segregation in a polyploid species may cause introgression

like effects, but polyploid series are absent from Quercus.

5. Intergradation may also produce introgression-like situations, and this represents an important criticism. Intergradation consists of rapidly altering character gradients between populations and it is possible to recognise two different types - primary intergradation in which the character gradient develops slowly and whilst the populations are in continuous contact, and secondary intergradation in which the differences are evolved in completely separated populations but which are today in contact. Using the wider view of hybridisation, secondary intergradation would be seen to involve hybridisation and recombination, whilst primary intergradation would only involve recombination. Barber and Jackson (1957) have argued that where ecological change is particularly great, simultaneous clinal variation in the frequencies of genes at a number of loci might be expected. Selection would have the effect of producing in such a system the loose association of characters considered important by Anderson, and thus primary intergradation might produce situations comparable to and possibly confused with introgression. The highly variable situations recorded in Scotland by Cousens (1963), Cornwall by Wigston (1971) and Wales by the present author may represent similar effects, where ecological gradients are particularly steep, and have led to the highly variable populations described by Barber and Jackson (1957). It is not possible to counter this argument as far as Scotland or Cornwall is concerned, but the situation in Wales is such that populations of suspected hybrid status, for which pollen viabilities were measured argues strongly that individuals of these populations of intermediate status are hybrids. Furthermore, the very variable populations recognised by Barber and Jackson (1957) in Eucalyptus were not found in oak populations studied for this thesis, with the exception of the 'intermediate' populations. All suspected introgressed populations

contained relatively few hybrid trees, and in a large majority of the cases, introgressed populations also contained alien trees of the other species which may have represented the original source of 'infection' of the trees of the population. The intermediate populations themselves are more problematical but since they contain a small proportion of 'pure' individuals, a hybrid origin for these populations seems more appropriate, particularly as the intermediate population assessed for pollen viability (DI) showed a high proportion of individuals with low pollen viabilities.

6. Heiser (1973) also points out a further situation which may involve difficulties - the hybrid swarm. Heiser (1973) emphasises the differences between the hybrid swarm situation where, although backcross individuals occur, the swarm usually occupies a restricted area, is transitory and no gene transfer takes place outside of the area and introgression which involves repeated backcrossing is not a transitory effect and gene transfer from outside is an essential part of the process. The dividing line between hybrid swarms and introgressed populations, although possible to define reasonably exactly, has been transgressed many times in publications, purely hybrid swarm situations being described as introgression, although rarely vice-versa. Examples of hybrid swarms might well be the intermediate populations, and indeed the argument proposed in Chapter 6 that these occurred in hybrid habitats between the habitats of the parental types supports this view.

7. Lastly, introgressive hybridisation derivatives may be no more than F_1 hybrids. This would be a reasoned argument in oaks, where the large range of variability observable in the pure species poses problems for the delimitation of the pure species and recognition of hybrid types. F_1 individuals of oaks are also likely to show a wide range of variation, due to factors such as position within the population, siting of the

population, etc. If only F_1 hybrids were present in British oak populations, and backcross derivatives absent, then pollen viability might be expected to show only two states - high viability for the pure species and low viability for the F_1 hybrids. The fact that a range of viabilities exist correlated with morphological type would dismiss such explanations unless reproductive potential were as variable as morphological variability. This is considered unlikely.

General criticisms aside, there remain the conceivably more important criticisms levelled against introgression within British oaks. These are:

1. The natural variability of the species
2. The apparently low fertility between the species
3. The time factor

Jones (1959, 1968) has argued that the large number of hybrid oaks recorded in the British flora is due to a misunderstanding of the important characters and their range of expression in the pure species. Criticisms such as this are entirely justified, when differentiation of the species is attempted using only two characters, as in the case of the PSD and where hybridisation is recognised only by six characters, eg. Cousens (1963). One population sampled and scored by Cousens (1965), Monks Wood Nature Reserve, was also (accidentally) sampled during the present work. Using only four characters, the so-called secondary characters, some 22% of the individuals of the population as scored by Cousens (1965) were classified as intermediate and therefore of hybrid origin. A sample of double the size by the present author of the same population (population BBA) failed to produce any evidence of hybridisation, the mean hybrid index being 97.7 (in a range of 0-340). Two possible reasons for this would be sampling in different parts of the same population or it is more likely due to a wider range of characters used in the present study (17 characters), and the multivariate approach to

the analysis of the data. Larger population samples, use of more characters and analysis with multivariate means would possibly produce different results for the Scottish populations from those recorded by Cousens (1963). Consequently, provided phenotypic plastic responses are taken into account, and provided a sufficiently wide range of characters is used and analysed in concert by multivariate analysis, the criticisms by Jones although not completely negated are lessened. Indeed, the complications of plasticity, since they confer on the individual the ability to survive, may be exceptionally important in understanding the variational responses observed in oak woodlands. Bradshaw (1965) and Cook (1968) have discussed the importance of phenotypic plasticity and its bearing on evolutionary processes.

The low fertility between the species has been cited by Jones (1959) as evidence of the difficulty of supporting large hybrid numbers, but counter arguments have already been produced (see Chapter 8).

The time scale for introgression to take place has possibly been since the last glaciation and must, therefore, have included a large number of generations, although Cousens (1965) maintains that his later researches indicate that Q. robur was already a very variable species before it arrived in Scotland, it having carried alien genes along with it from its origin after the last glaciation. The shorter generation time under coppice management would also have enhanced population change. The comments of Solbrig (1970) on naturally occurring disturbed habitats mentioned earlier in this chapter are also of relevance here, since disturbed areas of possible hybrid type would have been numerous after the last glaciation.

Davis and Heywood (1963) make a very important point concerning the recognition of hybrids:

"The main danger of the failure to recognise hybrids (as with .. Quercus ..)

is that the circumscription of the species will be amplified to include interspecific hybrids, quite apart from the justifiable inclusion of a limited amount of variation that may be brought into a species through introgression. It should be borne in mind that the more closely species resemble one another, the harder hybridisation will be to detect but the more likely it may be to occur."

This, I believe, is an adequate answer to the view of Jones (1968) who would take a broad view of the species and fail to recognise hybrids.

The levels of introgression in British oakwoods

Some mention of this has already been made (see Chapter 5) but this would seem an appropriate point to enlarge further the comments made previously. Assessment of hybridity in the area studied for this thesis would argue for a level of between 7% and 12% over the whole area, with the majority of this hybridity concentrated in the populations to the west. Cousens (1965) in trying to obtain 'good' species material for comparison with the Scottish oakwoods sampled 17 populations in the eastern half of England from Doncaster to the south coast (average population size - 21 trees). Of these, 87 trees out of the 357 trees sampled, or 24.4%, were regarded as being intermediate in status. Samples from the same area, but based on a wider range of characters, in the present work gave 34 trees out of 1283 in the range 150-189 as possible hybrids or 2.7%, and 65 trees or 5.1% in the range 140-199. There would appear to be an order of magnitude of some 5 or 10-fold difference between these results and those of Cousens(1965). As discussed above, large discrepancies also occurred between the present author's sample of Monkswood and that by Cousens (1965). If the original hybrid indices had been incorrectly set, then the major effect would have been to extend either the hybrid zone or the parental species part of the hybrid index. One important estimate of effects of this type would be seen in the scatters of pollen viability against morphological indices.

An exaggerated parental zone or hybrid zone would have shown clearly in these results. As it is, the zones would appear well defined and of equal proportions. It is from this evidence, and these scatter diagrams (Figure 7.5-7.9), that arguments for concluding that trees in the zone 150-189 are of hybrid origin can be accepted. Overall, the level would lie at between 7% and 12%, although evidence from pollen viability studies suggests a lower rather than a higher figure. The discrepancies between these results and Cousens (1965) for the same area, although admittedly different woodlands, seems to suggest that discrepancies might also arise should the techniques applied in this thesis be applied to Scottish oak populations.

Cousens (1965) has argued that there is evidence of a cline of increasing introgression northwards in England and Scotland, and he suggests that a possible explanation for this might lie in the more frequent cross-pollination between the species in the north due to a shorter growing season and therefore greater overlap of flowering times. Jones (1959) fails, however, to record any difference in flowering time between the species, and personal observation over four years would indicate that in mixed forests no differences occur in flowering time, but that differences between different populations of the same species might be quite great - up to two or three weeks different. If hybrid oaks are found in greater numbers in Scotland than elsewhere in the United Kingdom, it is thought more likely to be due to different selection pressures operating at the periphery of the species range from those in the centre. Agnew (1968b) has shown that populations at the extreme edge of a species range may be as variable or less variable than those at the centre, the reasons being that at the periphery, environmental stress will tend to select for few genotypes, whilst optimal environmental conditions at the centre of the range will allow a wider range of

genotypes to survive. In Quercus the wide variability observed at the periphery in both Wales and Scotland indicates possible selection against the genotypic norm and selection for atypical genotypes. For comparison, it would be useful to investigate populational variation in Q. robur and Q. petraea at their northern and southern extremities where they remain sympatric, in order to test the above hypothesis.

Future Work

As already mentioned, progress is underway in building a populational model of introgression, but other work is also required if an answer is to be found to the 'hybrid controversy' (Gardiner, 1970). Although attempts at producing the hybrid Q. robur x Q. petraea were made during the course of this thesis, I think that these should be repeated, possibly on a much larger scale, and including suspected hybrid individuals. The pollen viability work could also be usefully extended to other areas.

One area of investigation not yet successfully attempted, however, is that of chemotaxonomy. Several studies exist in which chemical constituents of plants have been useful in understanding relationships between populations. Tigerstedt (1973) has, for example, used iso-enzyme differences (esterase, acid phosphatase and leucine-amino-peptidase) to study variation between marginally situated and central populations of Picea abies. Hanover and Wilkinson (1970) have used chromatographic analysis of phenolic compounds to detect introgressive hybridization in the genus Picea and this would appear to be one of the few remaining approaches left for a definitive answer to the hybrid controversy.

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A General Statistical Note

The analyses for much of the data processing for this thesis follows the worked examples of Sokal and Rohlf (1969). For convenience and speed, these were programmed in either 4100, 1900 ALGOL or BASIC. Significance levels for the analyses presented in the following appendices are taken from Rohlf and Sokal (1969).

APPENDIX 1

Appendix 1 lists the means, standard deviations and analysis of the leaf characters at different canopy sites discussed in Chapter 2.

The first part records the means, standard deviations and least significant difference of means of leaf characters at three canopy heights (upper, middle, lower), in different woodland types (open and closed) for different aspects (north and south, N and S) and different degrees of exposure (sun and shade, S and Sh). The first figure for each tabular cell is the mean, the second (underneath) the standard deviation..

The second part records the three-way analysis of variance of the above data, the main effects being canopy heights, species/woodland types, and aspects/exposure. The table gives the variance ratio for each effect and interaction between them, together with the error variance and significance levels for appropriate combinations of degrees of freedom.

	Q. robur				Q. petraea			
	SS	SSh	NS	NSh	SS	SSh	NS	NSh
Upper Open	97.2	78.3	88.9	75.4	93.0	75.4	78.3	68.7
	4.7	3.6	5.6	4.6	4.2	3.7	4.6	3.7
Closed	75.7	62.8	69.3	60.0	86.9	70.1	74.8	65.2
	4.9	3.1	7.6	4.4	3.4	3.3	5.7	1.6
Middle Open	87.6	72.3	79.1	69.7	84.1	75.2	76.1	68.4
	5.6	4.9	4.3	3.7	5.8	4.4	3.5	5.2
Closed	73.4	60.4	65.0	57.9	79.0	72.1	69.7	60.4
	6.3	3.9	5.2	4.3	4.2	2.3	2.9	2.6
Lower Open	77.4	68.4	75.1	65.3	78.9	71.0	73.2	63.9
	3.3	6.1	3.2	4.6	3.9	5.0	3.0	6.5
Closed	69.0	59.8	63.8	55.1	72.8	70.0	68.7	55.3
	3.4	3.7	4.1	3.7	2.1	2.1	2.4	1.9

Total lamina thickness

(x 2.35 = μ)

Least significant difference of means: 0.1% - 4.0; 1% - 3.1; 5% - 2.4

Upper Open	51.2	36.6	43.7	34.3	45.4	34.1	37.1	30.3
	2.9	4.3	2.9	4.6	2.9	3.4	3.1	4.1
Closed	35.9	26.7	32.3	26.1	41.9	30.4	34.4	26.9
	3.3	2.5	4.2	3.0	1.9	1.9	2.8	0.9
Middle Open	42.2	31.5	35.3	29.6	40.4	32.8	33.1	26.8
	3.7	3.7	4.8	3.2	4.2	3.3	2.9	2.2
Closed	35.2	24.9	28.9	23.6	37.2	29.9	29.1	23.9
	3.9	2.0	3.0	2.1	2.7	2.4	1.6	1.2
Lower Open	36.8	29.2	32.3	26.0	35.6	28.4	31.2	24.7
	1.9	1.8	3.6	2.2	1.5	1.4	1.1	0.8
Closed	30.7	23.4	26.9	20.6	32.6	26.8	28.1	20.2
	1.9	1.8	3.6	2.2	1.5	1.4	1.1	0.8

Palisade thickness

(x 2.35 = μ)

Least significant difference of means: 0.1% - 2.9; 1% - 2.2; 5% - 1.7

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	26.8	23.0	25.4	25.0	27.7	23.4	23.0	24.8	
	2.9	4.5	3.6	3.3	3.8	3.3	5.4	3.0	
Closed	23.5	20.5	21.3	21.0	25.9	23.2	23.5	24.6	
	4.5	2.9	3.9	2.8	2.1	3.4	4.6	1.7	
Middle Open	25.3	24.9	26.6	23.7	25.5	27.2	23.0	26.4	
	3.0	3.6	3.2	2.9	3.6	3.4	3.9	4.7	Spongy Mesophyll Thickness
Closed	23.0	22.0	21.1	21.6	26.7	30.6	25.8	24.4	
	3.7	2.7	2.9	2.0	4.6	3.9	2.4	2.2	(x 2.35 = μ)
Lower Open	25.4	26.4	28.8	25.8	28.9	27.7	28.9	27.5	
	3.4	2.8	3.7	3.4	2.4	3.3	2.9	2.9	
Closed	25.7	26.2	24.4	24.2	21.7	32.9	28.4	24.2	
	2.5	3.1	2.2	4.1	1.4	1.8	2.3	2.0	

Least significant difference of means: 0.1% - 3.1; 1% - 2.4; 5% - 1.9

Upper Open	19.2	18.6	19.7	15.9	19.8	17.7	18.1	13.5	
	2.5	3.0	2.6	1.9	2.6	2.4	1.9	3.5	
Closed	16.1	15.4	14.9	12.8	19.0	16.3	16.7	13.6	
	2.4	2.1	2.7	1.8	1.5	2.2	2.6	1.5	
Middle Open	20.0	15.6	17.0	16.2	18.0	15.1	19.9	15.1	
	2.2	3.1	2.6	3.4	2.9	2.4	3.2	2.2	Epidermal Thickness
Closed	15.0	12.6	13.8	13.4	14.9	11.5	14.7	12.0	
	2.5	2.0	1.8	2.4	3.4	2.3	1.6	1.6	(x 2.35 = μ)
Lower Open	15.0	12.6	13.8	13.4	14.2	14.7	13.0	11.5	
	1.9	1.8	3.2	2.8	2.3	3.2	2.6	2.3	
Closed	12.6	9.9	12.4	10.6	12.9	10.1	12.0	10.8	
	1.5	1.4	2.1	2.7	1.2	1.2	1.5	1.4	

Least significant difference of means: 0.1% - 2.3; 1% - 1.8; 5% - 1.3

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	3.15	2.52	2.36	2.08	3.07	2.74	2.48	1.98	
	0.42	0.50	0.53	0.29	0.47	0.46	0.60	0.42	
Closed	2.80	2.20	1.96	1.80	3.08	2.52	2.04	1.68	
	0.40	0.57	0.67	0.40	0.50	0.35	0.37	0.43	
Middle Open	3.02	2.31	2.28	1.89	2.88	2.26	2.11	1.78	
	0.46	0.44	0.44	0.30	0.30	0.32	0.55	0.39	Number of cell layers in palisade tissue
Closed	2.80	2.20	2.16	1.56	2.76	2.20	2.10	1.55	
	0.40	0.40	0.55	1.24	0.40	0.43	0.51	1.32	
Lower Open	2.84	2.01	1.97	1.75	2.62	1.90	1.86	1.62	
	0.44	0.60	0.32	0.42	0.32	0.43	0.43	0.31	
Closed	2.56	1.76	1.88	1.48	2.48	1.92	1.72	1.32	
	0.50	0.43	0.43	0.51	0.27	0.45	0.37	0.35	

Least significant difference of means: 0.1% - 0.47; 1% - 0.37; 5% - 0.28

Upper Open	17.4	15.6	12.2	10.9	15.6	13.4	11.4	9.1	
	1.2	1.1	0.8	1.2	1.3	1.6	1.3	1.6	
Closed	12.8	9.6	10.6	8.6	12.7	9.1	9.9	7.8	
	1.8	1.7	1.4	1.1	1.9	1.5	0.9	1.0	
Middle Open	15.2	13.6	12.2	10.3	15.5	10.7	10.3	8.2	
	1.2	1.4	1.2	1.5	1.5	1.3	1.4	1.4	Stomatal density
Closed	11.3	10.1	10.2	8.0	11.9	9.3	9.3	6.7	
	0.9	0.9	0.9	1.1	1.4	1.5	1.3	1.2	(x 23 = per mm^2)
Lower Open	12.6	13.6	12.0	9.9	12.9	10.6	10.2	7.8	
	0.8	0.8	0.7	0.9	1.1	1.4	1.3	0.9	
Closed	9.5	9.0	9.1	8.6	10.5	7.0	8.7	6.4	
	0.8	0.8	0.7	0.9	0.8	1.4	1.0	1.1	

Least significant difference of means: 0.1% - 1.2; 1% - 1.0; 5% - 0.7

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	.276	.294	.286	.332	.298	.311	.294	.362	
	.019	.021	.021	.016	.019	.037	.012	.019	
Closed	.311	.327	.308	.351	.298	.332	.315	.377	
	.041	.035	.036	.021	.047	.027	.032	.026	
Middle Open	.289	.364	.337	.341	.304	.362	.347	.387	Spongy Mesophyll ratio
	.024	.022	.017	.017	.019	.029	.029	.019	
Closed	.314	.365	.326	.374	.339	.424	.371	.405	
	.033	.032	.042	.027	.033	.035	.029	.028	
Lower Open	.329	.387	.384	.395	.367	.391	.395	.432	
	.023	.027	.032	.028	.022	.022	.024	.028	
Closed	.373	.434	.383	.439	.390	.346	.395	.596	
	.034	.022	.019	.027	.034	.039	.036	.024	

Least significant difference of means: 0.1% - 0.026; 1% - 0.021; 5% - 0.016

Upper Open	.198	.238	.222	.212	.213	.236	.369	.197	
	.027	.025	.022	.013	.027	.022	.026	.026	
Closed	.214	.247	.215	.213	.219	.233	.224	.209	
	.035	.039	.046	.027	.032	.034	.037	.039	
Middle Open	.229	.217	.216	.233	.215	.202	.262	.221	
	.022	.026	.021	.031	.019	.022	.022	.028	Epidermal ratio
Closed	.204	.222	.229	.218	.189	.160	.212	.199	
	.024	.025	.038	.027	.027	.033	.027	.025	
Lower Open	.195	.185	.185	.206	.181	.208	.178	.181	
	.024	.021	.026	.029	.022	.023	.021	.023	
Closed	.183	.167	.194	.193	.178	.145	.175	.195	
	.024	.024	.025	.024	.014	.027	.031	.027	

Least significant difference of means: 0.1% - 0.026; 1% - 0.020; 5% - 0.015

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	.526	.468	.492	.456	.489	.453	.474	.441	
	.019	.017	.015	.013	.019	.016	.019	.019	
Closed	.474	.426	.467	.435	.482	.434	.461	.414	
	.029	.033	.033	.034	.007	.011	.013	.009	
Middle Open	.482	.437	.447	.426	.481	.436	.436	.392	
	.030	.029	.015	.021	.011	.013	.022	.018	Palisade ratio
Closed	.477	.413	.446	.408	.472	.416	.418	.396	
	.029	.016	.024	.018	.004	.004	.019	.015	
Lower Open	.476	.428	.431	.399	.452	.401	.427	.387	
	.015	.011	.014	.016	.009	.023	.021	.016	
Closed	.445	.391	.421	.374	.449	.384	.416	.367	
	.018	.015	.032	.032	.010	.013	.010	.009	

Least significant difference of means: 0.1% - 0.018; 1% - 0.014; 5% - 0.011

Upper Open	0.12	0.46	0.62	0.96	3.31	3.28	3.35	3.46	
	0.72	0.30	0.41	0.85	1.05	1.15	0.53	1.25	
Closed	0.22	0.48	0.76	0.86	3.22	3.22	3.42	3.52	
	0.51	0.76	0.89	1.11	0.55	1.09	0.88	0.84	
Middle Open	0.27	0.86	0.56	0.88	3.28	3.42	3.52	3.81	
	0.59	0.83	0.61	0.81	0.80	1.12	0.50	0.85	Basal shape of lamina
Closed	0.48	0.94	0.66	0.94	3.54	3.74	3.72	3.84	
	0.76	1.04	0.89	1.13	0.79	0.60	0.57	0.42	(Index)
Lower Open	0.34	0.88	0.74	1.02	3.34	3.56	3.72	3.80	
	0.82	0.92	0.93	0.42	0.63	0.86	0.76	0.73	
Closed	0.58	0.96	0.82	1.24	3.62	3.82	3.80	3.94	
	0.91	1.05	1.00	1.10	0.73	0.48	0.45	0.24	

Least significant difference of means: 0.1% - 0.75; 1% - 0.59; 5% - 0.45

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	0.17	0.36	0.42	0.53	2.98	3.42	3.54	3.67	
	0.62	0.50	0.71	0.62	1.21	0.84	0.76	0.91	
Closed	0.26	0.70	0.66	0.98	3.14	3.34	3.44	3.70	
	0.66	0.76	0.89	0.96	0.97	0.85	0.91	0.61	
Middle Open	0.15	0.51	0.58	0.86	3.06	3.51	3.61	3.58	
	0.89	0.91	0.82	0.40	0.94	1.11	0.93	1.03	Auricle development
Closed	0.48	0.80	0.70	1.20	3.40	3.64	3.78	3.84	
	0.79	1.01	0.95	1.01	0.78	0.63	0.51	0.42	(Index)
Lower Open	0.32	0.88	0.99	1.31	3.36	3.84	3.72	3.69	
	0.53	0.70	0.65	0.70	1.31	0.89	0.91	0.88	
Closed	0.60	0.94	0.96	1.28	3.74	3.72	3.90	3.96	
	0.83	1.02	1.05	1.07	0.53	0.61	0.36	0.73	

Least significant difference of means: 0.1% - 0.78; 1% - 0.61; 5% - 0.46

Upper Open	0.96	0.45	0.47	0.27	3.89	3.84	3.61	3.47	
	0.44	0.41	0.77	0.57	1.21	0.74	0.63	0.91	
Closed	0.70	0.32	0.32	0.22	3.94	3.72	3.60	3.52	
	0.86	0.59	0.62	0.47	0.24	0.57	0.73	0.79	
Middle Open	0.88	0.32	0.28	0.16	3.92	3.68	3.42	3.16	
	0.55	0.40	0.70	0.42	0.66	0.51	0.50	0.51	Leaf hairiness
Closed	0.48	0.12	0.32	0.05	3.88	3.72	3.58	3.34	
	0.76	0.39	0.59	0.20	0.39	0.57	1.58	0.92	(Index)
Lower Open	0.91	0.34	0.27	0.08	3.76	3.51	3.40	3.20	
	0.34	0.61	0.62	0.71	0.66	0.83	0.80	0.74	
Closed	0.48	0.06	0.04	0.00	3.80	3.52	3.62	3.24	
	0.79	0.34	0.20	0.00	0.40	0.74	0.67	1.06	

Least significant difference of means: 0.1% - 0.63; 1% - 0.49; 5% - 0.37

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	5.26	5.07	5.15	4.83	6.85	5.42	5.62	5.41	
	0.52	0.44	0.66	0.65	0.64	0.72	0.62	0.63	
Closed	5.22	4.80	4.88	4.52	6.92	5.32	5.70	5.22	
	0.55	1.45	0.44	0.76	0.53	0.74	0.76	0.55	
Middle Open	5.15	4.99	5.02	4.71	5.99	5.35	5.41	5.36	
	0.59	1.02	0.64	0.86	0.73	0.79	0.69	0.72	Lobe number
Closed	5.02	4.78	4.80	4.48	6.30	5.18	5.64	5.02	
	0.98	0.55	0.61	0.74	0.81	0.56	0.66	0.65	
Lower Open	4.95	4.62	4.56	4.43	5.71	5.09	5.28	5.09	
	0.60	0.71	0.74	0.71	0.66	0.85	0.80	0.96	
Closed	5.00	4.10	4.02	3.52	5.86	4.88	5.18	4.84	
	1.01	0.61	0.62	0.54	0.61	0.59	0.63	0.62	

Least significant difference of means: 0.1% - 0.67; 1% - 0.62; 5% - 0.40

Upper Open	67.4	48.4	35.2	20.1	10.4	9.8	7.9	6.3	
	10.4	6.1	12.4	9.6	5.1	2.6	3.6	5.9	
Closed	54.0	36.6	39.9	21.6	4.9	2.9	0.0	0.0	
	14.4	10.9	11.4	9.9	6.0	4.7	0.0	0.0	
Middle Open	51.6	46.2	38.2	22.0	8.7	8.2	5.9	6.0	
	7.4	8.2	10.0	8.4	4.8	4.1	4.9	5.9	Venation
Closed	50.8	43.1	40.1	23.1	6.2	0.0	5.2	0.0	(Percentage)
	10.2	7.5	6.9	7.2	7.5	0.0	4.5	0.0	
Lower Open	48.6	35.6	28.5	19.6	8.0	6.4	5.0	5.0	
	11.1	10.2	10.8	12.1	3.9	6.2	1.8	6.8	
Closed	32.8	27.7	26.0	17.8	0.9	0.0	1.1	0.0	
	9.4	7.5	6.6	5.9	2.7	0.0	3.2	0.0	

Least significant difference of means: 0.1% - 6.9; 1% - 5.4; 5% - 4.1

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	22.7	27.7	32.5	36.3	28.3	32.7	38.7	42.6	
	2.7	3.4	3.6	1.2	2.4	3.4	3.9	2.7	
Closed	20.8	28.5	30.1	37.7	31.4	40.2	40.2	44.7	
	2.1	2.3	2.3	2.2	2.6	2.7	3.0	3.3	
Middle Open	27.3	29.4	30.6	35.4	28.7	37.6	40.4	43.1	
	2.8	2.1	2.6	2.7	3.8	3.3	3.4	2.2	Leaf area
Closed	24.9	30.1	32.4	40.8	34.7	40.4	40.6	45.1	
	2.5	1.6	2.7	2.1	2.5	2.2	2.4	3.4	(sq. cms)
Lower Open	30.7	30.5	33.2	37.6	34.2	38.9	40.0	44.7	
	4.0	2.3	3.9	2.3	3.8	3.7	4.7	3.2	
Closed	30.1	35.0	38.1	40.0	39.1	43.0	40.0	46.9	
	2.4	2.3	2.6	2.1	2.0	2.7	2.1	3.1	

Least significant difference of means: 0.1% - 2.7; 1% - 2.1; 5% - 1.6

Upper Open	73.7	82.5	78.6	78.4	92.5	93.2	107.3	109.2	
	3.9	4.5	2.4	4.1	3.6	3.6	4.1	3.8	
Closed	78.8	84.1	81.9	92.1	98.7	107.1	109.9	117.7	
	2.3	1.6	1.7	1.7	3.3	1.8	3.6	2.0	
Middle Open	85.9	88.7	89.6	92.6	93.4	103.7	108.2	115.8	
	3.6	3.3	3.5	4.3	4.2	4.3	4.8	2.7	Lamina length
Closed	86.0	91.9	94.5	103.0	104.7	115.6	117.3	124.6	
	2.1	2.0	2.2	3.3	2.5	2.3	3.2	2.7	(mm)
Lower Open	95.2	95.6	97.6	103.0	98.6	109.2	120.6	122.7	
	2.9	3.5	3.1	3.3	4.6	2.1	3.1	2.1	
Closed	101.8	106.8	108.8	110.0	117.4	124.9	123.6	129.9	
	3.4	2.4	2.3	2.4	3.6	2.1	3.1	2.6	

Least significant difference of means: 0.1% - 2.9; 1% - 2.3; 5% - 1.7

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	2.0	3.8	4.3	5.3	11.1	12.4	16.1	18.4	
	1.3	0.8	2.2	1.2	1.5	1.9	1.6	1.4	
Closed	3.0	4.5	4.1	6.3	15.0	17.0	17.0	20.2	
	1.2	0.9	0.8	1.7	1.9	2.4	1.8	2.1	
Middle Open	2.9	3.8	5.5	5.0	12.4	17.3	16.6	19.3	
	1.3	1.7	0.8	1.3	2.4	1.4	1.5	1.5	Petiole length
Closed	3.6	4.5	6.5	7.5	17.6	20.1	21.9	22.9	
	1.2	1.0	1.2	1.5	2.0	2.0	1.6	1.5	(mm)
Lower Open	2.8	5.4	7.0	7.4	15.6	19.0	17.6	20.7	
	0.9	1.8	1.7	1.1	1.8	2.1	1.6	2.7	
Closed	7.1	9.1	8.4	9.8	19.1	23.6	22.9	25.1	
	1.5	1.1	1.1	1.3	0.9	2.2	2.2	1.5	

Least significant difference of means: 0.1% - 1.5; 1% - 1.2; 5% - 0.9

Upper Open	36.6	22.9	19.5	15.7	9.4	7.5	7.7	6.9	
	3.0	4.6	5.2	3.6	0.9	1.4	0.9	0.6	
Closed	31.0	20.3	22.4	16.2	7.6	7.6	7.6	6.9	
	15.8	2.5	5.1	4.3	1.0	0.8	0.7	0.7	
Middle Open	30.2	24.2	17.4	18.7	8.5	7.0	7.5	7.0	
	3.1	4.7	2.9	4.4	0.6	0.4	1.1	1.0	Petiole ratio
Closed	27.0	24.8	16.1	15.9	7.0	6.7	6.3	6.5	
	14.1	5.3	2.6	3.3	0.4	0.6	0.5	0.4	
Lower Open	35.3	18.6	14.9	14.9	7.3	6.7	7.8	6.9	
	3.0	4.0	5.2	4.3	0.5	0.7	1.1	0.9	
Closed	16.4	12.7	14.3	12.2	7.2	6.5	6.6	6.2	
	2.9	1.2	1.9	1.5	0.3	0.6	0.6	0.4	

Least significant difference of means: 0.1% - 3.7; 1% - 2.9; 5%-2.2

	Q. robur				Q. petraea				
	SS	SSh	NS	NSh	SS	SSh	NS	NSh	
Upper Open	1.85	1.62	1.66	1.52	2.23	1.95	2.17	2.09	
	0.05	0.07	0.09	0.04	0.10	0.09	0.07	0.08	
Closed	1.79	1.56	1.64	1.54	2.11	1.81	2.06	1.91	
	0.09	0.08	0.03	0.04	0.05	0.05	0.05	0.05	
Middle Open	1.58	1.65	1.42	1.63	2.17	1.87	2.08	1.92	
	0.06	0.05	0.07	0.05	0.08	0.07	0.08	0.09	Obversity
Closed	1.81	1.61	1.66	1.65	2.02	1.75	1.91	1.81	
	0.06	0.05	0.04	0.03	0.06	0.05	0.05	0.04	
Lower Open	1.63	1.58	1.48	1.47	1.96	1.83	1.89	1.79	
	0.03	0.05	0.07	0.09	0.10	0.08	0.09	0.10	
Closed	1.75	1.55	1.44	1.35	1.96	1.79	1.84	1.81	
	0.02	0.04	0.04	0.04	0.04	0.04	0.04	0.03	

Least significant difference of means: 0.1% - 0.06; 1% - 0.05; 5% - 0.04

Upper Open	1.86	1.91	2.05	2.25	2.53	2.75	2.93	3.18	
	0.07	0.08	0.12	0.11	0.11	0.08	0.05	0.09	
Closed	1.92	2.29	2.08	2.42	2.90	3.27	3.21	3.39	
	0.09	0.07	0.09	0.10	0.11	0.14	0.10	0.12	
Middle Open	1.99	2.23	2.35	2.42	2.61	2.97	3.18	3.24	
	0.09	0.08	0.12	0.08	0.09	0.10	0.09	0.13	Lobe depth ratio
Closed	2.34	2.51	2.62	2.70	3.00	3.51	3.45	3.65	
	0.05	0.06	0.05	0.03	0.07	0.05	0.03	0.03	
Lower Open	2.12	2.33	2.49	2.57	2.78	3.22	3.32	3.40	
	0.10	0.06	0.07	0.05	0.10	0.07	0.10	0.09	
Closed	2.51	2.71	2.66	2.78	3.31	3.55	3.65	3.73	
	0.05	0.03	0.03	0.05	0.04	0.04	0.04	0.06	

Least significant difference of means: 0.1% - 0.08; 1% - 0.06; 5% - 0.05

VARIANCE RATIOS FOR THE DIFFERENT EFFECTS AND ERROR VARIANCE - CANOPY DATA

Effects	Aspects/ Exposure	Canopy Height	Species/ Woodlands	A/E*CH	CH*Sp/W	A/E*Sp/W	A/E*CH*Sp/W	Error Variance
Degrees of Freedom	3	2	3	6	6	9	18	1152
Total lamina thickness	875.494	372.770	588.883	22.600	18.650	12.350	3.185	18.426
Palisade thickness	890.627	518.907	365.421	12.343	16.204	6.773	1.877	9.766
Spongy mesophyll thickness	8.428	72.006	63.625	20.136	6.529	12.716	4.418	11.138
Epidermal thickness	104.991	320.583	122.022	11.468	7.825	3.059	3.544	5.861
No. of cell layers in palisade	256.204	70.204	17.482	1.835	2.035	1.176	0.602	0.260
Stomatal density	632.385	182.774	517.183	19.123	2.483	31.165	6.186	1.714
Mesophyll ratio	379.087	914.984	144.693	38.353	8.306	30.154	27.947	0.000801
Epidermal ratio	38.140	272.575	49.683	32.073	16.409	28.241	18.976	0.000752
Palisade ratio	669.249	578.283	135.933	7.996	6.132	5.289	3.422	0.000381
Basal shape of lamina	18.757	14.753	1239.489	0.692	0.630	0.907	0.321	0.660
Auricle development	25.039	19.840	1194.013	0.023	0.728	0.853	0.429	0.694
Leaf hairiness	38.147	8.400	2333.800	0.203	0.235	1.282	0.261	0.452
Lobe number	87.960	64.435	127.120	1.544	1.575	5.806	1.658	0.517
Venation	267.010	90.012	1892.702	9.622	18.592	62.952	3.940	55.042
Leaf area	874.965	215.974	705.990	21.235	7.993	15.204	5.528	8.216
Lamina length	1082.635	3084.867	4460.797	6.772	56.813	80.880	18.223	9.948

Effects	Aspects/ Exposure	Canopy Height	Species/ Woodlands	A/E*CH	CH*Sp/W	A/E*Sp/W	A/E*CH*Sp/W	Error Variance
Degrees of Freedom	3	2	3	6	6	9	18	1152
Petiole length	428.638	544.760	6758.755	7.996	26.818	13.856	7.575	2.583
Petiole ratio	199.376	67.085	1175.961	8.861	25.328	64.035	6.705	16.117
Obversity	638.394	570.287	3148.106	44.843	61.089	59.402	22.999	0.003936
Lobe depth ratio	1891.130	2298.669	12667.934	23.693	32.837	53.487	14.093	0.006614

Significance levels	2*1152	3*1152	6*1152	9*1152	18*1152
5%	3.00	2.60	2.10	1.88	1.60
1%	4.61	3.32	2.80	2.41	1.92
0.1%	5.42	4.62	3.74	3.10	2.35

APPENDIX 2

Appendix 2 lists the means, standard deviations and analysis of the leaf characters measured during leaf expansion discussed in Chapter 3.

The first part records the means, standard deviations and least significant difference of means for the leaf characters measured in two woodland sites (open and closed; O and C), at two canopy sites (sun and shade; S and Sh), for ten sampling times (0 days, 6 days, 8 days, etc.), for the two species (R and P). The first figure in each tabular cell records the mean, the second (underneath) the standard deviation.

The second part records the three-way analysis of variance of the above data, the main effects being harvest times, species and canopy sites/woodland sites. The table gives the variance ratio for each effect and interactions between them, together with the error variance and significance levels for appropriate combinations of degrees of freedom.

SITE	0	6	12	18	24	30	36	42	48	54
RSO	44.2	43.2	44.6	43.6	45.6	49.3	61.3	68.2	75.8	76.7
	3.9	3.3	4.3	4.2	4.1	5.1	5.1	5.3	5.4	5.4
RShO	45.4	42.6	43.7	46.7	48.8	53.2	60.7	65.7	64.7	66.2
	3.4	4.6	4.4	4.1	4.3	5.3	6.3	4.8	5.1	5.5
RSC	46.2	45.3	45.8	44.7	48.2	55.3	62.3	66.7	69.7	68.7
	5.3	3.7	3.3	3.4	4.7	5.6	5.7	5.2	6.6	6.2
RShC	42.5	44.2	43.5	44.4	47.7	52.1	54.2	56.2	55.2	56.2
	4.4	5.1	4.0	3.4	4.8	4.5	5.1	5.4	5.6	6.0
PSO	43.2	44.8	44.2	45.3	46.7	56.5	65.1	75.0	78.6	78.0
	4.7	4.7	5.6	4.7	4.6	5.2	6.2	6.8	4.0	5.1
PShO	44.8	42.3	45.2	46.0	49.7	59.2	63.7	64.2	66.6	65.0
	5.3	3.6	3.6	3.6	5.6	5.1	5.2	5.2	6.7	6.7
PSC	42.8	45.7	44.2	45.6	50.2	60.2	68.2	71.3	72.5	72.5
	4.3	5.4	4.1	4.3	4.9	6.4	5.8	5.6	5.1	6.0
PShC	43.6	43.7	42.7	45.6	51.0	58.3	57.2	59.6	57.8	58.6
	5.1	4.3	4.3	4.2	4.4	5.1	5.0	5.7	5.4	5.6

Total lamina thickness

(x 2.35 = μ)

Least significant difference:
0.1% - 3.3
1% - 2.6
5% - 2.0

RSO	17.6	17.4	17.8	17.6	18.1	21.3	27.3	31.5	36.5	36.5
	2.9	2.1	3.7	3.1	3.7	3.1	3.7	4.7	3.1	4.1
RShO	19.5	17.2	17.6	18.8	19.3	21.0	23.8	25.8	25.3	26.4
	2.7	3.3	2.6	4.1	4.1	3.6	4.6	3.4	3.9	3.1
RSC	18.9	17.8	18.3	17.7	19.0	22.8	26.9	29.1	31.2	30.1
	2.8	3.1	3.3	4.7	3.4	4.7	4.4	4.4	4.1	3.9
RShC	16.5	17.2	17.2	17.2	18.4	19.8	20.0	20.9	20.1	20.7
	3.7	2.4	3.7	2.6	4.1	4.3	4.1	4.3	3.0	4.8
PSO	17.5	18.1	17.8	18.1	18.9	23.8	28.4	33.7	35.6	35.0
	3.4	4.9	3.4	3.1	4.6	3.7	4.6	4.1	3.9	3.4
PShO	17.8	16.7	17.7	18.1	19.3	22.8	23.9	23.7	25.0	24.2
	2.1	3.8	3.1	3.4	3.9	3.4	3.7	3.6	4.8	3.2
PSC	17.2	18.1	17.5	18.5	20.1	25.6	29.9	31.6	32.1	32.3
	3.6	3.0	3.6	4.3	3.3	3.7	3.1	3.7	4.6	4.9
PShC	17.1	17.2	16.6	17.8	19.6	21.3	20.9	21.5	21.0	21.1
	2.4	2.0	3.4	3.3	4.7	3.1	4.3	3.1	4.1	4.1

Palisade thickness

(x 2.35 = μ)

Least significant difference:
0.1% - 2.5
1% - 1.9
5% - 1.5

SITE	0	6	12	18	24	30	36	42	48	54
RSO	16.9	16.9	17.8	17.3	17.2	17.9	21.8	23.3	25.1	25.7
	2.7	2.1	3.4	3.3	3.1	3.1	3.7	3.1	4.5	4.0
RShO	20.4	17.0	17.4	18.7	19.0	20.6	23.2	25.2	24.7	24.8
	2.1	2.8	3.1	3.6	4.7	4.3	3.1	3.0	4.4	4.3
RSC	18.2	18.4	18.5	18.1	18.3	21.8	23.6	25.1	25.8	26.2
	3.8	3.6	3.3	3.7	2.7	4.7	4.9	3.3	4.3	3.7
RShC	16.5	17.1	16.9	17.3	19.1	22.1	23.7	25.1	24.3	25.2
	4.9	2.4	3.9	3.8	3.6	3.1	4.2	4.9	4.6	3.4
PSO	16.7	17.4	17.3	17.8	18.0	21.2	24.1	27.2	28.7	28.2
	3.1	3.7	3.1	3.8	3.3	3.6	3.6	4.4	4.4	4.8
PShO	17.9	16.8	17.9	18.1	19.2	23.8	27.2	27.5	28.2	28.1
	3.4	3.6	4.1	4.8	4.6	4.6	4.6	4.3	4.3	4.8
PSC	17.0	18.0	17.8	18.2	19.5	23.6	26.4	27.1	27.3	27.8
	3.2	2.1	3.8	3.3	4.2	4.3	4.0	4.8	4.7	4.3
PShC	16.4	16.7	16.4	17.4	21.0	25.8	25.4	26.1	25.8	25.8
	3.1	3.3	3.7	4.8	3.2	4.4	3.0	4.7	4.9	3.2

Spongy Mesophyll Thickness

(x 2.35 = μ)

Least significant difference:
0.1% - 2.6
1% - 2.0
5% - 1.5

RSO	9.9	9.0	9.0	8.7	10.3	10.1	12.2	13.5	14.2	14.5
	2.1	2.7	2.1	3.2	2.9	3.1	2.3	2.8	2.6	3.3
RShO	8.7	8.3	8.7	9.2	10.5	11.6	13.7	14.7	14.7	15.0
	2.1	2.7	1.7	3.6	2.0	2.3	1.4	1.8	2.4	2.7
RSC	9.5	9.1	9.0	8.9	10.9	10.7	11.8	12.5	12.7	12.4
	2.0	2.0	1.2	1.8	3.1	2.7	3.1	2.7	2.8	1.6
RShC	8.6	9.9	9.4	9.9	10.2	10.2	10.5	10.2	10.8	10.3
	2.2	2.4	3.7	2.1	1.6	2.6	3.0	2.9	1.7	3.3
PSO	9.1	9.3	9.1	9.4	9.8	11.5	12.6	14.1	14.3	14.8
	2.4	2.8	2.4	2.7	2.0	1.9	2.3	1.7	2.3	2.9
PShO	9.1	8.8	9.6	9.8	11.2	12.6	12.6	13.0	13.4	12.7
	3.4	2.0	2.3	2.4	3.3	2.4	2.3	2.2	3.3	3.3
PSC	8.6	9.6	8.9	8.7	10.6	11.0	11.9	12.6	13.1	12.5
	1.5	2.3	2.6	1.7	3.4	3.1	3.0	2.1	1.6	2.1
PShC	10.2	9.8	9.7	10.4	10.4	11.1	10.9	12.0	11.0	11.7
	1.8	2.1	3.9	2.6	3.1	2.3	3.4	3.1	3.3	3.4

Epidermal Thickness

(x 2.35 = μ)

Least significant difference:
0.1% - 1.7
1% - 1.4
5% - 1.0

SITE	0	6	12	18	24	30	36	42	48	54	
RSO	1.30	1.31	1.32	1.28	1.39	1.54	1.98	2.43	2.64	2.77	
	0.49	0.41	0.42	0.41	0.43	0.51	0.52	0.47	0.45	0.49	
RShO	1.31	1.16	1.23	1.21	1.29	1.47	1.69	1.72	1.70	1.68	Number of cell
	0.41	0.46	0.40	0.37	0.56	0.47	0.43	0.44	0.57	0.48	layers in
RSC	1.53	1.36	1.42	1.44	1.54	2.10	2.27	2.54	2.69	2.57	palisade
	0.44	0.47	0.31	0.49	0.41	0.35	0.45	0.37	0.41	0.56	
RShC	1.14	1.27	1.19	1.36	1.29	1.46	1.53	1.52	1.47	1.57	
	0.43	0.38	0.43	0.34	0.44	0.48	0.45	0.46	0.50	0.48	
PSO	1.19	1.37	1.34	1.34	1.42	1.82	2.27	2.36	2.71	2.63	
	0.37	0.49	0.46	0.46	0.57	0.56	0.49	0.47	0.52	0.47	Least significant
PShO	1.16	1.06	1.26	1.14	1.29	1.56	1.54	1.59	1.64	1.57	difference:
	0.44	0.44	0.47	0.56	0.41	0.40	0.55	0.59	0.42	0.46	0.1% - 0.3
PSC	1.33	1.43	1.28	1.37	1.54	1.89	2.27	2.51	2.46	2.71	1% - 0.23
	0.48	0.31	0.52	0.41	0.43	0.53	0.44	0.44	0.47	0.44	5% - 0.18
PShC	1.17	1.22	1.27	1.16	1.33	1.39	1.33	1.26	1.41	1.36	
	0.42	0.43	0.35	0.44	0.52	0.46	0.31	0.51	0.45	0.39	
RSO	54.5	48.4	31.3	22.4	13.8	12.6	12.4	13.3	12.4	13.3	
	1.1	1.6	1.9	1.4	1.2	1.7	1.7	1.0	1.7	1.6	
RShO	46.4	32.0	18.4	12.5	10.1	10.3	10.7	10.4	10.9	10.0	Stomatal density
	1.6	1.4	1.8	0.8	1.3	1.4	1.4	1.7	1.7	1.7	
RSC	43.3	47.0	26.3	21.2	12.4	11.0	12.7	11.2	12.0	11.2	(x 23 = per sq.mm.)
	1.4	0.9	1.7	1.3	1.6	1.9	1.1	1.3	1.5	1.3	
RShC	39.3	23.0	15.4	10.3	8.8	9.6	9.4	8.8	9.2	9.6	
	2.3	1.7	1.1	1.6	1.4	1.8	1.2	1.3	1.0	1.3	
PSO	49.2	44.0	27.8	18.9	15.2	11.4	10.7	11.0	11.0	10.9	
	1.9	1.3	1.8	1.7	1.4	1.4	1.8	1.6	1.3	1.2	Least significant
PShO	37.6	37.4	19.3	10.6	8.2	9.8	8.0	8.4	7.9	8.2	difference:
	1.7	1.3	1.4	1.1	1.5	1.8	1.0	1.1	1.5	1.4	0.1% - 1.0
PSC	37.2	35.7	24.0	14.3	9.4	10.7	9.6	9.9	9.0	10.2	1% - 0.8
	1.7	1.4	0.9	1.9	1.4	1.3	1.4	1.8	1.2	1.1	5% - 0.6
PShC	38.4	15.6	9.0	7.3	7.3	6.2	7.5	6.6	6.7	6.8	
	1.1	1.6	1.6	1.4	1.3	1.1	1.4	1.2	1.8	1.6	

SITE	0	6	12	18	24	30	36	42	48	54	
RSO	.396	.401	.399	.402	.396	.431	.446	.464	.479	.477	
	.019	.020	.023	.032	.023	.023	.021	.022	.027	.019	
RShO	.403	.404	.402	.401	.395	.395	.391	.393	.391	.397	Palisade
	.023	.027	.023	.027	.024	.024	.019	.027	.021	.023	ratio
RSC	.399	.393	.401	.395	.393	.412	.431	.436	.447	.438	
	.028	.026	.019	.026	.026	.023	.024	.021	.026	.021	
RShC	.388	.388	.394	.386	.386	.380	.368	.372	.364	.368	
	.018	.024	.027	.012	.018	.023	.017	.011	.018	.026	
PSO	.405	.405	.403	.399	.403	.421	.435	.449	.453	.448	
	.024	.016	.019	.024	.023	.017	.026	.024	.024	.027	Least significant
PShO	.395	.395	.391	.393	.389	.385	.374	.369	.375	.373	difference:
	.029	.026	.020	.021	.019	.026	.029	.019	.023	.024	0.1% - .015
PSC	.401	.397	.396	.405	.400	.425	.439	.442	.443	.445	1% - .012
	.018	.017	.026	.019	.021	.024	.023	.026	.028	.020	5% - .009
PShC	.392	.392	.389	.390	.383	.365	.364	.359	.362	.358	
	.015	.021	.024	.020	.027	.027	.023	.029	.022	.020	
RSO	.381	.391	.399	.395	.377	.362	.355	.340	.331	.335	
	.017	.019	.022	.033	.021	.042	.032	.024	.020	.021	Spongy
RShO	.405	.401	.397	.399	.390	.387	.382	.384	.381	.374	Mesophyll ratio
	.019	.027	.027	.032	.029	.028	.036	.026	.027	.029	
RSC	.393	.406	.405	.402	.380	.395	.379	.376	.369	.370	
	.027	.028	.024	.021	.018	.047	.017	.040	.034	.046	
RShC	.389	.385	.388	.389	.401	.424	.436	.446	.443	.449	
	.021	.025	.023	.019	.017	.031	.019	.024	.024	.038	
PSO	.385	.387	.391	.393	.385	.375	.369	.362	.365	.360	
	.031	.016	.041	.042	.034	.029	.039	.028	.021	.037	Least significant
PShO	.401	.397	.395	.394	.385	.403	.425	.427	.422	.432	difference:
	.022	.039	.019	.029	.024	.036	.028	.031	.034	.034	0.1% - .019
PSC	.397	.394	.402	.397	.388	.391	.387	.381	.377	.384	1% - .015
	.025	.026	.029	.030	.022	.047	.020	.026	.020	.023	5% - .011
PShC	.375	.381	.383	.381	.411	.441	.444	.436	.446	.441	
	.036	.038	.021	.020	.021	.018	.022	.027	.029	.021	

SITE	0	6	12	18	24	30	36	42	48	54	
RSO	.223	.208	.202	.203	.227	.207	.199	.196	.190	.188	
	.015	.015	.016	.024	.027	.028	.026	.027	.021	.028	
RShO	.192	.195	.201	.200	.215	.218	.227	.223	.228	.22)	Epidermal
	.017	.017	.014	.027	.013	.031	.024	.017	.020	.014	ratio
RSC	.208	.201	.194	.203	.227	.193	.190	.188	.184	.192	
	.014	.027	.044	.021	.021	.020	.027	.024	.028	.02)	
RShC	.203	.227	.218	.225	.213	.196	.196	.182	.193	.183	
	.027	.020	.037	.018	.024	.024	.023	.021	.028	.02)	
PSO	.210	.208	.206	.208	.212	.204	.196	.189	.182	.192	
	.036	.026	.017	.021	.028	.020	.019	.036	.020	.014	Least significant
PShO	.204	.208	.214	.213	.226	.212	.201	.204	.203	.195	difference:
	.023	.021	.019	.022	.028	.016	.019	.014	.019	.036	0.1% - .016
PSC	.202	.209	.202	.198	.212	.184	.174	.177	.180	.171	1% - .012
	.024	.030	.026	.024	.023	.028	.024	.024	.022	.027	5% - .009
PShC	.233	.227	.228	.229	.206	.194	.192	.205	.192	.201	
	.016	.021	.023	.026	.039	.021	.021	.023	.024	.021	
RSO	0.61	0.50	0.47	0.63	0.35	0.51	0.21	0.33	0.45	0.31	
	0.51	0.79	0.46	0.97	0.67	0.79	0.71	0.74	0.71	0.71	
RShO	0.14	0.39	0.25	0.46	0.52	0.77	1.24	0.46	0.56	0.53	Basal shape of
	0.47	0.47	0.77	0.96	0.66	0.77	0.86	0.60	0.74	0.67	lamina (Index)
RSC	0.35	0.14	0.33	0.89	0.75	0.84	0.56	0.61	0.61	0.75	
	0.31	0.87	0.86	0.93	0.41	0.63	0.82	0.74	0.62	0.53	
RShC	0.47	0.61	0.74	1.19	1.34	1.21	1.04	1.21	1.21	1.18	
	0.80	0.74	0.84	0.93	0.41	0.63	0.61	0.61	0.55	0.63	
PSO	3.24	3.00	3.26	3.04	3.11	3.06	2.99	3.24	3.11	3.04	
	0.87	0.36	0.78	0.84	0.56	0.84	0.63	0.41	0.44	0.64	Least significant
PShO	2.82	2.84	3.41	3.24	3.53	3.76	3.83	3.64	3.97	3.75	difference:
	0.94	0.86	0.47	1.21	0.54	0.41	0.77	0.83	0.48	0.66	0.1% - 0.46
PSC	3.17	3.21	3.01	3.11	3.38	3.27	3.31	3.08	3.46	3.22	1% - 0.36
	0.70	1.17	0.81	0.52	0.63	0.77	0.60	0.61	0.56	0.72	5% - 0.28
PShC	2.93	3.46	3.81	3.83	3.78	3.96	3.67	3.82	3.74	3.61	
	1.03	0.61	0.73	0.51	0.38	0.42	0.56	0.43	0.66	0.77	

SITE	0	6	12	18	24	30	36	42	48	54	
RSO	0.21	0.11	0.35	0.11	0.11	0.38	0.11	0.21	0.37	0.13	
	1.11	0.90	0.78	0.52	0.66	0.74	0.71	1.01	1.21	0.73	
RShO	0.34	0.54	0.55	0.64	0.60	1.18	0.86	1.15	0.81	0.84	Auricle
	0.97	0.71	1.04	0.75	0.74	0.76	0.63	0.94	1.14	0.77	development
RSC	0.42	0.27	0.22	0.39	0.33	0.11	0.31	0.54	0.41	0.33	(Index)
	0.88	1.08	0.76	0.96	0.59	1.05	0.81	0.89	0.62	0.66	
RShC	0.25	0.47	0.88	1.04	1.14	0.97	1.17	1.01	1.21	1.05	
	0.62	0.66	0.74	1.07	0.60	0.66	0.67	0.89	0.75	0.66	
PSO	3.24	2.94	3.03	3.06	3.05	2.98	3.19	2.85	2.95	2.81	
	0.76	0.77	0.57	0.61	0.67	0.87	1.06	0.78	0.89	0.92	Least significant
PShO	2.98	3.00	3.12	3.19	3.33	3.44	3.71	3.52	3.52	3.71	difference:
	0.74	0.74	0.66	0.93	0.68	1.11	0.78	0.69	0.89	1.11	0.1% - 0.54
PSC	3.17	3.24	2.91	3.37	3.11	3.26	3.31	3.31	3.25	3.25	1% - 0.42
	0.73	0.83	0.87	0.74	0.71	0.97	1.66	0.42	0.65	0.74	5% - 0.32
PShC	3.16	3.41	3.81	3.76	3.65	3.81	3.64	3.63	3.84	3.88	
	0.69	0.69	0.73	0.83	0.73	0.74	0.51	0.71	0.54	0.73	
RSO	0.14	0.55	0.26	0.03	0.14	0.43	0.23	0.54	0.33	0.83	
	0.51	0.47	0.67	0.71	0.51	0.73	0.73	0.73	0.51	0.52	
RShO	0.45	0.26	0.63	0.54	0.55	0.14	0.53	0.23	0.73	0.24	Leaf hairiness
	0.89	0.73	0.61	0.62	0.66	0.64	0.63	0.64	0.64	0.50	(Index)
RSC	0.63	0.69	0.51	0.42	0.43	0.28	0.37	0.11	0.13	0.62	
	0.77	0.66	0.41	0.71	0.74	0.60	0.67	0.53	0.78	0.67	
RShC	0.04	0.38	0.40	0.30	0.30	0.61	0.12	0.40	0.50	0.51	
	0.74	0.63	0.52	0.71	0.66	0.82	0.71	0.73	0.59	0.61	
PSO	2.74	2.65	2.77	2.54	2.63	2.52	2.84	3.73	3.65	3.81	
	0.60	0.62	0.74	0.83	0.80	0.73	0.52	0.62	0.64	0.70	Least significant
PShO	2.35	2.55	2.34	2.34	2.46	2.96	3.07	2.87	3.00	3.34	difference:
	0.30	0.74	0.74	0.87	0.83	0.61	0.51	0.51	0.67	0.73	0.1% - 0.44
PSC	2.67	2.81	2.53	2.75	2.82	2.76	3.44	3.53	3.88	3.66	1% - 0.35
	0.63	0.84	0.53	0.63	0.62	0.81	0.63	0.77	0.64	0.72	5% - 0.26
PShC	2.54	2.41	3.58	3.22	3.17	3.26	3.31	3.03	3.27	3.22	
	0.41	0.91	0.64	0.74	0.71	0.76	0.74	0.64	0.76	0.55	

SITE	0	6	12	18	24	30	36	42	48	54	
RSO	4.70	5.12	4.91	4.98	5.02	4.86	4.97	4.89	5.05	4.94	
	0.41	0.76	0.41	0.71	0.71	0.99	0.77	0.77	0.71	0.71	
RShO	4.22	4.35	4.49	4.34	4.33	4.49	4.36	4.45	4.36	4.49	Lobe number
	0.77	0.88	0.77	0.47	0.70	0.92	0.73	0.72	0.71	0.76	
RSC	5.02	5.26	5.03	4.90	5.10	4.97	4.90	5.02	4.98	5.02	
	1.11	0.40	0.91	0.79	0.71	0.76	0.77	0.76	0.73	0.77	
RShC	3.78	3.76	3.68	3.81	3.64	3.70	3.82	3.85	3.78	3.90	
	0.87	0.57	0.77	0.68	0.87	0.56	0.92	0.67	0.73	0.85	
PSO	5.50	5.78	5.62	5.64	5.58	5.82	5.74	5.65	5.73	5.70	
	0.84	0.99	0.72	1.03	0.77	1.20	0.84	0.61	0.65	0.64	Least significant
PShO	4.91	4.98	5.10	5.17	4.85	5.10	5.06	4.82	4.90	5.14	difference:
	0.46	0.90	0.82	0.76	0.84	0.87	0.73	0.76	0.64	0.64	0.1% - 0.49
PSC	5.62	5.54	5.49	5.52	5.66	5.60	5.54	5.54	5.50	5.49	1% - 0.39
	0.64	0.44	0.76	0.47	0.63	0.84	0.61	0.73	0.82	0.85	5% - 0.29
PShC	4.42	4.46	4.42	4.46	4.46	4.34	4.46	4.30	4.46	4.43	
	0.77	0.46	0.84	0.44	0.56	0.71	0.66	0.64	0.77	0.72	
RSO	45.3	42.8	45.3	45.1	47.8	43.3	44.2	44.0	48.2	45.0	
	6.4	9.7	9.9	7.4	9.1	9.2	7.1	7.6	8.5	7.5	
RShO	17.2	19.1	18.5	21.4	18.3	18.0	19.1	19.3	18.2	17.2	Venation (%)
	9.6	9.6	8.6	7.7	8.7	8.7	6.3	9.9	8.5	9.2	
RSC	31.6	35.4	35.0	34.6	35.3	32.7	34.6	32.4	31.7	32.2	
	8.3	8.0	9.1	8.1	7.4	8.4	7.0	9.0	7.4	6.6	
RShC	17.2	19.4	18.5	21.3	18.4	18.1	19.2	19.3	18.3	17.2	
	7.0	9.1	6.7	7.0	6.1	8.9	7.0	7.1	7.1	6.6	
PSO	10.0	11.2	10.1	9.3	11.2	10.3	11.2	8.9	12.4	10.3	
	3.9	4.5	4.4	4.6	6.6	4.6	4.4	6.4	4.0	4.9	Least significant
PShO	5.7	8.2	8.4	10.4	9.4	9.6	12.2	9.4	9.5	8.4	difference:
	5.1	7.4	8.5	6.2	9.5	5.4	5.2	6.9	6.7	4.7	0.1% - 4.7
PSC	0.9	3.4	5.6	3.6	5.7	3.4	4.6	4.7	2.3	4.4	1% - 3.7
	4.4	5.3	8.0	5.3	8.1	6.1	5.7	6.3	5.1	5.0	5% - 2.8
PShC	1.5	0.7	3.6	2.4	1.4	0.5	2.0	1.4	0.7	3.4	
	6.2	6.6	5.3	7.4	6.3	5.3	5.1	5.8	4.2	4.1	

SITE	0	6	12	18	24	30	36	42	48	54
RSO	1.2	3.1	11.2	18.6	29.2	31.2	33.2	31.7	33.3	32.7
	1.9	2.9	3.0	4.0	4.6	4.6	4.1	4.3	4.9	4.7
RShO	4.3	10.7	23.7	30.3	36.4	36.0	36.6	36.2	37.2	36.3
	2.0	2.8	3.4	4.6	4.4	4.1	3.4	2.9	4.7	4.4
RSC	2.0	2.3	13.6	21.2	31.4	33.7	32.7	33.6	34.2	33.7
	2.3	3.6	4.4	4.6	4.4	4.4	4.1	4.9	4.2	4.0
RShC	5.7	17.3	29.2	36.6	41.2	41.4	41.0	41.3	41.7	41.2
	1.4	3.4	3.1	4.4	3.4	4.1	3.4	4.6	4.6	4.7
PSO	2.7	4.6	12.2	17.7	30.3	34.2	33.7	34.7	33.6	34.8
	1.6	3.1	3.3	4.4	3.7	4.4	4.7	4.7	4.8	4.7
PShO	6.2	9.3	21.4	38.2	45.4	44.2	45.2	44.8	45.0	44.8
	3.7	3.7	4.6	3.7	4.3	5.3	4.6	3.2	4.4	4.4
PSC	6.4	10.0	17.6	33.2	40.0	39.3	40.2	41.7	40.2	39.8
	1.7	3.1	3.7	3.1	4.7	3.6	4.3	4.4	3.5	3.6
PShC	7.3	28.0	39.6	50.0	48.1	49.2	48.0	49.0	48.2	50.3
	1.8	2.0	4.0	4.3	4.1	4.2	4.6	4.3	4.5	4.7

Leaf area (sq. cm.)

Least significant difference:
0.1% - 2.6
1% - 2.0
5% - 1.6

RSO	29.4	28.3	35.6	55.8	67.9	91.4	94.0	91.4	93.4	99.5
	3.7	3.1	4.9	3.6	4.1	5.4	5.4	3.9	4.7	4.0
RShO	31.0	48.7	64.9	79.6	101.9	97.1	101.1	97.1	101.3	105.7
	4.4	3.3	4.0	3.9	4.9	4.4	4.6	4.9	4.7	4.4
RSC	24.8	32.3	38.1	45.1	61.0	81.7	86.7	94.4	87.7	95.7
	4.0	4.7	4.7	5.7	4.4	4.8	5.4	4.4	5.2	4.2
RShC	26.6	60.8	74.2	94.3	108.0	108.8	113.1	110.6	118.6	110.6
	4.9	3.8	3.7	6.3	4.7	4.9	4.4	4.8	6.5	4.1
PSO	33.2	39.1	41.3	51.7	71.8	87.7	83.9	82.7	82.4	91.1
	4.6	4.6	3.1	4.1	4.8	4.7	4.1	4.9	4.8	5.8
PShO	34.1	41.0	62.3	69.4	92.9	105.6	107.8	107.7	115.1	112.4
	5.1	4.4	4.4	4.4	4.6	5.4	4.4	5.4	5.8	4.8
PSC	36.9	37.7	57.4	75.7	83.1	101.7	115.6	113.6	111.6	117.3
	4.4	3.9	3.8	4.6	4.1	4.1	4.3	4.3	4.4	5.7
PShC	38.4	80.7	110.7	131.8	127.1	133.7	129.2	131.3	127.4	130.6
	3.7	4.9	3.4	4.3	3.3	4.7	4.1	4.6	4.4	4.7

Lamina length (mm)

Least significant difference:
0.1% -3.0
1% - 2.4
5% -1.8

SITE	0	6	12	18	24	30	36	42	48	54	
RSO	2.5	2.6	2.5	1.2	2.7	2.2	1.7	2.7	1.7	2.7	
	0.8	1.8	1.2	1.8	2.1	1.1	1.0	2.4	2.9	2.2	
RShO	1.0	2.7	3.7	3.2	4.7	3.7	4.7	4.7	3.5	4.7	Petiole length
	1.8	1.6	0.9	0.7	1.4	2.0	1.7	1.4	2.9	2.6	(mm)
RSC	0.2	1.2	1.4	1.8	3.5	7.4	6.2	7.2	6.5	7.2	
	1.3	2.7	1.6	1.9	1.3	1.1	1.3	1.4	2.6	2.3	
RShC	1.7	3.5	5.7	7.5	9.9	10.0	10.1	9.2	10.2	10.1	
	1.2	1.3	1.4	3.1	1.2	2.7	2.1	2.7	2.4	2.4	
PSO	4.7	5.2	6.5	9.7	11.5	15.6	13.1	15.5	14.2	16.2	
	1.4	1.1	1.1	1.8	1.4	1.3	1.6	2.1	1.8	1.7	Least significant
PShO	5.7	10.2	13.7	17.2	20.1	21.4	20.4	20.0	20.7	20.7	difference:
	1.1	1.4	1.7	1.8	2.4	1.4	1.4	1.1	1.3	2.1	0.1% - 1.3
PSC	3.7	4.2	8.7	12.4	17.5	18.5	16.7	18.1	17.7	19.6	1% - 1.0
	1.9	1.1	2.4	1.7	1.2	1.7	1.1	2.6	2.1	2.9	5% - 0.7
PShC	6.3	15.1	20.7	25.3	23.2	24.0	23.2	23.1	25.0	24.2	
	1.7	1.3	1.1	1.6	1.1	2.7	2.4	2.1	2.3	2.8	
RSO	12.6	15.1	15.0	45.1	25.4	41.1	54.7	34.1	54.2	36.9	
	4.1	4.6	3.2	3.1	5.1	4.3	4.7	4.7	4.6	4.3	
RShO	32.7	18.4	18.1	25.6	22.3	28.6	26.2	21.4	29.9	23.1	Petiole ratio
	3.7	3.9	2.7	4.3	3.6	5.6	4.1	4.6	4.0	4.2	
RSC	97.4	26.6	39.1	26.0	18.5	12.6	14.7	14.4	14.4	14.2	
	5.1	4.4	4.6	3.1	2.9	4.2	3.4	4.7	4.8	4.4	
RShC	15.9	18.2	13.9	13.8	13.0	11.8	12.3	12.9	12.8	12.0	
	3.3	3.3	4.4	4.4	3.7	4.4	4.3	4.8	3.4	4.6	
PSO	7.9	8.4	7.3	6.2	7.1	6.8	7.3	6.6	6.7	6.6	
	0.8	0.8	0.8	0.9	1.4	1.7	0.9	1.3	1.1	1.2	Least significant
PShO	6.8	5.0	5.5	5.0	5.6	6.0	6.3	6.3	6.5	6.3	difference:
	0.7	1.2	0.7	0.9	0.8	1.1	1.5	0.9	1.6	1.9	0.1% - 2.1
PSC	10.6	9.7	7.5	7.2	5.7	6.4	7.8	7.2	7.2	7.1	1% - 1.6
	1.1	0.9	1.8	1.6	0.8	1.7	1.4	1.1	1.9	1.7	5% - 1.2
PShC	7.0	6.3	6.5	6.2	6.5	6.5	6.6	5.6	6.7	6.3	
	1.3	1.7	0.9	1.3	0.9	1.1	1.6	1.3	1.6	0.9	

SITE	0	6	12	18	24	30	36	42	48	54	
RSO	2.43	2.41	2.37	2.27	2.25	2.20	2.19	2.15	2.23	2.15	
	0.15	0.16	0.11	0.17	0.10	0.11	0.09	0.16	0.15	0.17	
RShO	2.85	2.80	2.69	2.65	2.49	2.55	2.51	2.54	2.49	2.53	Lobe depth
	0.17	0.09	0.13	0.14	0.19	0.16	0.14	0.14	0.12	0.11	ratio
RSC	2.77	2.74	2.77	2.71	2.53	2.51	2.46	2.41	2.47	2.47	
	0.10	0.17	0.14	0.20	0.11	0.17	0.11	0.13	0.18	0.14	
RShC	2.74	2.71	2.83	2.86	2.92	3.00	3.01	2.96	2.98	2.95	
	0.11	0.13	0.11	0.16	0.13	0.11	0.13	0.14	0.15	0.17	
PSO	2.58	2.55	2.61	2.61	2.70	2.78	2.75	2.79	2.82	2.76	
	0.18	0.11	0.17	0.11	0.18	0.19	0.19	0.17	0.12	0.16	Least significant
PShO	2.71	2.83	3.03	3.19	3.35	3.41	3.35	3.39	3.47	3.41	difference:
	0.19	0.14	0.16	0.15	0.11	0.14	0.18	0.11	0.18	0.14	0.1% - 0.10
PSC	2.81	2.77	2.87	2.91	3.11	3.25	3.39	3.31	3.35	3.31	1% - 0.08
	0.13	0.16	0.09	0.17	0.17	0.13	0.17	0.11	0.12	0.11	5% - 0.06
PShC	2.89	3.05	3.31	3.69	3.63	3.71	3.67	3.71	3.65	3.69	
	0.17	0.14	0.13	0.12	0.14	0.11	0.23	0.13	0.15	0.16	
RSO	1.61	1.64	1.62	1.65	1.64	1.65	1.62	1.65	1.60	1.65	
	0.08	0.06	0.07	0.06	0.05	0.06	0.07	0.08	0.07	0.07	
RShO	1.72	1.69	1.60	1.67	1.53	1.51	1.47	1.45	1.46	1.49	Obversity
	0.08	0.05	0.05	0.07	0.05	0.05	0.07	0.07	0.07	0.07	
RSC	1.63	1.61	1.58	1.62	1.60	1.63	1.63	1.61	1.62	1.63	
	0.07	0.06	0.06	0.07	0.08	0.07	0.07	0.09	0.07	0.07	
RShC	1.66	1.65	1.63	1.53	1.46	1.47	1.45	1.47	1.48	1.45	
	0.06	0.06	0.06	0.07	0.08	0.07	0.06	0.09	0.08	0.06	
PSO	1.69	1.72	1.76	1.79	1.85	1.91	1.91	1.95	1.91	1.93	
	0.09	0.08	0.08	0.06	0.04	0.08	0.09	0.08	0.07	0.06	Least significant
PShO	1.67	1.67	1.66	1.71	1.75	1.76	1.73	1.76	1.73	1.76	difference:
	0.07	0.09	0.08	0.06	0.07	0.07	0.08	0.06	0.06	0.06	0.1% - 0.05
PSC	1.65	1.71	1.68	1.72	1.71	1.77	1.85	1.83	1.85	1.84	1% - 0.04
	0.06	0.09	0.07	0.06	0.08	0.08	0.07	0.07	0.07	0.08	5% - 0.03
PShC	1.70	1.73	1.78	1.80	1.78	1.82	1.79	1.80	1.78	1.83	
	0.08	0.07	0.07	0.06	0.07	0.09	0.09	0.06	0.09	0.09	

VARIANCE RATIOS FOR THE DIFFERENT EFFECTS AND THE ERROR VARIANCE - LEAF DEVELOPMENT DATA

Effects	Harvest Times	Canopy types/ sites	Species	HT*CT/S	CT/S* Sp	HT*Sp	Ht*CT/S*Sp	Error Variance
Degrees of Freedom	9	3	1	27	3	9	27	3920
Total lamina thickness	1695.572	282.575	142.774	60.282	4.748	17.900	2.552	25.524
Palisade thickness	629.563	423.835	12.795	58.653	8.725	5.262	1.697	14.100
Mesophyll thickness	421.573	15.745	97.669	5.156	2.531	14.104	1.255	15.220
Epidermal thickness	161.435	34.292	5.495	10.974	6.032	1.392	2.493	6.902
No. of cell layers in palisade	250.827	399.334	8.977	30.568	4.692	1.340	1.866	0.208
Stomatal density	25539.667	6953.952	3329.827	576.564	99.658	77.961	88.389	2.317
Palisade ratio	45.447	955.571	45.007	76.812	26.808	6.832	2.583	0.000528
Mesophyll ratio	3.034	389.226	67.978	52.609	22.605	15.977	4.772	0.000822
Epidermal ratio	48.023	82.227	7.567	16.965	17.041	9.514	6.532	0.000583
Basal shape of lamina (index)	19.675	119.596	15058.707	7.768	11.248	2.670	2.867	0.493
Auricle development (index)	8.514	133.747	11284.286	4.467	5.314	0.918	1.253	0.673
Leaf hairiness (index)	32.420	13.228	14866.991	7.661	21.754	24.378	8.191	0.450
Lobe number	0.999	589.661	710.849	1.041	3.117	0.645	0.512	0.561
Venation	1.564	1145.391	10196.834	2.234	657.149	1.409	1.011	50.468
Leaf area	4380.813	2415.736	1915.719	50.692	139.654	18.456	12.980	15.936

Effects	Harvest Times	Canopy types/ sites	Species	HT*CT/S	CT/S*Sp	HT*Sp	Ht*CT/S*Sp	Error Variance
Degrees of freedom	9	3	1	27	3	9	27	3920
Lamina length	14319.302	11178.250	5117.010	308.154	1755.409	28.174	98.130	21.290
Petiole length	1311.413	2845.313	35022.973	61.061	391.328	368.464	27.356	3.625
Petiole ratio	419.447	1938.509	33234.920	702.208	1594.211	329.744	52.425	9.852
Lobe depth ratio	114.583	4067.892	13631.508	38.988	79.895	377.627	26.546	0.021
Obversity	7.663	316.174	6399.594	37.455	121.744	190.129	10.534	0.005656

Significance levels	1*3920	3*3920	9*3920	27*3920
5%	3.84	2.60	1.88	1.49
1%	6.63	3.78	2.41	1.74
0.1%	10.80	5.42	3.10	2.06

APPENDIX 3

Appendix 3 lists the means, standard deviations and analysis of the seedling leaf characters discussed in Chapter 4.

The first part records the means, standard deviations and least significant difference of means for the five groups of seedlings (100, 75, 50, 25 and GH) for the three harvests of the experiment, collected after the seedlings had been under uniform greenhouse conditions (1969, GH), different light intensities in the growth chamber (1970, GC) and finally under uniform greenhouse conditions (1971, GH). This table also records the results from the second batch of seedlings grown only under different growth chamber light conditions (1971, GC). The first figure in each tabular cell records the mean, the second (underneath) records the standard deviation.

The second part records the three-way analysis of variance of the above data, the main effects being seedling groups (at some stage under different light intensities), harvests and species. The table gives the variance ratio for each effect and interactions between them, together with the error variance and significance levels for appropriate combinations of degrees of freedom.

The last part records the two-way analysis of variance of the results for the second batch of seedlings with species and light intensities as the main effects. The analyses are presented in the same format as that described above.

	Q. robur					Q. petraea					
Light intensity	100	75	50	25	GH	100	75	50	25	GH	
1969 GH	70.7	72.9	60.0	71.4	68.5	68.1	65.5	69.3	67.0	66.5	Total lamina
	5.4	5.5	4.4	5.0	4.9	5.5	4.0	4.6	5.9	4.8	thickness (x 2.35 = μ)
1970 GC	70.0	62.1	50.4	45.0	70.1	69.2	59.6	55.2	49.0	69.4	
	5.8	6.5	4.8	4.0	4.8	5.2	5.0	4.5	4.5	4.0	LSD: 1 2
1971 GH	72.9	67.7	68.4	66.7	71.1	70.4	68.3	71.0	67.4	69.5	
	5.5	6.2	5.5	4.6	5.5	4.1	5.1	3.9	3.6	4.8	5% 1.88 1.50
1971 GC	65.4	63.1	50.3	43.3	42.3	62.0	56.4	51.6	47.4	40.2	1% 2.47 1.98
	3.5	5.0	4.1	4.2	4.7	3.0	3.6	3.5	2.9	4.8	0.1% 3.13 2.52
1969 GH	34.3	33.7	32.5	33.9	31.8	32.1	29.5	32.9	31.1	30.1	Palisade thickness
	3.2	3.4	2.6	3.0	3.1	2.5	4.3	3.7	5.3	3.1	(x 2.35 = μ)
1970 GC	33.2	26.8	20.8	16.7	32.9	32.5	26.2	24.2	19.3	31.1	
	3.7	3.7	4.1	3.0	2.9	2.1	3.4	4.3	5.0	3.2	LSD: 1 2
1971 GH	34.7	31.8	30.9	32.0	32.1	31.1	30.5	32.8	32.1	33.3	
	4.2	4.9	4.4	3.5	4.3	3.2	2.4	4.4	5.1	4.2	5% 1.43 1.34
1971 GC	29.6	27.3	20.2	15.6	14.9	28.8	25.9	22.4	18.8	14.4	1% 1.89 1.77
	2.0	3.2	3.1	3.5	4.2	3.2	4.7	3.1	4.7	2.9	0.1% 2.40 2.25
1969 GH	24.5	23.0	23.2	25.1	23.4	21.7	21.7	22.1	23.2	19.9	Spongy mesophyll
	2.8	3.0	2.2	2.5	3.0	3.5	4.9	5.0	2.9	3.1	thickness (x 2.35 = μ)
1970 GC	21.5	22.9	21.8	20.3	22.5	22.1	21.2	19.7	21.9	22.3	
	4.1	3.7	4.5	4.5	2.8	2.9	3.1	3.2	3.1	2.9	LSD: 1 2
1971 GH	24.9	22.0	24.2	21.5	23.7	23.0	20.3	22.3	23.1	23.2	
	3.5	3.9	3.2	2.6	3.2	3.1	4.2	3.9	2.9	3.6	5% 1.31 0.90
1971 GC	23.4	23.0	19.2	18.2	15.3	18.7	20.4	19.1	20.0	16.5	1% 1.72 1.19
	1.9	3.0	2.1	2.1	3.4	1.4	2.7	1.6	2.4	2.5	0.1% 2.19 1.51

	Q. robur					Q. petraea					
Light intensity	100	75	50	25	GH	100	75	50	25	GH	
1969 GH	11.9	16.2	13.3	12.5	13.2	14.6	14.2	14.3	12.8	16.5	Epidermal thickness
	3.7	3.5	4.5	5.2	3.4	3.4	3.6	3.0	3.3	3.6	(x 2.35 = μ)
1970 GC	15.3	12.3	7.8	8.0	14.7	14.7	12.2	11.3	7.8	16.1	
	3.0	2.9	3.3	2.5	3.9	2.7	2.5	2.4	2.6	2.9	LSD: 1 2
1971 GH	13.4	14.0	12.3	13.2	15.2	16.3	17.5	15.9	12.2	13.0	
	3.1	2.8	4.0	3.5	3.7	3.0	3.3	3.3	2.5	3.6	5% 1.26 0.97
1971 GC	12.5	12.8	10.9	9.5	12.1	14.6	10.1	10.0	8.7	9.3	1% 1.66 1.27
	3.0	3.0	3.2	2.7	2.9	2.3	2.1	2.0	1.9	2.0	0.1% 2.11 1.62
1969 GH	1.92	1.82	1.85	1.79	1.97	1.78	1.85	1.54	1.94	1.84	No. of cell layers
	0.41	0.36	0.39	0.36	0.33	0.32	0.41	0.39	0.50	0.32	in palisade
1970 GC	2.10	1.85	1.54	1.17	2.06	2.01	1.87	1.43	1.34	2.11	
	0.34	0.25	0.37	0.31	0.30	0.46	0.32	0.41	0.26	0.51	LSD: 1 2
1971 GH	2.44	2.21	2.08	2.06	2.54	2.30	2.41	2.21	2.02	2.41	
	0.44	0.51	0.56	0.54	0.53	0.44	0.46	0.56	0.47	0.45	5% 0.158 0.080
1971 GC	1.62	1.36	1.12	1.02	1.00	1.56	1.48	1.32	1.14	1.00	1% 0.208 0.105
	0.30	0.27	0.05	0.13	0.11	0.17	0.26	0.25	0.16	0.26	0.1% 0.265 0.134
1969 GH	13.5	14.0	13.2	14.0	14.0	13.9	12.9	14.0	12.6	12.8	Stomatal density
	1.4	1.6	1.3	1.5	1.3	1.3	1.1	1.5	1.6	1.7	(x 23 = per sq. mm)
1970 GC	12.5	11.4	9.9	10.4	10.8	12.8	11.9	10.7	12.0	13.0	
	1.2	1.4	1.1	1.0	1.3	1.3	1.3	1.0	1.6	1.1	LSD: 1 2
1971 GH	11.2	10.9	11.9	10.5	11.2	11.3	11.0	11.4	11.3	11.7	
	1.5	1.2	1.3	1.5	1.0	1.1	1.4	1.2	1.3	1.1	5% 0.495 0.500
1971 GC	14.8	11.6	12.1	12.3	9.2	13.0	12.9	12.3	10.5	8.8	1% 0.651 0.658
	1.2	1.1	1.3	1.4	1.5	1.2	1.2	1.1	1.5	1.7	0.1% 0.831 0.839

	Q. robur					Q. petraea							
Light intensity	100	75	50	25	GH	100	75	50	25	GH			
1969 GH	.485	.463	.471	.474	.465	.472	.451	.475	.464	.453	Palisade ratio		
	.048	.026	.042	.033	.054	.039	.026	.028	.033	.024			
1970 GC	.474	.432	.413	.372	.469	.469	.440	.438	.394	.447			
	.037	.046	.042	.034	.033	.033	.022	.022	.030	.026	LSD:	1	2
1971 GH	.476	.469	.452	.480	.452	.441	.447	.462	.477	.417			
	.040	.039	.034	.032	.024	.029	.046	.038	.029	.033	5%	.013	.013
1971 GC	.452	.433	.401	.361	.353	.464	.459	.435	.395	.359	1%	.017	.017
	.042	.047	.034	.026	.036	.021	.019	.024	.034	.045	0.1%	.022	.022
1969 GH	.347	.315	.336	.251	.342	.314	.332	.319	.346	.299	Mesophyll ratio		
	.051	.042	.038	.031	.039	.053	.042	.038	.025	.043			
1970 GC	.307	.369	.432	.451	.321	.319	.356	.357	.447	.321			
	.053	.047	.034	.029	.037	.031	.041	.038	.046	.028	LSD:	1	2
1971 GH	.341	.324	.354	.322	.334	.327	.297	.314	.342	.333			
	.052	.048	.037	.037	.045	.030	.038	.032	.044	.023	5%	.015	.014
1971 GC	.357	.364	.381	.421	.362	.301	.362	.371	.421	.411	1%	.020	.018
	.033	.032	.040	.039	.038	.036	.041	.046	.026	.031	0.1%	.025	.023
1969 GH	.168	.222	.193	.175	.193	.214	.218	.207	.190	.248	Epidermal ratio		
	.044	.052	.047	.041	.051	.037	.044	.042	.035	.047			
1970 GC	.219	.199	.155	.177	.210	.216	.204	.205	.159	.232			
	.033	.042	.046	.038	.038	.032	.030	.043	.046	.048	LSD:	1	2
1971 GH	.183	.207	.194	.198	.214	.232	.256	.224	.182	.187			
	.061	.059	.057	.050	.053	.067	.065	.049	.047	.048	5%	.018	.012
1971 GC	.191	.203	.218	.218	.285	.235	.179	.194	.183	.231	1%	.023	.015
	.031	.041	.035	.024	.027	.024	.031	.032	.031	.027	0.1%	.030	.019

	Q. robur					Q. petraea					
Light intensity	100	75	50	25	GH	100	75	50	25	GH	
1969 GH	3.74	3.68	3.92	3.61	3.74	3.81	3.75	3.82	3.60	3.58	Basal shape of lamina (index)
	1.10	1.53	0.92	1.76	0.88	1.21	0.67	0.84	0.65	1.11	
1970 GC	3.54	3.49	3.62	3.71	3.34	3.72	3.64	3.99	3.98	3.95	
	1.42	1.33	0.97	0.95	1.76	1.01	0.99	0.98	1.11	0.78	LSD: 1 2
1971 GH	2.74	2.56	2.83	2.95	2.63	3.82	3.72	3.86	3.94	3.71	
	1.57	1.31	0.89	0.99	1.02	0.84	0.64	0.53	0.74	1.01	5% 0.41 0.44
1971 GC	3.86	3.91	3.72	3.87	3.94	3.62	3.81	3.51	3.42	3.32	1% 0.54 0.58
	1.14	1.34	1.27	1.14	1.29	1.20	1.11	1.15	0.98	0.95	0.1% 0.69 0.74
1969 GH	3.25	3.41	3.16	2.97	3.35	3.84	3.51	3.64	3.82	3.99	Auricle development (index)
	0.98	1.12	1.04	0.74	0.86	0.98	0.87	1.04	0.62	0.74	
1970 GC	2.04	2.52	2.33	2.48	2.13	3.84	3.98	3.91	3.42	3.72	
	0.78	1.21	1.41	0.99	0.96	1.02	0.77	0.67	0.98	0.89	LSD: 1 2
1971 GH	2.04	1.99	2.41	2.52	2.37	3.60	3.52	3.80	3.92	3.52	
	0.78	0.94	0.86	0.99	1.04	0.64	0.78	0.72	0.98	0.77	5% 0.35 0.40
1971 GC	3.48	3.51	3.23	3.49	3.62	3.60	3.61	3.79	3.97	3.42	1% 0.46 0.52
	1.02	1.23	0.96	1.04	1.21	0.98	1.21	0.97	0.85	0.82	0.1% 0.58 0.66
1969 GH	1.09	1.40	1.21	0.91	0.71	3.87	3.92	3.52	3.71	3.91	Leaf hairiness (index)
	0.88	0.73	0.94	1.19	1.06	0.76	0.98	1.15	1.01	0.74	
1970 GC	1.33	1.03	0.72	1.29	1.21	3.91	3.87	3.74	3.22	3.64	
	0.81	0.98	1.27	1.34	0.69	0.62	0.89	0.97	0.84	0.72	LSD: 1 2
1971 GH	1.44	1.50	0.85	0.76	1.25	3.82	3.94	3.71	3.64	3.78	
	0.93	1.24	1.17	0.95	1.15	0.47	0.98	1.23	0.92	0.92	5% 0.36 0.39
1971 GC	1.78	1.46	1.57	1.37	1.28	3.32	3.43	3.59	3.29	3.15	1% 0.48 0.52
	0.97	0.82	1.31	1.24	1.34	1.21	1.17	0.98	0.72	0.42	0.1% 0.61 0.66

	Q. robur					Q. petraea					
Light intensity	100	75	50	25	GH	100	75	50	25	GH	
1969 GH	4.32	4.57	4.82	4.64	4.54	4.03	4.47	4.21	4.82	4.76	Lobe number
	0.63	0.71	0.59	0.58	0.73	0.76	1.01	0.64	0.63	0.84	
1970 GC	4.98	4.89	4.72	4.31	4.81	5.00	5.09	4.84	4.63	4.82	
	0.64	0.58	0.75	0.84	0.61	0.59	0.78	0.69	0.57	0.88	LSD: 1 2
1971 GH	4.87	5.01	4.93	4.84	4.72	5.21	5.37	5.04	4.98	4.97	
	0.41	0.37	0.54	0.61	0.54	0.42	0.38	0.71	0.69	0.65	5% 0.250 0.204
1971 GC	4.41	4.52	4.34	4.21	3.01	4.52	4.84	4.22	4.20	3.27	1% 0.328 0.269
	0.42	0.31	0.37	0.39	0.44	0.82	0.61	0.72	0.56	0.54	0.1% 0.419 0.342
1969 GH	25.1	22.9	35.5	33.4	30.2	4.2	6.7	3.0	5.0	10.2	Venation (%)
	10.9	10.0	9.4	11.9	14.7	7.7	10.5	8.0	9.0	16.2	
1970 GC	33.1	35.4	41.8	33.9	32.4	4.1	8.3	2.1	10.2	9.0	
	12.1	11.9	9.9	9.8	8.5	6.7	7.6	9.0	8.1	9.0	LSD: 1 2
1971 GH	44.2	38.1	37.7	44.8	39.4	4.2	7.1	8.0	3.2	8.0	
	7.3	8.4	10.1	11.2	11.3	8.2	7.7	9.0	6.0	7.1	5% 3.71 3.03
1971 GC	33.8	34.9	25.4	38.3	29.3	6.0	5.4	6.7	2.3	1.1	1% 4.88 3.99
	8.0	8.9	9.4	12.5	7.7	9.0	6.1	7.9	3.0	3.0	0.1% 6.23 5.09
1969 GH	7.8	8.0	7.2	7.3	8.1	8.5	9.1	8.9	9.5	8.9	Leaf area (sq. cm.)
	1.8	1.8	1.9	1.7	1.7	1.1	2.2	1.9	1.5	1.4	
1970 GC	9.9	10.7	12.5	10.7	9.4	12.1	14.6	18.5	14.8	12.9	
	1.8	1.9	2.3	2.3	2.1	1.2	1.4	1.1	1.0	1.9	LSD: 1 2
1971 GH	13.0	15.3	12.1	14.3	15.0	22.4	22.4	25.2	23.0	24.0	
	3.5	3.6	3.3	3.1	3.5	3.0	3.0	3.7	2.5	3.2	5% 0.89 0.46
1971 GC	7.6	8.4	10.4	8.3	4.5	7.8	8.3	8.6	6.9	5.2	1% 1.17 0.60
	1.4	1.2	1.3	1.1	1.5	0.9	1.4	0.9	1.2	1.1	0.1% 1.49 0.76

	Q. robur					Q. petraea							
Light intensity	100	75	50	25	GH	100	75	50	25	GH			
1969 GH	29.7	29.6	27.4	26.2	30.1	27.4	29.4	30.0	30.1	31.3	Lamina length (mm)		
	3.1	4.5	3.2	3.5	5.2	3.0	3.0	3.2	3.2	3.0			
1970 GC	29.1	32.4	35.7	31.2	28.2	40.0	46.6	58.4	47.2	39.4			
	3.2	3.5	3.5	3.2	2.5	3.9	4.1	5.5	4.5	4.1	LSD:	1	2
1971 GH	55.0	49.7	56.3	52.5	54.5	75.9	69.4	83.2	78.0	70.0			
	6.4	7.7	7.7	7.8	8.4	10.4	11.0	12.7	11.7	11.0	5%	2.40	0.83
1971 GC	26.2	27.0	28.4	22.7	14.2	25.8	27.2	26.0	21.3	15.3	1%	3.16	1.09
	2.9	1.5	2.7	1.2	2.5	1.9	2.2	2.5	2.0	1.9	0.1%	4.03	1.39
1969 GH	3.13	4.08	3.03	3.53	3.35	3.02	3.46	3.44	3.10	3.88	Petiole length (mm)		
	1.09	1.54	1.72	0.98	0.99	1.52	1.46	0.98	1.64	1.24			
1970 GC	3.15	4.34	5.03	5.64	3.80	3.18	4.02	5.04	7.02	3.24			
	1.33	1.41	0.97	0.89	1.21	1.72	0.95	1.45	2.07	1.42	LSD:	1	2
1971 GH	4.33	4.00	4.96	4.79	4.77	7.42	6.86	8.92	7.33	6.99			
	1.36	1.42	1.27	1.54	1.36	1.43	2.09	1.78	1.66	1.51	5%	0.54	0.44
1971 GC	3.38	3.36	5.33	5.09	3.55	2.74	2.72	2.51	2.89	1.78	1%	0.71	0.58
	1.09	2.01	0.98	0.96	1.21	0.87	1.21	0.98	0.78	1.21	0.1%	0.91	0.75
1969 GH	10.5	8.3	10.1	7.4	10.0	10.1	9.5	9.7	10.7	9.1	Petiole ratio		
	2.3	2.5	2.7	2.6	3.0	1.9	2.2	1.5	1.7	1.8			
1970 GC	10.2	8.5	8.1	6.5	8.4	13.6	12.6	12.6	7.0	13.2			
	2.4	2.6	2.7	2.2	2.3	1.9	2.2	1.9	1.7	1.8	LSD:	1	2
1971 GH	12.7	13.4	12.4	12.0	12.4	11.2	11.1	10.3	11.6	11.0			
	2.2	2.3	2.4	2.3	2.3	1.6	2.3	2.2	1.9	1.7	5%	0.83	0.69
1971 GC	8.7	9.0	6.3	5.5	5.0	10.4	11.0	11.4	8.4	9.6	1%	1.09	0.91
	2.3	2.2	2.2	2.0	1.9	1.2	1.3	1.5	1.5	1.7	0.1%	1.39	1.16

	Q. robur					Q. petraea							
Light intensity	100	75	50	25	GH	100	75	50	25	GH			
1969 GH	1.75	1.69	1.98	2.00	1.81	1.91	1.85	1.76	2.11	2.01	Obversity		
	0.24	0.24	0.23	0.25	0.24	0.24	0.24	0.24	0.24	0.23			
1970 GC	1.76	1.79	2.00	2.01	1.95	1.90	1.92	1.96	2.10	1.79			
	0.24	0.25	0.22	0.22	0.26	0.23	0.23	0.23	0.24	0.24	LSD:	1	2
1971 GH	1.74	1.70	1.59	1.81	1.71	1.94	2.00	1.86	1.84	1.89			
	0.24	0.25	0.25	0.25	0.24	0.24	0.26	0.25	0.25	0.26	5%	0.091	0.092
1971 GC	1.74	1.89	1.75	1.64	1.76	1.86	1.79	2.20	1.95	2.01	1%	0.120	0.121
	0.26	0.25	0.24	0.24	0.23	0.23	0.24	0.25	0.24	0.25	0.1%	0.153	0.155
1969 GH	2.80	2.76	2.69	2.73	2.84	2.82	2.61	2.74	2.85	2.95	Lobe depth ratio		
	0.21	0.20	0.22	0.22	0.21	0.28	0.30	0.28	0.31	0.29			
1970 GC	2.40	2.62	2.65	2.85	2.33	2.48	2.68	2.94	3.06	2.54			
	0.22	0.22	0.21	0.21	0.20	0.29	0.30	0.30	0.28	0.28	LSD:	1	2
1971 GH	2.21	2.44	2.38	2.20	2.15	2.30	2.52	2.41	2.40	2.30			
	0.24	0.27	0.25	0.29	0.27	0.26	0.29	0.30	0.30	0.29	5%	0.099	0.088
1971 GC	2.75	2.91	2.88	3.20	3.40	2.95	3.21	3.24	3.46	3.65	1%	0.130	0.116
	0.20	0.22	0.22	0.22	0.23	0.22	0.24	0.26	0.25	0.28	0.1%	0.166	0.148

LSD 1 = Least significant differences for three year old seedlings

LSD 2 = Least significant differences for one year old seedlings

VARIANCE RATIOS FOR THE DIFFERENT EFFECTS AND THE ERROR VARIANCE - SEEDLING DATA

Effects	Seedling Groups (light intensities)	Harvests	Species	SG*H	H*Sp	SG*Sp	SG*H*Sp	Error Variance
Degrees of Freedom	4	2	1	8	2	4	8	1590
Total lamina thickness	184.046	603.447	9.414	139.127	26.182	15.599	4.493	24.807
Palisade thickness	109.360	423.549	11.305	98.968	17.308	15.513	4.264	14.309
Spongy mesophyll thickness	4.557	20.737	42.053	9.405	9.080	5.203	3.790	11.980
Epidermal thickness	71.038	77.235	37.201	28.759	0.519	7.930	12.802	11.150
No. of cell layers in palisade	68.583	240.457	1.167	18.355	1.852	4.855	2.787	0.175
Stomatal density	17.229	462.104	16.157	19.276	50.086	3.350	13.582	1.721
Palisade ratio	24.951	122.209	14.702	53.280	14.389	14.423	1.958	0.001224
Mesophyll ratio	53.109	226.220	17.521	91.485	9.662	44.021	19.898	0.001554
Epidermal ratio	32.857	6.161	57.395	9.212	2.988	9.457	11.410	0.002236
Basal shape of lamina	2.021	28.509	69.104	0.827	35.210	0.086	0.508	1.201
Auricle development	0.643	51.798	616.461	4.210	43.841	1.178	2.694	0.850
Leaf hairiness	8.891	0.771	2968.272	1.102	0.961	0.645	4.585	0.946
Lobe number	4.183	71.043	6.633	11.210	10.557	3.487	2.836	0.438
Venation	4.681	47.772	3513.124	3.156	43.492	13.518	9.926	96.734
Leaf area	23.276	2627.029	1753.461	24.321	423.476	21.030	7.133	5.547
Lamina length	51.636	4438.487	1578.980	32.246	370.620	24.900	4.245	40.566

Effects	Seedling Groups (light intensities)	Harvests	Species	SG*H	H*Sp	SG*Sp	SG*H*Sp	Error Variance
Degrees of Freedom	4	2	1	8	2	4	8	1590
Petiole length	40.365	462.870	196.900	32.552	184.871	4.345	6.094	2.054
Petiole ratio	41.183	160.131	57.519	26.230	173.220	1.193	17.663	4.840
Obversity	21.819	30.216	74.744	10.793	17.213	8.348	9.384	0.058
Lobe depth ratio	27.122	419.863	63.294	40.379	8.986	6.065	1.933	0.069

Significance levels	1*1590	2*1590	4*1590	8*1590
5%	3.84	3.00	2.37	1.94
1%	6.63	4.61	3.32	2.51
0.1%	10.8	6.91	4.62	3.27

VARIANCE RATIOS FOR THE DIFFERENT EFFECTS AND THE ERROR VARIANCE - THE SEEDLING DATA FOR THE SINGLE HARVEST

Effect	Species	Light intensities	LI*Sp	Error Variance	
Degrees of Freedom	1	4	4	530	
	15.488	609.575	29.545	15.838	Total lamina thickness
	2.998	318.930	8.712	12.648	Palisade thickness
	17.536	94.762	34.204	5.720	Spongy mesophyll thickness
	21.179	44.237	16.175	6.580	Epidermal thickness
	17.682	142.550	6.626	0.045	No. of cell layers in palisade
	64.168	223.740	11.600	1.754	Stomatal density
	58.094	175.912	3.816	0.001166	Palisade ratio
	1.454	90.803	28.035	0.001341	Mesophyll ratio
	49.564	77.702	39.393	0.000942	Epidermal ratio
	10.475	0.845	0.867	1.353	Basal shape of lamina
	5.628	0.818	2.402	1.078	Auricle development
	420.649	2.256	0.870	1.115	Leaf hairiness
	5.793	131.164	3.091	0.292	Lobe number
	1640.749	10.329	15.966	64.627	Venation
	21.907	218.918	21.411	1.456	Leaf area
	9.488	633.590	10.785	4.786	Lamina length
	254.317	27.000	18.156	1.383	Petiole length
	425.830	60.778	19.028	3.330	Petiole ratio
	95.666	10.016	19.726	0.060	Obversity
	181.319	136.390	1.753	0.055	Lobe depth ratio

Significance levels:	5%	1%	0.1%
1*530	4.04	7.19	12.30
4*530	2.57	3.74	5.50

APPENDIX 4

Appendix 4 lists the basic population data, together with information concerning the populations from Chapters 5 and 6. It is divided into four tables:-

1. A list of population code numbers, grid references, names, average hybrid index, hybrid number, and the results of the population characterisation.
2. A list of the means for each character for each population for the raw data, i.e. not converted to hybrid index form. These characters are in the following order from left to right - leaf regularity (LR), basal shape of lamina (BS), auricle development (AU), simple hairs (SPH), stellate hairs (STH), number of lobe pairs (LN), number of sinuses with veins (SN), venation (V), petiole length (PL), lamina + petiole length (LP), petiole ratio (PTR), lamina length to widest part (TWP), lamina length (LL), lamina shape, obversity (OB), depth of sinus (DS), lamina width (LW), and lobe depth ratio (LDR). All measurements are expressed in millimetres.
3. A list of environmental variables for each population analysed in Chapter 6 - height above sea level (Ht) feet; soil pH (pH); soil base status (B) milli-equivalents; east-west position (EW); north-south position (NS); February minimum temperature (FMIN) ^{o}C; January mean temperature (JANM) ^{o}C; July mean temperature (JULM) ^{o}C; annual rainfall (R) inches; humidity (H) - a ratio.
4. A list of species found in each population. The species are represented by code numbers which correspond to those in Table 6.2.

POPULATION DESCRIPTION

Code	Name of Woodland	Grid Reference	Average Hybrid Index	Hybrid Number	Character of Population
AA	Uffmoor Wood	SO 950 815	104.6	104.6	Pure R
AB	Pepper Wood	SO 938 744	110.6	99.4	Pure R + 2 P trees
AC	Santery Hill Wood	SO 915 737	205.8	130.4	Introgressed P (heavily)
AD	Hilcot Wood	SO 990 158	100.2	100.2	Pure R
AE	Flaxley Woods	SO 693 169	95.1	85.8	Pure R + 2 P trees
AF	Blaisdon Wood	SO 694 172	109.9	94.4	Pure R + 3 P trees
AG	nr. Walters Farm	ST 095 686	115.7	115.4	Pure R
AH	Hensol Forest	ST 040 757	89.1	89.1	Pure R
AI	nr. Tredegar	SS 635 993	162.0	133.2	Intermediate
AJ	North of AI	SS 634 995	126.6	113.5	Introgressed R (medium) + 3 P trees
AK	nr. Whitland Abbey	SN 214 181	207.8	114.9	Introgressed P (medium) + 5 R trees
AL	rr. Whitland Abbey	SN 212 174	131.2	126.1	Introgressed R (medium) + 1 P tree
AM	Minwear Wood	SN 046 137	216.4	121.0	Introgressed P (medium) + 1 R tree
AN	nr. Blackpool	SN 064 146	181.4	131.2	Introgressed P (medium) + 3 R trees
AO	Canaston Wood	SN 076 141	206.5	131.3	Introgressed P (heavily)
AP	Canaston Wood	SN 074 139	184.9	136.1	Intermediate
AQ	nr. Pontfaen	SN 032 339	208.2	129.3	Introgressed P (medium) + 1 R tree
AR	nr. Pontfaen	SN 024 343	188.4	144.1	Intermediate
AS	Llannerch	SN 057 356	177.7	144.4	Intermediate
AT	Llannerch	SN 056 353	189.8	142.3	Intermediate
AU	nr. Afon Wyddon	SN 833 424	196.2	136.1	Introgressed P (medium)
AV	nr. Aberedw	SO 085 470	223.5	116.5	Pure P
AW	The Gaer	SO 084 547	170.4	123.3	Mixed + 3 hybrid trees
AX	The Gaer	SO 085 548	186.3	113.9	Introgressed P (medium) + 5 R trees

AY	Nash Wood	SO 313 630	235.5	104.5	Pure P
BA	Hay Wood	SP 206 708	98.9	98.9	Pure R
BB	Oakley Wood	SP 302 597	105.0	97.6	Pure R + 5 P trees
BC	Hazelborough Wood	SP 654 426	93.4	93.4	Pure R
BD	Hartwell Clear Copse	SP 793 515	83.5	83.5	Pure R
BE	Monk's Arbour Wood	SP 839 860	92.6	92.6	Pure R
BF	King's Wood	SP 867 874	177.4	125.1	Mixed + 4 hybrid trees
BG	nr. Kentford	TL 684 660	97.2	97.2	Pure R
BH	Link Wood	TL 897 606	84.3	84.3	Pure R
BI	Blackthorpe	TL 892 634	87.4	87.4	Pure R
BJ	Bruisyard Wood	SM 333 673	96.9	96.9	Pure R
BK	Oak Wood	TM 133 524	90.2	90.2	Pure R
BL	Wolves Wood	TM 056 436	88.4	88.4	Pure R
BM	nr. Takeley	TL 565 216	105.0	105.0	Pure R
BN	Quendon Wood	TL 514 300	86.0	86.0	Pure R
BO	Quendon Wood	TL 514 300	83.3	83.3	Pure R
BP	nr. Bridge Green	TL 473 370	109.7	109.7	Pure R
BQ	nr. Bridge Green	TL 473 370	116.1	116.1	Introgressed R (slight)
BR	Holly Wood	SP 588 100	96.2	96.2	Pure R
BS	Holly Wood	SP 588 100	97.8	97.8	Pure R
BT	Holly Wood	SP 588 100	118.3	113.1	Introgressed R (medium)
BU	Finstock Wood	SP 372 159	97.0	97.0	Pure R
BV	Wolford Wood	SP 240 333	102.9	102.9	Pure R
BW	Rough Hill Wood	SP 056 640	213.5	115.7	Introgressed P (medium) + 5 R trees
BX	Pitcher Oak Wood	SP 023 671	111.2	110.2	Introgressed R (slight)
BY	Foxlydiate Wood	SP 022 673	131.8	122.7	Introgressed R (medium) + 1 P tree
CA	Hopwas Hays Wood	SK 166 054	122.2	110.3	Introgressed R (medium) + 4 P trees
CB	Bradley Wood	SK 198 463	109.0	102.7	Introgressed R (slight) + 3 P trees
CC	Robin Hood's Stride	SK 222 619	113.7	113.7	Pure R
CD	Padley Wood	SK 256 798	221.6	118.1	Pure P (pseudo-pure)

CE	Padley Wood	SK 255 795	228.4	111.6	Pure P (pseudo-pure)
CF	nr. Knaith Park	SK 844 858	112.8	105.7	Pure R + 3 P trees
CG	Mill House Farm	TF 173 826	111.3	110.7	Pure R (pseudo-pure)
CH	Willoughby Wood	TF 459 706	92.2	92.2	Pure R
CI	Shire Wood	TF 293 616	102.2	102.2	Pure R
CJ	White Hills Wood	TF 699 249	88.8	88.8	Pure R
CK	Roydon	TF 695 245	93.1	93.1	Pure R
CL	Weasenham Plant-ation	TF 855 199	84.6	84.6	Pure R
CM	opposite Shakers Wood	TL 814 965	87.1	87.1	Pure R
CN	Snake Wood	TL 812 906	88.0	88.0	Pure R
CO	Newell Wood	TF 008 144	116.6	107.1	Introgressed R (medium) + 1 P tree
CP	Newell Wood	TF 007 142	80.7	80.7	Pure R
CQ	Elsea Wood	TF 098 185	98.4	98.4	Pure R
CR	Math Wood	TF 096 185	105.8	105.8	Pure R
CS	Park Wood	TF 104 161	84.8	84.8	Pure R
CT	Collyweston Great Wood	TF 014 008	114.0	109.8	Introgressed R (slight)
CU	Collyweston Great Wood	TF 013 007	111.3	104.2	Pure R + 2 P trees
CV	Tugby Wood	SK 768 020	96.7	96.7	Pure R
CW	Hawcliff Hill	SK 569 152	100.3	100.3	Pure R
CX	Swithland Wood	SK 540 126	204.2	132.3	Introgressed P (heavily) + 1 R tree
DA	Star Crossing Halt	SJ 181 678	204.5	127.4	Introgressed P (medium) + 1 R tree
DB	nr. Llyn Helyg	SJ 113 769	200.8	128.5	Introgressed P (heavily) + 3 R trees
DC	Hafod Wood	SH 999 688	136.6	131.4	Introgressed R (heavily) + 2 P trees
DD	nr. Treflech	SH 945 685	159.4	143.2	Intermediate + 1 P + 3 R trees
DE	Glynisaf	SH 768 730	163.4	143.1	Intermediate + 6 P + 17 R trees
DF	Coed Gwydir	SH 778 658	132.4	118.4	Introgressed R (heavily) + 4 P trees

DG	nr. Hafothy	SH 782 692	214.0	123.5	Introgressed P
DH	nr. Tyddyn	SH 479 402	160.0	123.0	Mixed + 3 hybrid trees
DI	Cabin Wood	SH 461 388	170.0	153.6	Intermediate
DJ	Coed Rhos-fawr	SH 375 395	194.8	137.5	Introgressed P (heavily) + 3 R trees
DK	nr. Rhyd-y-gwystl	SH 406 392	146.1	137.0	Introgressed R (heavily) + 1 P tree
DL	nr. Tremadoc	SH 565 397	139.3	121.8	Introgressed R (heavily)
DM	nr. Erw-wen	SH 598 326	198.6	139.4	Introgressed P (heavily)
DN	Geuos	SH 665 185	202.8	134.0	Introgressed P (heavily) + 1 R tree
DO	Nescliff Hill	SJ 385 198	197.8	132.0	Introgressed P (heavily) + 4 R trees
DP	nr. Westcott	SJ 398 019	117.6	106.2	Introgressed R (slight) + 4 P trees
DQ	Stoneyhill	SJ 664 058	214.0	125.7	Introgressed P (slight)
DR	The Ercall	SJ 646 103	210.8	120.5	Introgressed P (heavily) + 5 R trees
DS	Chetwynd Heath	SJ 712 222	102.1	100.8	Pure R + 2 P trees
DT	Hungry Hatton	SJ 675 268	102.9	99.9	Pure R + 3 P trees
DU	Fullmoor Wood	SJ 942 116	103.7	103.7	Pure R
DV	Bilberry Wood	SJ 312 642	111.2	107.5	Introgressed R (medium) + 1 P tree
DW	Little Budworth Common	SJ 588 658	107.9	107.9	Pure R
DX	Little Budworth Common	SJ 584 652	103.6	103.6	Pure R
DY	Moss Cott	SJ 730 802	110.0	110.0	Pure R
DZ	Alderley Edge	SJ 856 773	115.7	114.9	Introgressed R (slight)
AAA	nr. Rhydowen	SN 452 443	200.3	139.7	Pure P (pseudo-pure)
AAB	nr. Bwlchyddwyallt	SN 702 634	217.7	120.1	Introgressed P (heavily)
AAC	nr. Pant Mawr	SN 850 824	216.8	121.9	Introgressed P (medium)

AAD	nr. Cross Ash	SO 414 197	131.1	126.6	Introgressed R (medium) + 1 P tree
AAE	Whitwich Manor	SO 609 460	92.7	92.7	Pure R (psuedo-pure)
BBA	Monks Wood	TL 205 797	97.7	97.7	Pure R
BBB	Sheerhatch Wood	TL 127 468	137.9	102.6	Mixed + 2 hybrid trees
BBC	Hitch Wood	TL 196 232	177.2	111.2	Mixed + 10 hybrid trees
CCA	Cotgrave Wood	SK 650 340	104.1	104.1	Pure R
CCB	Old orchard	SK 909 696	84.9	84.9	Pure R
CCC	Nr. Aunsly	TF 044 385	98.4	98.4	Pure R
CCD	Aylsham	TG 165 254	96.3	96.3	Pure R
CCE	New Witton Park	TG 318 315	147.5	123.3	Mixed + 5 hybrid trees
CCF	Wood nr. Wroxham	TG 309 135	99.4	99.4	Pure R
DDA	Pentreheylin Hall	SJ 251 195	129.8	129.6	Pure R
A1	Austy Wood	SP 165 625	112.4	107.4	Introgressed R (slight) + 2 P trees
A2	Austy Wood	SP 173 633	99.9	99.9	Pure R
W1	Wyre Forest	SO 746 762	241.0	99.0	Pure P
W2	Wyre Forest	SO 762 764	240.7	99.3	Pure P
W3	Wyre Forest	SO 743 762	225.9	101.4	Pure P + 4 R trees
W4	Wyre Forest	SO 760 765	244.4	92.0	Pure P + 1 R tree
W5	Wyre Forest	SO 748 763	231.2	97.8	Pure P + 3 R trees
W6	Wyre Forest	SO 719 767	120.3	112.6	Introgressed P (slight) + 2 R trees
W7	Wyre Forest	SO 741 756	238.5	101.5	Pure P (pseudo-pure)
W8	Wyre Forest	SO 743 762	194.3	97.7	Mixed + 2 hybrid trees
W9	Wyre Forest	SO 743 769	234.4	105.2	Pure P
W10	Wyre Forest	SO 742 761	226.9	106.9	Pure P + 1 R tree
W11	Wyre Forest	SO 742 761	191.6	121.2	Introgressed P (medium) + 5 R trees
A	Wyre Forest	SO 749 758	218.9	120.5	Introgressed P (slight)
B	Wyre Forest	SO 760 763	238.9	101.1	Pure P
C	Wyre Forest	SO 760 765	224.2	113.2	Pure P + 1 R tree

D	Austy Wood	SP 165 625	117.5	106.0	Introgressed R (medium) + 5 P trees
F	Sutton Park	SP 103 970	135.8	109.5	Introgressed R (medium) + 9 P trees
G	Wyre Forest	SO 718 768	122.8	114.8	Pure P + 3 R trees
H	Wyre Forest	SO 756 765	226.7	106.4	Pure P + 2 R trees

POPULATIONAL RAW DATA - CHARACTER MEANS

Code	LR	BS	AU	SPH	STH	LN	SN	V	PL	LP	PTR	TWP	LL	CB	DS	LW	LDR
AA	5.1	5.1	5.1	1.1	0.5	21.4	18.2	43.5	5.8	96.6	6.1	59.4	90.8	1.55	16.2	25.8	1.63
AB	5.8	3.8	5.0	2.2	1.6	22.9	20.1	46.5	6.8	102.9	6.6	58.4	96.1	1.71	18.2	30.2	1.79
AC	12.0	13.8	15.1	12.6	9.1	26.2	8.4	17.5	11.5	120.4	9.4	60.0	108.9	1.83	13.8	31.2	2.42
AD	8.2	5.2	7.0	1.6	0.3	21.9	23.4	54.5	3.7	99.3	3.6	59.8	95.6	1.62	17.1	28.7	1.75
AE	4.8	1.8	2.8	2.2	1.2	23.0	28.5	26.0	6.0	105.9	5.5	64.5	99.9	1.56	18.1	30.4	1.75
AF	6.6	5.7	6.6	2.5	2.2	23.8	25.8	56.9	6.2	101.8	5.9	57.9	95.6	1.67	18.4	29.3	1.71
AG	11.6	3.9	4.3	3.8	1.2	20.9	15.3	36.9	5.2	103.4	5.1	61.9	98.2	1.61	17.9	31.4	1.85
AH	4.4	2.4	3.2	0.4	0.0	22.8	29.2	64.9	5.6	110.6	5.1	67.6	105.4	1.57	19.6	31.9	1.66
AI	12.7	10.3	10.6	7.0	6.2	25.1	12.4	26.3	7.5	100.8	7.0	52.0	93.3	1.83	14.3	27.2	2.12
AJ	11.7	8.8	9.8	3.3	2.3	23.4	18.9	42.0	5.0	89.0	5.2	51.2	85.4	1.68	14.7	25.2	1.84
AK	13.5	13.4	13.8	12.1	11.4	27.6	7.6	14.9	11.2	116.1	9.4	58.1	105.3	1.83	13.4	29.0	2.93
AL	11.1	5.1	6.1	4.4	3.3	23.8	16.5	35.1	5.0	104.5	4.8	61.4	99.5	1.64	15.6	28.9	2.06
AM	13.8	11.4	12.2	14.3	15.2	26.0	8.8	17.3	14.2	119.1	12.0	57.6	107.4	1.39	12.9	32.9	2.79
AN	11.7	10.5	12.1	9.8	11.2	26.9	10.2	21.4	10.3	120.6	8.3	63.2	110.3	1.78	17.8	33.7	1.99
AO	12.6	11.5	11.9	12.0	13.8	28.9	11.7	20.7	14.1	129.3	10.9	60.1	115.2	1.95	15.2	33.5	2.23
AP	12.5	8.9	10.2	11.6	11.8	27.4	15.3	28.9	11.9	119.3	9.9	60.9	107.4	1.78	15.3	31.4	2.21
AQ	12.9	15.0	14.8	11.5	14.4	26.0	12.7	25.3	11.1	137.5	8.0	71.7	126.4	1.80	15.2	37.5	2.72
AR	11.2	10.6	12.1	12.1	9.5	25.7	12.9	25.0	10.9	128.1	8.4	70.8	117.3	1.68	16.0	33.2	2.21
AS	10.8	9.4	10.9	13.8	13.4	24.4	11.9	25.8	10.0	108.4	9.1	50.1	98.4	2.00	15.2	30.5	1.97
AT	12.1	11.0	11.7	14.6	13.7	24.2	15.7	34.3	11.2	115.7	9.5	52.0	104.4	2.05	14.6	30.7	2.20
AU	13.8	13.2	14.0	10.0	9.2	27.5	10.3	19.1	11.6	126.1	9.0	73.3	114.5	1.57	15.9	34.4	2.30
AV	14.2	12.4	13.1	13.5	13.6	28.3	6.0	10.8	17.4	114.1	15.0	59.7	96.8	1.63	11.7	28.9	2.60
AW	13.3	10.7	11.2	5.2	5.8	24.3	13.5	30.3	11.3	124.9	8.9	62.7	113.6	1.83	19.3	36.7	2.09
AX	11.6	11.2	12.6	9.2	8.4	25.4	9.1	20.3	11.2	123.2	9.0	60.6	112.0	1.87	19.1	36.3	2.17
AY	16.1	15.0	14.9	14.1	14.5	27.8	5.8	10.7	15.7	137.9	11.3	67.6	122.2	1.83	15.0	35.4	2.49
BA	7.0	4.4	5.6	1.3	0.1	23.4	22.7	48.8	3.5	96.4	3.4	56.9	92.9	1.67	17.6	26.7	1.58
BB	8.2	3.7	5.4	2.8	2.5	22.5	24.7	56.6	5.4	96.7	5.4	57.8	91.4	1.59	15.7	27.6	1.85
BC	5.4	5.1	5.5	0.5	0.1	21.6	23.6	55.6	3.9	98.5	4.0	60.7	94.5	1.58	17.4	28.3	1.70
BD	4.2	1.2	2.0	1.8	0.6	22.1	26.3	60.8	4.2	93.7	4.5	55.7	89.8	1.63	17.1	27.4	1.67
BE	5.8	3.0	4.0	1.1	0.2	21.5	22.4	52.5	4.6	95.6	4.7	57.3	91.1	1.62	17.4	28.0	1.70
BF	9.6	14.8	15.5	9.3	7.6	29.3	15.8	29.6	8.0	120.4	6.6	61.2	112.4	1.88	18.9	31.0	1.85
BG	5.0	4.3	5.7	0.6	0.1	23.7	23.1	49.5	5.4	109.0	4.9	63.7	103.7	1.66	21.3	33.6	1.62

CODE	LR	BS	AU	SPH	STH	LN	SN	V	PL	LP	PTR	TWP	LL	OB	DS	LW	LDR
BH	6.6	2.8	3.5	1.3	0.1	23.5	29.1	63.1	2.9	90.3	3.2	57.0	87.4	1.55	16.8	26.6	1.68
BI	6.9	1.2	2.2	1.4	0.6	24.0	30.3	64.3	3.8	93.8	4.0	56.0	90.0	1.63	16.7	26.2	1.63
BJ	6.1	5.4	6.4	1.1	0.0	23.0	25.2	56.3	3.3	98.0	3.4	61.9	94.7	1.54	16.4	27.2	1.78
BK	6.1	2.0	3.3	2.2	0.9	21.2	24.5	58.9	5.6	99.3	5.6	60.1	93.7	1.58	19.0	29.6	1.65
BL	4.9	3.6	4.1	0.9	0.1	23.7	23.5	50.3	4.3	102.0	4.2	61.4	97.8	1.62	21.1	32.3	1.57
BM	5.5	4.2	5.3	1.1	0.3	23.2	21.2	47.8	5.4	109.8	5.0	66.1	104.4	1.61	19.2	32.2	1.74
BN	6.2	1.6	2.6	1.2	0.6	23.2	24.2	52.8	4.2	91.7	4.7	55.2	87.4	1.60	18.6	28.5	1.62
BO	8.1	3.1	4.5	0.4	0.0	22.9	28.4	62.7	1.8	83.3	2.2	50.4	81.4	1.62	14.6	22.9	1.67
BP	7.2	5.5	6.3	1.3	0.2	27.0	23.6	45.5	5.4	113.0	4.8	66.8	107.6	1.64	21.8	34.2	1.64
BQ	8.3	9.1	9.9	1.4	0.1	24.1	19.2	40.7	4.8	102.1	4.5	60.2	97.3	1.66	18.9	31.9	1.77
BR	5.0	2.6	3.4	0.2	0.2	25.1	23.5	46.6	5.0	100.3	5.0	61.6	95.2	1.56	17.9	28.4	1.65
BS	8.7	5.1	6.7	1.7	0.5	24.6	27.6	56.2	3.1	87.3	3.6	55.0	84.2	1.56	15.1	24.1	1.62
BT	11.4	7.1	8.4	4.2	2.4	24.6	20.9	45.0	3.5	94.8	3.5	54.6	91.2	1.71	15.9	26.8	1.72
BU	6.2	4.7	5.6	0.3	0.0	24.2	24.3	50.6	4.0	98.7	4.0	59.9	94.7	1.60	17.6	28.5	1.71
BV	6.2	2.8	3.6	2.3	1.8	21.4	19.8	46.7	4.9	99.1	4.9	58.7	94.2	1.62	16.2	28.0	1.79
BW	13.3	13.0	13.1	13.8	13.1	28.3	5.7	10.8	11.6	116.7	9.9	55.7	105.2	1.90	13.7	32.6	2.60
BX	7.8	6.3	7.5	2.8	1.8	24.0	22.8	48.8	4.2	92.7	4.5	58.4	88.5	1.54	15.0	26.3	1.85
BY	9.8	8.9	9.5	3.2	1.8	24.0	15.1	33.4	5.7	103.6	5.4	59.3	97.9	1.66	17.7	30.2	1.90
CA	9.7	5.7	6.5	4.4	4.7	22.6	17.9	40.4	5.1	99.1	4.9	59.3	93.9	1.59	16.3	28.3	1.84
CB	6.8	4.7	6.5	1.8	1.2	23.5	20.5	45.4	5.6	106.6	5.2	63.5	101.0	1.62	19.0	31.3	1.82
CC	9.6	4.6	5.8	3.0	2.5	22.4	18.1	41.6	4.9	88.9	5.6	52.7	84.0	1.61	14.4	25.7	2.02
CD	15.7	13.6	13.2	13.3	14.2	28.1	2.7	4.9	9.8	98.5	9.8	51.5	88.6	1.74	8.9	26.0	3.25
CE	15.9	14.3	15.3	12.6	13.7	29.1	3.2	5.5	10.5	108.9	9.5	57.6	98.6	1.74	9.7	28.5	3.22
CF	4.5	4.6	5.7	2.8	1.8	20.8	13.8	34.0	5.1	89.3	5.8	53.9	84.1	1.58	14.4	24.4	1.82
CG	5.2	5.7	7.6	3.1	2.1	21.0	17.1	41.9	5.0	96.7	5.3	55.5	91.6	1.68	16.7	27.6	1.74
CH	8.0	1.7	4.0	0.9	0.4	22.3	27.3	61.6	5.3	100.9	5.2	58.7	95.4	1.64	18.2	29.6	1.69
CI	7.7	5.9	6.7	0.4	0.0	23.9	26.9	57.1	5.0	101.1	5.0	60.4	96.1	1.61	17.5	28.9	1.75
CJ	8.2	2.6	3.5	0.7	0.2	21.9	26.1	60.8	4.2	97.4	4.3	57.1	97.2	1.65	18.5	29.2	1.67
CK	5.4	3.0	3.4	2.6	0.6	19.2	23.8	63.4	4.7	92.6	5.0	53.4	87.9	1.69	15.0	25.3	1.78
CL	6.0	2.2	3.5	2.5	1.0	21.5	24.4	58.2	3.8	89.4	4.3	52.3	85.6	1.67	18.3	28.1	1.62
CM	5.6	1.5	2.4	0.7	0.5	22.4	26.9	61.4	5.9	106.5	5.6	62.4	100.5	1.65	21.0	33.2	1.63
CN	3.3	1.9	2.4	3.4	2.2	17.9	18.1	51.0	4.5	78.4	5.7	42.4	73.9	1.79	15.5	25.0	1.72
CO	7.0	3.1	4.0	4.6	5.0	21.2	20.6	50.0	8.5	112.5	7.4	61.4	103.9	2.29	19.8	32.4	1.71
CP	3.2	1.8	2.2	0.2	0.0	24.8	23.3	48.6	3.2	97.9	3.3	63.2	94.7	1.51	20.4	29.6	1.49
CQ	7.0	3.6	4.4	0.3	0.0	26.6	26.6	50.7	4.6	116.3	4.0	72.1	111.7	1.57	22.6	37.8	1.76

CODE	LR	BS	AU	SPH	STH	LN	SN	V	PL	LP	PTR	TWP	LL	OP	DS	LW	LDR
CR	8.0	8.2	8.9	1.0	0.4	23.0	24.2	52.3	4.3	104.8	4.3	62.6	100.4	1.63	19.3	30.8	1.69
CS	5.7	2.0	3.7	1.5	0.5	21.7	24.8	25.8	3.1	84.8	3.6	49.7	81.7	1.66	15.2	24.7	1.71
CT	6.6	4.8	6.1	3.0	1.9	24.3	21.4	44.4	6.8	113.9	6.0	70.8	107.1	1.53	20.8	33.7	1.68
CU	7.5	6.2	6.9	3.4	1.6	24.6	24.2	50.7	5.0	113.6	4.3	64.6	108.6	1.72	21.2	35.3	1.73
CV	4.9	3.1	4.6	1.5	0.7	21.7	21.0	50.2	6.2	98.6	6.4	58.0	92.3	1.62	18.3	29.8	1.67
CW	5.0	3.6	4.7	1.6	0.7	24.0	23.0	49.0	4.7	97.5	4.5	60.3	92.8	1.55	18.2	26.4	1.68
CX	14.2	12.0	13.0	15.6	14.0	29.7	9.2	16.9	9.5	105.7	8.9	56.4	96.3	1.74	13.0	29.0	2.40
DA	12.2	13.0	14.0	10.7	10.4	26.4	7.1	14.3	11.1	119.9	9.2	61.9	108.8	1.77	13.9	34.9	2.63
DB	13.5	13.5	13.9	8.8	8.1	30.2	6.3	11.2	9.4	115.7	8.0	65.9	106.3	1.63	13.7	32.7	2.59
DC	10.7	9.3	10.7	5.6	6.6	24.2	22.9	48.6	5.4	93.3	5.8	51.4	87.9	1.73	13.4	24.5	1.96
DD	9.3	11.3	12.0	6.7	5.2	24.8	13.1	28.0	11.0	110.6	9.8	62.0	99.6	1.62	17.5	32.5	1.92
DE	12.0	11.7	13.4	6.9	8.6	25.4	13.7	29.3	6.4	99.1	6.3	54.5	92.6	1.71	13.7	27.5	2.08
DF	9.9	7.0	9.0	5.6	6.3	24.6	17.4	37.6	6.1	100.5	6.6	56.1	93.5	1.69	17.1	28.8	1.73
DG	14.6	11.0	11.6	15.6	17.0	27.9	7.5	13.8	14.1	110.6	12.8	53.4	96.5	1.84	13.8	28.5	2.17
DH	11.4	6.5	7.8	9.2	12.7	25.4	20.7	42.5	8.8	112.9	7.7	61.6	104.1	1.73	14.7	29.0	2.08
DI	9.8	6.9	9.3	9.9	7.0	25.4	11.4	22.6	10.6	111.6	9.6	61.6	101.1	1.65	14.9	30.1	2.14
DJ	15.0	9.5	11.4	11.7	12.6	27.5	10.4	19.6	10.8	110.8	9.6	56.2	100.1	1.81	13.9	29.2	2.30
DK	10.3	6.7	9.1	5.9	6.2	24.1	18.8	40.3	8.1	109.1	7.6	55.8	101.0	1.86	16.3	30.9	1.99
DL	7.1	7.2	7.8	4.3	3.5	27.5	13.8	27.3	7.3	110.6	6.6	65.4	103.2	1.61	18.4	32.2	1.82
DM	13.1	12.7	13.5	14.4	13.8	26.5	11.4	22.0	13.1	113.1	11.6	59.3	99.9	1.72	15.6	30.7	2.06
DN	14.1	12.9	13.3	11.5	11.5	28.3	10.0	18.8	13.1	119.7	10.8	62.7	106.6	1.72	15.2	32.5	2.25
DO	12.5	11.0	12.4	8.3	8.4	28.6	9.4	17.0	14.4	116.9	12.1	57.5	102.5	1.81	13.9	30.0	2.30
DP	7.6	5.8	7.6	2.7	2.7	21.3	19.4	47.7	5.5	104.7	5.2	62.6	99.3	1.61	16.3	29.3	1.94
DQ	13.5	14.4	14.4	13.6	12.5	30.7	5.0	8.4	9.6	109.9	8.8	55.6	100.3	1.81	13.3	29.1	2.34
DR	16.1	12.4	12.9	11.5	10.9	28.2	7.5	14.1	12.4	117.3	10.6	57.1	104.9	1.86	14.1	13.7	2.43
DS	7.6	4.7	5.5	2.7	1.5	22.8	24.8	55.4	5.4	105.3	5.1	52.2	99.9	1.64	19.7	31.7	1.66
DT	7.2	4.7	5.1	1.7	0.7	22.9	23.7	53.1	4.9	98.3	5.0	58.0	93.4	1.63	17.2	28.6	1.76
DU	8.8	3.9	5.1	2.8	1.3	24.0	24.5	52.1	4.9	98.5	4.9	58.1	93.5	1.63	18.2	28.5	1.66
DV	10.7	5.0	6.3	4.0	2.4	24.7	24.2	50.3	4.4	94.7	4.5	57.8	90.3	1.60	15.9	27.1	1.75
DW	9.3	5.7	8.0	2.4	1.1	24.9	25.2	51.4	4.0	96.1	4.0	58.9	92.0	1.57	16.7	27.5	1.72
DX	11.8	4.3	5.0	1.4	0.9	24.6	25.7	52.6	4.8	105.9	4.4	61.4	101.2	1.66	19.9	31.9	1.65
DY	9.0	7.7	8.0	1.4	0.4	24.2	21.9	46.4	4.2	96.0	4.4	57.9	91.7	1.60	16.6	27.1	1.71
DZ	7.6	6.0	6.9	2.4	1.7	23.9	19.7	43.4	5.3	100.4	5.2	61.0	95.2	1.50	16.2	27.6	1.85
AAA	15.1	14.8	14.1	15.4	16.5	28.7	13.6	23.9	8.2	95.2	8.5	48.8	87.0	1.80	11.6	26.1	2.36
AAB	14.6	13.7	13.7	14.8	14.0	27.1	5.6	10.5	13.6	118.1	11.3	54.7	104.5	1.95	14.9	30.9	2.17

CODE	LR	BS	AU	SPH	STH	LN	SN	V	PL	LP	PTR	TWP	LL	OB	DS	LW	LDR
AAC	15.7	14.1	14.8	12.0	12.1	26.1	6.4	12.4	13.4	115.2	11.6	55.0	101.7	1.87	13.1	29.1	2.40
AAD	12.0	7.2	7.5	4.3	4.2	21.2	14.6	35.9	4.8	85.6	5.5	47.3	80.9	1.74	12.2	23.7	2.03
AAE	10.4	6.5	7.5	1.2	0.1	21.1	25.6	62.2	3.0	75.2	4.0	41.9	72.6	1.75	14.4	22.0	1.58
BBA	12.2	3.5	4.5	2.5	1.2	23.4	28.6	61.8	4.5	90.7	4.9	53.6	86.2	1.63	16.5	26.5	1.70
BBB	11.8	7.8	8.4	6.3	5.0	25.1	18.1	37.6	6.1	100.1	5.7	54.4	93.7	1.74	17.2	29.5	1.89
BBC	12.1	10.8	11.1	11.5	10.4	26.0	12.2	27.3	9.5	106.9	8.6	52.2	97.4	1.85	15.6	30.4	2.14
CCA	12.0	5.3	5.5	2.4	1.5	20.7	22.4	56.5	4.7	89.1	5.0	53.0	84.5	1.63	15.3	25.8	1.75
CCB	7.3	1.9	2.9	0.9	0.2	21.0	28.2	67.9	4.6	93.8	4.9	56.3	89.2	1.61	18.0	21.1	1.57
CCC	8.2	4.9	5.6	4.6	2.2	21.2	23.0	55.4	4.2	84.3	5.1	48.5	80.1	1.68	15.7	23.4	1.53
CCD	11.7	4.1	5.1	2.8	1.2	21.4	24.9	58.6	3.8	90.1	4.2	53.5	86.3	1.54	16.4	27.3	1.71
CCE	10.2	8.6	9.5	8.2	7.4	24.8	17.4	37.6	7.6	102.6	7.3	59.8	95.0	1.62	16.1	30.3	2.07
CCF	8.4	5.5	7.1	3.6	1.6	22.7	24.9	55.0	3.6	97.9	3.7	53.6	94.3	1.80	20.3	30.7	1.57
DDA	12.1	14.3	15.7	0.3	0.0	25.5	25.0	49.5	6.4	117.8	5.5	65.4	111.4	1.72	23.3	33.6	1.48
A1	8.4	5.1	7.1	1.8	1.1	25.0	22.6	46.2	5.6	110.9	5.0	65.0	105.3	1.63	20.9	33.9	1.73
A2	7.2	5.8	6.6	1.1	0.7	22.6	23.8	53.7	5.0	102.8	4.8	61.8	97.8	1.63	19.2	31.0	1.68
W1	16.0	17.2	17.6	14.4	13.8	28.2	3.0	6.0	16.7	129.4	12.8	59.9	112.7	1.90	14.5	34.4	2.78
W2	15.0	17.1	17.5	12.1	12.5	30.0	3.7	5.9	15.2	129.7	11.3	64.6	114.5	1.81	13.3	32.5	2.64
W3	14.5	16.7	17.2	12.3	11.8	29.6	6.6	12.7	14.3	126.2	11.2	62.0	111.5	1.80	14.5	32.6	2.33
W4	15.2	17.7	18.1	11.1	11.7	27.0	3.4	6.7	19.5	134.0	14.4	62.1	114.5	1.85	12.9	33.4	2.73
W5	14.0	16.0	16.1	10.9	10.5	26.3	6.1	13.3	19.6	143.1	13.6	67.2	124.2	1.87	15.2	35.4	2.55
W6	8.6	9.0	9.3	2.9	1.3	22.9	23.3	52.9	6.9	126.6	5.2	72.1	119.6	1.68	24.7	38.7	1.63
W7	13.8	15.8	16.3	13.0	15.4	27.9	3.5	6.8	18.0	128.8	13.9	57.5	110.8	1.96	14.3	34.4	2.53
W8	12.0	13.5	13.8	9.2	10.7	24.9	12.4	27.8	12.4	126.1	9.6	60.0	113.8	1.94	16.6	33.7	2.26
W9	14.7	15.8	15.8	13.9	15.9	28.4	5.7	10.1	17.2	129.9	13.3	60.4	112.6	1.89	15.2	33.9	2.35
W10	13.3	12.8	13.4	12.9	12.7	27.1	7.7	15.5	23.0	145.1	15.9	64.1	122.1	1.92	16.9	35.7	2.24
W11	13.3	14.4	15.0	8.2	7.2	28.4	11.2	21.9	11.1	121.4	8.9	64.4	110.3	1.74	16.4	32.1	2.07
A	14.6	9.7	13.2	11.6	13.8	28.5	5.0	9.2	13.3	117.8	11.3	61.8	104.4	1.74	11.8	31.5	3.13
B	14.9	12.7	15.5	16.6	16.7	27.9	5.0	9.4	14.1	116.5	12.1	54.8	102.4	1.96	10.2	29.8	3.41
C	15.6	14.1	16.3	15.0	15.2	29.7	9.5	16.6	10.8	114.9	9.3	63.0	104.2	1.68	11.2	28.7	2.80
D	10.1	3.7	6.8	4.1	3.0	22.3	21.1	49.8	6.2	93.9	6.3	53.7	87.7	1.68	15.6	28.6	2.00
F	11.5	4.7	7.5	6.8	6.9	22.6	23.2	54.4	7.7	96.7	7.8	55.8	88.9	1.64	13.6	26.2	2.16
G	9.8	6.2	7.3	3.7	3.0	21.4	24.4	58.4	6.3	105.6	5.8	62.7	99.3	1.64	13.3	30.0	2.30
H	14.5	11.3	14.6	15.5	16.9	25.4	9.2	18.5	18.6	122.6	15.1	54.4	102.5	1.97	12.4	27.9	3.07

Characters LR, BS, AU, SPH, STH, LN, SN: average given per tree, i.e. summed for five leaves

Other characters: average given per leaf

POPULATIONAL ENVIRONMENTAL VARIABLES

CODE	Ht	pH	B	EW	NS	FMIN	JANM	JULM	R	H
AA	525	6.94	12.5	157	73	34.8	40.2	62.0	31.0	180
AB	520	6.21	10.1	158	79	34.8	40.3	62.1	29.0	180
AC	450	5.41	8.8	159	80	34.9	40.3	62.1	29.0	180
AD	825	6.52	13.4	157	115	35.3	40.2	62.2	34.0	185
AE	150	6.82	15.7	182	115	36.2	41.2	62.2	40.0	195
AF	275	7.13	21.4	183	116	36.2	41.1	62.2	40.0	195
AG	175	6.34	13.9	214	145	37.9	42.4	61.6	35.0	260
AH	400	6.52	10.1	216	140	38.2	42.3	61.6	35.0	265
AI	250	5.78	15.4	241	126	37.7	42.9	61.0	43.0	330
AJ	200	6.81	13.1	241	127	37.7	42.9	61.0	43.0	330
AK	300	7.21	14.7	266	114	37.6	42.8	60.0	44.0	355
AL	225	6.43	13.6	265	115	37.6	42.8	60.0	44.0	355
AM	75	6.21	7.7	278	116	38.2	43.0	59.5	48.0	360
AN	50	6.51	10.1	277	115	38.2	43.0	59.5	48.0	360
AO	200	5.21	4.4	277	114	38.2	43.0	59.5	48.0	360
AP	240	5.48	9.7	276	117	38.2	43.0	59.5	48.0	360
AQ	350	6.21	3.2	279	104	37.9	42.9	59.5	45.0	360
AR	300	6.43	7.7	280	104	37.9	42.9	59.5	45.0	360
AS	600	5.58	5.5	278	103	37.9	42.9	59.5	45.0	360
AT	450	7.03	6.8	279	103	37.9	42.9	59.5	45.0	360
AU	550	5.31	5.7	232	103	36.0	41.9	60.9	58.0	305
AV	560	5.94	12.3	214	96	35.2	41.3	61.0	60.0	260
AW	800	5.87	4.4	214	90	35.2	41.2	60.9	59.0	265
AX	900	6.01	6.9	215	89	35.2	41.2	60.9	58.0	265
AY	525	4.87	2.0	198	85	34.9	40.8	61.1	42.0	195
BA	420	6.34	14.4	145	78	34.8	40.2	62.2	32.0	165
BB	225	6.41	11.3	137	89	34.7	39.9	62.3	28.0	160
BC	480	7.22	24.7	115	97	34.2	39.7	62.1	27.0	145
BD	425	8.31	23.1	106	94	33.8	39.7	61.9	26.0	140
BE	400	6.91	19.8	103	72	33.6	39.7	61.7	25.0	155
BF	400	6.54	11.7	102	71	33.6	39.7	61.7	25.0	155
BG	150	7.02	15.4	52	85	33.4	39.5	62.0	24.0	125
BH	250	6.33	10.8	37	87	33.2	39.5	62.2	23.0	130
BI	190	5.99	14.7	38	84	33.4	39.5	62.2	23.0	130
BJ	160	7.23	18.7	10	83	34.9	39.8	61.8	37.0	140
BK	200	7.11	12.6	23	91	34.2	39.7	61.9	27.0	130
BL	200	5.98	8.7	28	98	34.1	39.7	62.0	26.0	125
BM	320	6.22	11.4	60	110	33.9	39.8	62.6	27.0	100
BN	300	6.93	14.9	61	106	33.8	39.8	62.6	27.0	100
BO	300	7.33	32.8	61	106	33.8	39.8	62.6	27.0	100
BP	400	7.04	29.6	64	102	33.7	39.7	62.4	26.0	110
BQ	375	6.22	13.7	64	102	33.7	39.7	62.4	26.0	110
BR	225	6.47	16.4	120	118	34.8	40.0	62.4	29.0	160
BS	225	6.53	9.3	120	118	34.8	40.0	62.4	29.0	160
BT	225	6.47	10.8	120	118	34.8	40.0	62.4	29.0	160
BU	450	5.88	13.1	135	116	34.7	39.9	62.2	32.0	165
BV	420	6.27	7.8	139	105	34.9	39.9	62.3	32.0	170
BW	400	5.34	5.1	154	85	34.9	40.3	62.1	29.0	175
BX	450	5.78	3.6	155	84	35.0	40.3	62.1	30.0	180
BY	450	6.22	8.7	155	83	34.9	40.3	62.1	30.0	180
CA	375	6.37	10.3	145	59	34.3	39.9	61.9	32.0	170
CB	500	6.97	14.7	144	34	33.8	39.7	61.5	36.0	185

CODE	Ht	pH	B	EW	NS	FMIN	JANM	JULM	R	H
CC	725	6.64	8.5	142	23	34.0	39.7	61.3	38.0	185
CD	900	5.33	3.7	140	14	34.3	39.8	61.3	39.0	190
CE	950	5.03	1.1	140	13	34.3	39.8	61.3	39.0	190
CF	40	5.87	7.7	103	8	34.2	39.5	61.1	25.0	175
CG	200	7.22	9.6	84	11	34.5	39.2	60.7	24.0	195
CH	90	6.23	14.6	65	19	34.8	39.1	60.0	28.0	205
CI	50	5.99	7.2	75	24	34.3	39.0	60.8	29.0	195
CJ	40	5.33	3.1	51	47	33.8	39.0	61.0	25.0	185
CK	50	6.22	5.3	53	48	33.9	39.0	61.0	25.0	185
CL	215	5.27	3.7	40	50	33.7	39.2	61.4	28.0	180
CM	110	6.47	15.7	42	65	32.8	39.3	61.9	24.0	180
CN	110	6.19	11.7	42	67	32.8	39.3	61.9	24.0	180
CO	250	6.07	14.1	93	53	33.5	39.2	61.2	25.0	170
CP	250	6.07	13.9	93	53	33.5	39.2	61.2	25.0	170
CQ	20	7.34	34.8	87	51	33.4	39.1	61.1	24.0	175
CR	30	5.89	11.4	88	52	33.2	39.2	61.1	24.0	175
CS	35	6.43	12.4	87	55	33.5	39.2	61.1	26.0	175
CT	250	6.07	12.1	93	61	33.2	39.3	61.3	27.0	165
CU	250	5.74	13.4	94	61	33.3	39.3	61.3	27.0	165
CV	500	6.43	14.4	109	61	33.9	39.7	61.7	27.0	160
CW	260	7.22	20.6	123	35	34.2	39.8	61.7	28.0	165
CX	325	5.87	10.5	124	55	34.2	39.8	61.7	28.0	165
DA	550	4.33	0.8	208	22	36.2	41.2	50.3	39.0	250
DB	600	5.87	8.7	210	14	36.2	41.6	60.1	38.0	255
DC	650	5.07	2.6	218	21	36.8	41.8	60.2	44.0	290
DD	950	6.49	5.4	284	21	37.0	41.8	60.2	44.0	295
DE	200	6.49	4.7	233	18	37.5	42.4	60.0	47.0	320
DF	300	5.24	4.6	233	23	37.5	42.2	60.1	62.0	330
DG	100	4.33	1.2	232	21	37.4	42.4	60.0	62.0	330
DH	230	6.22	12.5	253	38	37.6	42.8	59.4	55.0	370
DI	150	4.83	1.7	253	36	37.7	42.8	59.4	54.0	360
DJ	250	4.77	2.9	258	38	38.2	42.8	59.4	49.0	370
DK	150	7.04	9.7	256	38	37.8	42.9	59.3	53.0	360
DL	50	7.74	18.4	247	37	37.5	42.6	59.7	47.0	340
DM	200	6.77	10.3	244	43	37.1	42.3	60.0	37.0	330
DN	50	5.00	5.6	240	51	37.0	42.2	59.9	47.0	325
DO	350	4.87	2.1	194	52	34.9	40.7	60.8	37.0	195
DP	675	5.67	5.1	194	61	34.9	40.8	60.9	39.0	195
DQ	500	4.98	4.0	177	60	34.6	40.2	61.0	29.0	185
DR	500	5.47	1.7	178	57	34.6	40.2	61.0	29.0	185
DS	300	7.20	11.7	174	48	34.7	40.1	61.0	32.0	190
DT	360	5.97	3.1	177	45	34.8	39.9	61.1	32.0	185
DU	340	6.07	7.1	161	55	34.3	39.8	61.4	34.0	175
DV	400	7.21	17.4	198	23	35.8	41.1	60.2	39.0	200
DW	250	6.98	7.4	182	22	35.5	40.8	60.5	36.0	190
DX	220	7.37	14.7	182	22	35.5	40.8	60.5	37.0	190
DY	165	5.37	4.4	172	13	35.3	40.2	61.1	39.0	195
DZ	530	6.98	17.9	165	14	35.1	39.9	61.2	40.0	190
AAA	50	5.22	6.3	253	97	37.2	42.3	60.2	52.0	335
AAB	550	4.71	2.9	237	86	36.5	41.8	60.2	53.0	310
AAC	1050	3.97	0.1	229	73	36.3	41.8	60.2	62.0	305
AAD	630	6.33	10.4	191	114	36.2	41.2	62.1	51.0	195
AAE	305	6.41	10.9	182	96	34.9	40.8	61.9	34.0	190
BBA	130	8.27	37.4	81	77	33.7	39.5	61.7	36.0	130
BBB	215	7.73	14.7	85	97	33.9	39.7	62.0	24.0	125
BBC	410	6.97	12.7	83	110	34.2	39.9	62.5	30.0	125
CCA	255	6.47	11.0	115	42	33.8	39.5	61.6	27.0	165

CODE	Ht	pH	B	EW	NS	FMIN	JANM	JULM	R	H
CCB	60	5.88	13.7	100	20	34.0	39.4	61.1	28.0	175
CCC	170	6.37	13.3	91	39	33.7	39.2	61.1	26.0	160
CCD	125	7.34	22.9	19	46	34.9	39.5	61.4	27.0	190
CCE	125	5.87	6.9	10	43	35.4	39.8	61.0	30.0	200
CCF	75	6.34	11.7	12	54	34.8	39.7	61.7	29.0	195
DDA	375	5.21	3.0	204	51	35.0	40.8	60.8	42.0	205
A1	250	6.52	13.4	145	85	34.8	40.2	62.2	28.0	160
A2	250	5.81	14.7	145	85	34.8	40.2	62.2	28.0	160
W1	300	6.94	17.6	171	77	34.7	40.5	61.8	30.0	185
W2	210	6.53	7.9	171	77	34.7	40.5	61.8	30.0	185
W3	160	6.74	15.9	171	77	34.7	40.5	61.8	30.0	185
W4	200	5.72	5.7	171	77	34.7	40.5	61.8	30.0	185
W5	240	6.21	5.3	171	77	34.7	40.5	61.8	30.0	185
W6	290	6.43	11.4	171	77	34.7	40.5	61.8	30.0	185
W7	380	7.21	16.7	171	77	34.7	40.5	61.8	30.0	185
W8	170	5.21	5.3	171	77	34.7	40.5	61.8	30.0	185
W9	220	5.81	2.8	171	77	34.7	40.5	61.8	30.0	185
W10	200	6.31	10.2	171	77	34.7	40.5	61.8	30.0	185
W11	200	5.98	7.7	171	77	34.7	40.5	61.8	30.0	185
A	150	5.46	4.4	171	77	34.7	40.5	61.8	30.0	185
B	210	6.41	10.1	171	77	34.7	40.5	61.8	30.0	185
C	200	5.93	7.4	171	77	34.7	40.5	61.8	30.0	185
D	310	6.47	18.4	145	85	34.8	40.2	62.2	28.0	160
F	450	5.21	3.7	150	65	34.5	40.0	61.9	33.0	170
G	290	7.21	13.7	171	77	34.7	40.5	61.8	30.0	185
H	200	6.31	6.2	171	77	34.7	40.5	61.8	30.0	185

SPECIES PRESENT (SEE TABLE 6.2 FOR SPECIES CODE NUMBERS)

AA	7 11 15 17 19 20 21 23 24 25 26 27 28 29 31 32 33 34 35 36 37
AB	3 7 11 15 16 17 19 23 24 25 26 27 29 30 32 33 34 35 37
AC	4 8 13 17 20 23 25 26 27 28 29 31 32 34 35 36 37
AD	3 19 21 23 25 26 27 29 30 31 32 33 35 36 37
AE	1 6 13 19 21 23 25 26 27 28 29 30 31 32 33 34 35 36 37
AF	4 19 24 26 27 28 29 30 32 33 35 36 37
AG	11 15 16 18 19 21 23 24 25 26 27 28 29 30 31 32 33 35 37
AH	7 9 11 19 21 25 26 27 28 29 30 31 32 33 35 37
AI	1 2 3 5 6 8 9 11 19 21 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
AJ	1 3 4 5 7 8 9 10 13 19 21 23 24 25 27 29 31 32 33 34 35 37
AK	2 3 7 9 11 14 20 21 25 26 27 29 30 33 34 35 36 37
AL	3 4 6 9 14 16 18 20 26 27 28 29 30 32 33 35 36 37
AM	1 2 3 4 5 6 8 9 10 13 61 20 23 24 25 28 29 32 34 35 36 37
AN	1 3 4 6 8 9 10 13 16 19 20 23 25 27 29 30 33 34 35 36
AO	1 3 4 5 6 8 10 13 16 19 21 23 24 28 29 30 32 33 34 35 37
AP	1 3 4 5 6 8 9 13 16 17 19 20 21 23 24 25 27 28 29 32 33 35 36 37
AQ	1 3 4 5 6 8 9 10 11 12 13 17 23 24 26 34 36
AR	1 2 5 6 7 9 10 11 12 13 19 21 23 24 34 35
AS	2 4 5 6 7 8 10 11 12 13 17 19 21 23 24 32 34 35 36
AT	2 4 5 6 8 10 11 12 13 21 23 24 26 32 34 35
AU	4 6 7 9 15 16 17 20 23 24 25 26 27 28 29 31 34 35 36 37
AV	1 2 3 4 5 6 8 10 13 16 17 19 23 24 25 28 31
AW	1 2 3 4 5 6 7 8 9 10 11 12 13 15 16 19 20 23 24 25 26 28 32 34 36
AX	1 2 3 5 6 8 10 12 17 20 24 25 26 29 32 36
AY	2 4 5 6 7 11 13 15 17 19 20 23 24 25 26 28 30 32 34 36 37
BA	9 15 16 18 19 20 21 25 26 27 29 31 32 34 35 36 37
BB	5 7 8 13 15 17 18 20 21 23 24 25 27 28 30 32 33 34 36
BC	8 18 20 23 24 25 26 28 29 30 31 32 33 35 36 37
BD	6 18 20 23 24 25 26 27 31 32 33 34 35 36
BE	11 15 20 23 25 26 27 29 30 32 33 34 35 36 37
BF	8 15 16 19 21 23 25 26 27 28 29 30 31 32 33 34 36
BG	13 16 21 22 23 25 26 27 30 32 33 36
BH	1 3 7 8 11 13 15 16 19 21 22 25 27 28 30 31 32 34 35 36

BI	1 3 7 8 11 13 15 16 17 20 25 26 28 30 32 33 34 36
BJ	3 5 7 8 9 13 16 17 21 22 23 24 25 26 27 28 31 34 35 36
BK	3 8 13 16 17 21 23 23 24 25 26 28 30 31 32 33 34 36
BL	8 15 16 22 25 26 28 29 30 31 33 37
BM	8 16 17 21 23 24 25 26 27 28 30 32 33 34 35 37
BN	3 13 21 22 25 26 28 29 30 32 33 34 35 36 37
BO	3 13 21 22 25 26 28 29 30 32 33 34 35 36 37
BP	21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
BQ	11 13 16 19 20 23 25 26 27 28 29 30 31 32 33 34 35 36 37
BR	2 14 20 22 25 26 28 30 31 32 33 35 36 37
BS	2 14 20 22 25 26 28 30 31 32 33 35 36 37
BT	2 14 20 22 25 26 28 30 31 32 33 35 36 37
BU	2 10 13 17 21 23 26 27 28 29 30 31 32 33 35 36 37
BV	15 20 22 26 27 28 29 30 32 34 35 36
BW	5 7 8 9 13 15 16 17 18 19 20 21 24 25 26 28 31 33 36
BX	1 7 13 17 19 20 26 28 29 32 34 37
BY	2 5 11 15 17 19 21 24 25 27 28 31 33 35 36 37
CA	1 2 4 9 10 11 12 13 15 61 19 20 22 25 26 27 28 29 31 32 33 35 36 37
CB	4 5 6 9 10 11 15 16 17 18 21 23 25 26 27 29 31 32 33 34 35 37
CC	2 3 7 8 11 15 18 19 20 21 23 24 25 26 27 28 29 32 33 36
CD	2 4 6 8 10 12 13 15 61 17 19 21 23 24 25 27 31 35
CE	2 4 6 8 10 12 13 15 16 17 19 21 23 24 25 27 31 35
CF	2 7 8 11 12 13 15 16 17 18 21 22 23 25 26 28 30 32 33 34 36 37
CG	1 2 4 5 6 7 8 10 11 13 16 17 20 21 22 26 28 29 31 35
CH	6 13 15 16 19 21 25 27 28 29 31 34 35 36
CI	1 2 5 6 7 8 9 13 15 19 20 26 33 34 36
CJ	1 2 3 4 7 8 9 10 12 13 16 18 19 21 23 27 29 31 35
CK	2 3 4 5 6 7 8 11 13 14 17 20 23 25 28 29 33 37
CL	1 3 4 5 7 9 12 14 18 21 28 30 32 35
CM	15 16 18 20 25 26 27 30 32 33 35 36
CN	16 20 23 25 26 28 30 32 33 35
CO	15 17 19 23 25 26 27 28 29 30 31 32 33 36 37
CP	15 17 19 23 25 26 27 28 29 30 31 32 33 36 37
CQ	18 19 23 24 25 26 27 28 29 31 32 34 35 36
CR	1 13 15 16 18 20 25 26 27 28 29 30 31 32 33 34 37
CS	1 15 16 25 26 27 28 30 32 36

CT	8 15 16 18 19 25 26 27 28 29 30 31 33 36
CU	8 15 18 21 25 26 27 28 29 30 31 32 34 37
CV	18 21 22 24 25 26 27 28 29 30 32 33 34 35 36 37
CW	2 3 7 8 10 15 18 23 24 25 26 27 28 29 31 32 33 36 37
CX	3 4 8 10 15 18 23 24 25 26 28 29 31 32 33 34 36 37
DA	1 2 4 5 6 8 9 11 13 16 17 19 21 22 26 28 30 32 35 37
DB	2 3 5 6 7 8 10 15 16 17 21 22 23 25 26 28 29 32 34 36
DC	2 4 5 6 9 11 13 15 17 20 23 25 28 36
DD	1 2 3 4 5 6 9 11 12 13 15 17 19 20 23 25 28 36
DE	1 2 3 4 5 6 7 8 9 10 11 12 13 19 23 24 31 36 37
DF	1 2 3 4 5 6 7 8 9 10 13 15 16 25 26 32
DG	1 2 3 4 5 6 7 8 9 10 13 15 16 17 26 31
DH	1 2 3 5 6 7 8 9 10 11 12 13 16 19 21 24 25 29 33 36
DI	1 2 3 4 5 6 7 8 9 10 11 12 13 15 16 17 20 23 24 25 26 27 29 30
DJ	1 2 3 4 5 6 7 8 9 10 11 12 13 15 17 25 27 29 33
DK	1 2 5 7 10 11 16 25 27 28 32 35
DL	1 2 3 5 6 7 8 9 10 11 12 13 15 23 24 25 27 32 35 36
DM	1 2 3 4 5 6 8 9 10 11 12 13 16 20 24 27 31 33 34
DN	1 2 3 4 5 6 7 8 9 10 12 13 16 21 23 25 26 27 28 29 30 31 36
DO	1 2 3 4 5 7 8 11 13 15 16 17 19 21 22 23 25 28 31 36
DP	1 3 4 5 6 7 8 9 10 11 13 15 17 19 21 23 26 27 30 33 36 37
DQ	1 2 3 4 5 7 8 10 11 13 15 16 17 19 20 22 24 25 26 28 29 32 34 36 37
DR	1 2 3 4 5 6 7 8 10 11 12 13 16 17 19 21 23 24 26 30 31 35 37
DS	1 5 8 9 11 13 15 16 18 20 21 23 26 27 31 32 34 36
DT	1 2 3 5 7 8 9 10 11 15 16 17 19 20 21 25 26 29 33 35
DU	2 4 6 7 9 10 12 15 16 17 19 21 23 24 25 26 28 32 34 36
DV	1 3 5 8 16 17 21 22 23 25 26 27 28 30 31 32 34 35 36
DW	1 7 12 15 19 23 25 26 28 29 30 32 34 35 36
DX	3 8 12 16 19 23 25 28 29 30 32 33 34 35 36
DY	2 3 4 5 7 8 10 11 13 15 16 17 19 20 22 23 24 27 29 32 36
DZ	3 7 8 13 16 19 21 24 25 26 27 32 34 35 36
AAA	1 4 5 7 8 9 10 12 13 16 23 24 25 34 35 36
AAB	1 2 3 6 7 8 9 10 13 15 17 21 23 25 27 28 32 33
AAC	1 2 3 4 6 7 8 9 10 15 16 17 23 25 27
AAD	16 20 23 24 25 27 28 30 32 33 34 35 37
AAE	10 16 18 20 21 23 25 26 27 28 30 31 33 34 35 36 37

BBA	18 21 23 24 25 26 27 28 30 32 33 34 35 36 37
BBB	10 13 18 21 23 24 26 27 29 31 32 33 35 36 37
BBC	15 17 21 22 23 25 26 27 28 29 30 31 32 33 34 35 36 37
CCA	15 19 20 22 24 25 26 27 28 29 31 32 35 36
CCB	1 2 5 7 8 9 10 11 12 13 15 16 17 18 20 21 22 23 25 28 33 35 36
CCC	8 18 19 22 23 24 26 28 29 31 32 33 34 35 36 37
CCD	2 3 11 16 18 21 22 23 24 26 28 30 31 34 36
CCE	2 8 10 11 13 17 19 20 22 23 25 27 28 29 32 33 34 37
CCF	1 4 8 13 15 16 17 19 20 21 22 23 24 25 28 29 32 34 36
DDA	1 2 4 5 6 8 9 10 11 13 15 17 18 20 23 25 28 30 33 35 37
A1	2 5 10 15 17 18 21 23 24 25 26 28 29 30 31 32 33 34 35 37
A2	7 15 16 18 21 25 27 28 29 30 31 32 33 35 36
W1	1 5 10 13 17 18 23 29 31 33 34 36 37
W2	1 2 4 7 10 14 16 18 20 21 22 24 27 30 32 35 37
W3	3 4 5 6 9 10 11 12 14 15 16 19 21 25 28 29 33
W4	1 5 8 10 11 14 16 18 20 29 30 31 32 34 35
W5	3 4 5 8 9 10 11 13 14 16 18 20 21 22 26 29 32
W6	2 4 6 9 10 11 12 14 17 19 22 23 25 27 29 31 33 37
W7	1 3 5 7 8 9 12 13 17 20 24 26 29 30 33 35
W8	2 3 6 8 11 15 18 22 25 26 27 28 32 34 36 37
W9	1 2 3 4 6 7 8 9 10 11 13 15 16 19 21 22 26 28 31 34
W10	1 4 5 6 8 12 13 15 18 20 23 30 32 35 37
W11	1 4 5 6 8 12 13 15 18 20 23 26 28 30 32 35
A	1 3 4 5 7 10 12 14 16 17 19 24 26 27 28 29 30 32 36
B	2 4 5 7 9 14 18 22 26 28 29 30 34
C	10 15 17 18 21 23 24 25 26 28 29 30 31 32 33 34 35 37
D	2 5 6 7 8 9 11 12 16 18 22 25 26 27 28 30 31 32 34 35 37
F	1 2 3 6 7 8 11 13 15 16 17 20 21 22 24 25 26 27 28 31 32 33 34 35 36
G	2 4 6 9 10 11 12 14 17 19 22 23 25 27 29 31 33 37
H	1 4 5 6 8 12 13 15 18 20 23 30 32 35

APPENDIX 5

ACTUALPLOT is an ALGOL procedure for plotting scatter diagrams, and, with slight modification, graphs of data on a computer line printer. The procedure can be used directly in the form presented here on 1900 machines, but it is necessary to note the following:-

1. ACTUALPLOT calls another procedure TEXT. Each point as it is plotted requires two pieces of information - a) the type of point to be plotted, eg. if a classification array is being plotted, different classes may be required to be plotted with different symbols; b) any duplication of points, i.e. do any points fall at the same point? TEXT is simply a procedure printing different symbols for different classes, and different numbers of overlapping points. A general call of TEXT would be TEXT (A,B); where B = classification of point to be plotted and A = number of overlapping points.
2. ACTUALPLOT is called from within other procedures, namely ARRAY, MATCOL, and MATROW. ACTUALPLOT destroys the data it has plotted and consequently it is convenient to copy data to be plotted into holding arrays which are used for the plotting procedure so that the original data is preserved. The copying takes place in procedures ARRAY, MATCOL and MATROW where the X and Y co-ordinates forming two arrays are placed into holding arrays (ARRAY) or where the X and Y co-ordinates form two columns of a matrix and are placed into holding arrays (MATCOL) or where the X and Y co-ordinates form two rows of a matrix and are placed into holding arrays (MATROW). At the end of each of these copying procedures, ACTUALPLOT may be called as ACTUALPLOT (X,Y,N), where N = number of points to be plotted, X = an array holding the X values, and Y similarly holding the Y values.
3. Modification of the procedure allows standardisation to fit pages of

any size (see 'comment' statements for where this occurs in the procedure). The procedure in the form presented here includes a scaling quantity RAT which draws the second axis in ratio to the first, for PCA scatters. For general graph plotting use, this is best deleted.

```
'PROCEDURE'ACTUAL PLOT(X,Y,N);
'VALUE'N; 'INTEGER'N; 'ARRAY'X,Y;
'BEGIN'
'INTEGER''ARRAY'XIN,YIN[1:N];
'INTEGER''ARRAY'AY[1:N];
'INTEGER'I,MAXYIN,Z,J,HOLD,T;
'INTEGER'COUNT,BETA,CO2,K,MINXIN,R,DELTA,HOLD2,PUSS;
'REAL'MAXX,MINX,MAXY,MINY;
'REAL' RAT;
MAXX:=MINX:=X[1];
MAXY:=MINY:=Y[1];
'FOR'I:=1'STEP'1'UNTIL'N'DO''BEGIN'
'IF'X[I]>MAXX'THEN'MAXX:=X[I];
'IF'X[I]<MINX'THEN'MINX:=X[I];
'IF'Y[I]>MAXY'THEN'MAXY:=Y[I];
'IF'Y[I]<MINY'THEN'MINY:=Y[I];
'END';
RAT:=(MAXY-MINY)/(MAXX-MINX);
'COMMENT'CALCULATES MAX. AND MIN. ELEMENTS OF THE ARRAYS ;
'FOR'I:=1'STEP'1'UNTIL'N'DO''BEGIN'
X[I]:=(((X[I]-MINX)*((76-11)/(MAXX-MINX)))+11);
Y[I]:=(((Y[I]-MINY)*((40-01)/(MAXY-MINY)))+01)*RAT;
XIN[I]:=ENTIER(X[I]+0.9);
YIN[I]:=ENTIER(Y[I]+0.9);
'END';
PAPERTHROW;
NEWLINE(1);
'FOR'I:=1'STEP'1'UNTIL'N'DO''BEGIN'
PRINT(I,3,0); PRINT(XIN[I],3,0); PRINT(YIN[I],3,0); NEWLINE(1);
'END';
'COMMENT'STANDARDISATION OF ARRAYS TO FIT STANDARD COMPUTER PAGE;
PAPERTHROW;
NEWLINE(1);
'FOR'I:=1'STEP'1'UNTIL'2'DO''BEGIN'
SPACE(8); WRITETEXT('('I')'); NEWLINE(1);
'END';
'COMMENT' START AXIS LINE;
'COMMENT'NEW PLOTTING;
'FOR'I:=1'STEP'1'UNTIL'N'DO''BEGIN'
COUNT:=0;
'FOR'J:=1'STEP'1'UNTIL'N'DO''BEGIN'
'IF'XIN[J]=0'THEN'COUNT:=COUNT+1;
'END';
'IF'COUNT=N'THEN''GOTO'OUT2;
'COMMENT'ANY MORE VALUES LEFT;
MAXYIN:=YIN[1]; Z:=1;
'FOR'J:=2'STEP'1'UNTIL'N'DO''BEGIN'
'IF'YIN[J]>MAXYIN'THEN''BEGIN'
MAXYIN:=YIN[J]; Z:=J;
'END';
'END';
'COMMENT'FIND MAX Y ;
BETA:=0;
'FOR'J:=1'STEP'1'UNTIL'N'DO''BEGIN'
AY[J]:=0;
'IF'YIN[J]=MAXYIN'THEN''BEGIN'
BETA:=BETA+1;
AY[J]:=1;
'END';
```

```
'END';
'COMMENT' HOW MANY MAX Y ;
'IF'BETA=1'THEN''BEGIN'
'IF'I=1'THEN''BEGIN'
PRINT(YIN[Z],3,0); SPACE(2); WRITETEXT('('I')'); SPACE(5);
SPACE(XIN[Z]); TEXT(1,Z); PRINT(Z,3,0); NEWLINE(1);
HOLD:=MAXYIN; YIN[Z]:=XIN[Z]:=MAXYIN:=0; AY[Z]:=0;
'END';
'IF'I>1'THEN''BEGIN'
'FOR'J:=1'STEP'1'UNTIL'HOLD-MAXYIN-1'DO''BEGIN'
SPACE(8); WRITETEXT('('I')'); NEWLINE(1);
'END';
PRINT(YIN[Z],3,0); SPACE(2); WRITETEXT('('I')'); SPACE(5);
SPACE(XIN[Z]); TEXT(1,Z); PRINT(Z,3,0); NEWLINE(1);
HOLD:=MAXYIN; YIN[Z]:=XIN[Z]:=MAXYIN:=0; AY[Z]:=0;
'END';
'END';
'COMMENT' PLOTTING IF ONLY 1 Y OF MAX VALUE;
'IF'BETA>1'THEN''BEGIN'
'FOR'J:=1'STEP'1'UNTIL'BETA'DO''BEGIN'
CO2:=0;
'FOR'K:=1'STEP'1'UNTIL'N'DO''BEGIN'
'IF'AY[K]>0'THEN''BEGIN'
'IF'XIN[K]=0'THEN'CO2:=CO2+1;
'END';
'END';
'IF'CO2=BETA'THEN''BEGIN'
NEWLINE(1);
'GOTO'NEXTI;
'END';
'COMMENT' ANY MORE X VALUES;
MINXIN:=10000; R:=0;
'FOR'K:=1'STEP'1'UNTIL'N'DO''BEGIN'
'IF'AY[K]=1'THEN''BEGIN'
'IF'XIN[K]<MINXIN'THEN''BEGIN'
MINXIN:=XIN[K];
R:=K;
'END';
'END';
'END';
'COMMENT' FIND MIN X ;
DELTA:=0;
'FOR'K:=1'STEP'1'UNTIL'N'DO''BEGIN'
'IF'AY[K]=1'THEN''BEGIN'
'IF'XIN[K]=MINXIN'THEN''BEGIN'
DELTA:=DELTA+1;
AY[K]:=2;
'END';
'END';
'END';
'COMMENT' HOW MANY MIN X;
'IF'I=1'THEN''BEGIN'
'IF'J=1'THEN''BEGIN'
PRINT(YIN[R],3,0); SPACE(2); WRITETEXT('('I')');
SPACE(5); SPACE(XIN[R]); TEXT(DELTA,R);
HOLD2:=MINXIN; MINXIN:=0;
HOLD:=MAXYIN; MAXYIN:=0;
'FOR'K:=1'STEP'1'UNTIL'N'DO''BEGIN'
```

```
'IF'AY[K]=2'THEN''BEGIN'
XIN[K]:=YIN[K]:=0;
AY[K]:=3;
'END';
'END';
'END';
'IF'J>1'THEN''BEGIN'
SPACE(MINXIN-HOLD2-1); TEXT(DELTA,R);
HOLD2:=MINXIN;
MINXIN:=0;
'FOR'K:=1'STEP'1'UNTIL'N'DO''BEGIN'
'IF'AY[K]=2'THEN''BEGIN'
XIN[K]:=YIN[K]:=0;
AY[K]:=3;
'END';
'END';
'END';
'END';
'IF'I>1'THEN''BEGIN'
'IF'J=1'THEN''BEGIN'
'FOR'K:=1'STEP'1'UNTIL'HOLD-MAXYIN-1'DO''BEGIN'
SPACE(8); WRITETEXT('('I')'); NEWLINE(1);
'END';
HOLD:=MAXYIN; MAXYIN:=0;
PRINT(YIN[R],3,0); SPACE(2); WRITETEXT('('I')');
SPACE(5); SPACE(XIN[R]); TEXT(DELTA,R);
HOLD2:=MINXIN; MINXIN:=0;
'FOR'K:=1'STEP'1'UNTIL'N'DO''BEGIN'
'IF'AY[K]=2'THEN''BEGIN'
XIN[K]:=YIN[K]:=0;
AY[K]:=3;
'END';
'END';
'END';
'IF'J>1'THEN''BEGIN'
SPACE(MINXIN-HOLD2-1); TEXT(DELTA,R); HOLD2:=MINXIN;
MINXIN:=0;
'FOR'K:=1'STEP'1'UNTIL'N'DO''BEGIN'
'IF'AY[K]=2'THEN''BEGIN'
XIN[K]:=YIN[K]:=0;
AY[K]:=3;
'END';
'END';
'END';
'END';
'IF'J=BETA'THEN'NEWLINE(1);
'END';
'END';
NEXTI: PUSS:=1;
'END';
OUT2: PUSS:=1;
'COMMENT' PLOTTING OF ACTUAL POINTS AND AXIS ;
'FOR'I:=1'STEP'1'UNTIL'2'DO''BEGIN'
SPACE(8); WRITETEXT('('I')'); NEWLINE(1);
'END';
SPACE(8); WRITETEXT('('------I--------I--------I--------I--------I
---------I--------I--------I--------I--------I--------I---')');
```

```
NEWLINE(1);
SPACE(8); WRITETEXT('('%%%%%0%%%%%%%%%10%%%%%%%%20%%%%%%%%30%%%%%%%%4
0%%%%%%%%50%%%%%%%%60%%%%%%%%70%%%%%%%%80%%%%%%%%90%%%%%%%%100:):);
NEWLINE(1);
PAPERTHROW;
'COMMENT'HORIZONTAL AXIS AND GRADUATIONS;
'END'ACTUAL PLOT;
```

APPENDIX 6

Analysis of Variance of the results of the artificial hybridisation reported in Chapter 8

KEY

One way analyses of variance -

TSS	Total sums of squares
TDF	Total degrees of freedom
BSS	Between groups sums of squares
BDF	Between groups degrees of freedom
WSS	Within groups sums of squares
WDF	Within groups degrees of freedom
BVAR	Between groups variance
WVAR	Within groups variance
V.RAT.	Variance ratio

	TSS	TDF	BSS	BDF	WSS	WDF	BVAR	WVAR	V.RAT.	PROBABILITY	
1969											
Q. robur selfed x Q. petraea selfed	12036.7	40	5981.3	1	6055.4	39	5981.3	155.3	38.5	0.001	***
Q. robur intra x Q. petraea intra	5455.1	33	101.9	1	5353.3	32	101.9	167.3	1.64	0.50-0.75	NS
Q. robur female x Q. petraea female	17.9	101	0.041	1	17.7	100	0.041	0.177	4.23	0.25-0.50	NS
Q. robur selfed x Q. robur intra	15415.8	36	11777.2	1	3638.6	35	11777.2	103.9	113.3	0.001	***
Q. petraea selfed x Q. petraea intra	8387.0	37	616.2	1	7771.0	36	616.2	215.9	2.85	0.05-0.10	NS
1970											
Q. robur selfed x Q. petraea selfed	50132.8	44	2988.4	1	47144.4	43	2988.4	1096.4	2.72	0.10-0.25	NS
Q. robur intra x Q. petraea intra	7391.5	46	364.5	1	7027.0	45	364.5	156.2	2.33	0.10-0.25	NS
Q. robur female x Q. petraea female	4.20	94	0.948	1	3.26	93	0.948	0.036	26.3	0.001	***
Q. robur selfed x Q. robur intra	11749.2	44	4946.78	1	6802.4	43	4946.78	158.2	31.3	0.001	***
Q. petraea selfed x Q. petraea intra	47392.2	46	23.10	1	47369.1	45	23.10	1052.7	45.6	0.10-0.25	NS
1971											
Q. robur selfed x Q. petraea selfed	7664.6	41	2156.9	1	5507.7	40	2156.9	137.7	15.7	0.001	***
Q. robur intra x Q. petraea intra	13108.5	48	213.9	1	12894.7	47	213.9	274.4	1.28	0.50-0.75	NS
Q. robur female x Q. petraea female	83.1	105	1.52	1	81.6	104	1.52	0.785	1.94	0.10-0.25	NS
Q. robur selfed x Q. robur intra	26214.5	46	15675.7	1	10538.8	45	15675.7	234.2	66.9	0.001	***
Q. petraea selfed x Q. petraea intra	11168.7	43	3305.2	1	7863.5	42	3305.2	187.2	17.7	0.001	***
Differences between years											
Q. robur selfed	12041.8	66	3269.2	2	10407.3	64	1634.6	165.2	9.89	0.001	***
Q. petraea selfed	54689.2	60	4716.6	2	52330.0	58	2358.3	918.1	2.57	0.05-0.10	NS
Q. robur intra specific crosses	23944.8	61	11737.7	2	18075.9	59	5868.9	311.7	18.8	0.001	***
Q. petraea intra specific crosses	29003.3	67	15934.6	2	21036.0	65	7967.3	328.7	24.2	0.001	***
Q. petraea female x Q. robur male	59.6	148	10.17	2	54.49	146	5.08	0.376	13.5	0.001	***
Q. robur female x Q. petraea male	64.6	153	11.42	2	58.87	151	5.71	0.561	10.2	0.001	***

APPENDIX 7

Appendix 7 describes the calculation of resistances to water vapour loss from model and actual leaves using the results of the 'cut-shoot' method detailed in Chapter 9.

THE CALCULATION OF LEAF RESISTANCES TO WATER VAPOUR LOSS FROM THE 'CUT-SHOOT' METHOD DESCRIBED IN CHAPTER 9

Laws of gaseous exchange are analogous to Ohm's law for electrical flow, i.e. $\text{Flux} = \frac{\text{Potential Difference}}{\text{Resistance}}$

Since leaves lose water we can substitute the following in the above equation:

Flux = evaporation or transpiration rate in $mg/cm^2/sec$

Potential Difference = vapour pressure difference between the surface and the air in density units, mg/cm^3, i.e. the water vapour gradient over which the water molecules are moving

Resistance = leaf resistance to water vapour diffusion in sec/cm

The leaf resistance may be partitioned into the resistance provided by the cuticle, by the stomata and by the boundary layer, i.e. that layer of air above a surface through which molecular exchange is limited by static diffusion and turbulent mixing. These different components of leaf resistance are diagrammed in Diagram A7.1

Water loss from a model filter paper leaf represents the simplest case and will be considered first. The model leaf has only one resistance to water vapour loss, that provided by the Boundary Layer (R_B).

Using Ohm's law, R_B can be calculated from: $R_B = \frac{P - P_A}{E_M}$

where P = saturation vapour pressure (i.e. the model is assumed to be saturated with water) at the model temperature (derived from tables)

P_A = saturation vapour pressure at air temperature, corrected for relative humidity

E_M = evaporation rate from the model

This represents total boundary layer resistance for the model which can be partitioned into a lower boundary layer resistance R_{BL} and an upper boundary layer resistance R_{BU} remembering that these are resistances in parallel:

DIAGRAM A7.1

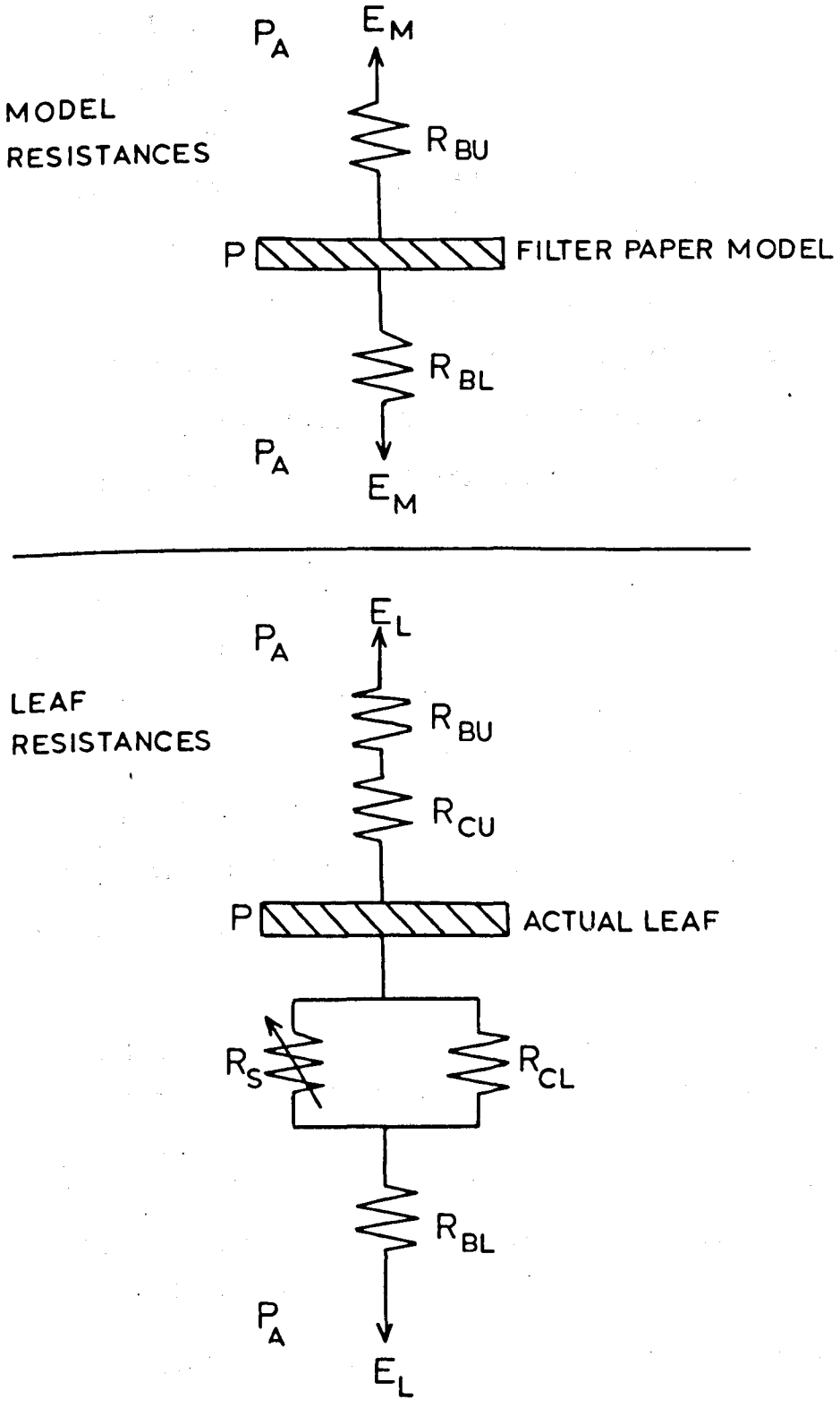

DIAGRAM A7.1 Resistances to water vapour loss from model and actual leaves. See Text for explanation.

$$\frac{1}{R_B} = \frac{1}{R_{BU}} + \frac{1}{R_{BL}}$$

Since both sides of the model are the same it is assumed that $R_{BU} = R_{BL}$ and therefore: $R_B = \frac{R_{BU}}{2} = \frac{R_{BL}}{2}$

Water loss from a real leaf can be considered in two stages, loss when the stomata are open, and loss when the stomata are closed. In this latter case, water loss from the leaf occurs only through the cuticle and consequently there are only two resistances in the 'circuit', the cuticular resistance and the boundary layer resistance beyond it. Figure 9.1 illustrates the rate of loss of water from a leaf, and three distinct zones can be identified:

A - B: water loss with stomata open

B - C: phase of stomatal closure

C - D: water loss with stomata closed, i.e. through cuticles alone

Consequently if the rate of loss of water from the leaf during CD is calculated from the graph, and if this is halved, i.e. it is assumed that during phase CD, equal quantities of water are lost from both the upper and lower surfaces then: $E_U = \frac{P - P_A}{R_U}$

where E_U = rate of water loss from the upper surface only

P_A = saturation vapour pressure at air temperature (as before)

P = saturation vapour pressure at leaf temperature during phase CD. This assumes that the evaporating surfaces of the leaf cells are at saturation vapour density (the cell osmotic tension would need to be very high to invalidate this).

R_U = sum of the upper leaf resistances, the only unknown

Consequently R_U can be calculated and since the upper resistances are in parallel: $R_U = R_{CU} + R_{BU}$

where R_{CU} = upper cuticular resistance

R_{BU} = as before, i.e. the leaf is assumed to have the same boundary layer properties as the model

It is assumed, possibly incorrectly, that $R_{CU} = R_{CL}$, i.e. the upper and lower cuticular resistances are equal.

During Phase AB, loss is through both the upper and lower surfaces, and through the cuticles and stomata. The total rate of loss of water during Phase AB = E, and $E = E_U + E_L$ where E_L = rate of loss through the lower surface, the unknown, E is known from the graph and E_U has been calculated above also from the graph, and therefore E_L can be derived. This part of the calculation assumes that E_U during the Phase AB is equal to E_U during Phase CD.

Therefore $$E_L = \frac{P - P_A}{R_L}$$

where R_L = sum of the lower resistances

R_L has three components, R_{BL}, R_{CL} and R_S, the stomatal resistance, but the latter two are in parallel to the third. R_S must be calculated therefore from: $$\frac{1}{R_L - R_{BL}} = \frac{1}{R_S} + \frac{1}{R_{CL}}$$

APPENDIX 8

Appendix 8 lists the means, standard deviations and analysis of the leaf resistance data discussed in Chapter 9.

The first part records the means, standard deviations and least significant difference of means for the three experimental treatments on the seven types of leaves (adult sun and shade leaves; seedling leaves, greenhouse and four light intensities 100, 75, 50 and 25) for boundary layer resistance (R_B), cuticular resistance (R_C) and stomatal resistance (R_S). The first figure in each tabular cell records the mean, the second (underneath) the standard deviation.

The second part records the three-way analysis of variance of the above data, the main effects being the types of leaves ('pre-treatment'), experimental conditions and species. The table gives the variance ratios for each effect and interaction between them, together with the error variance and significance levels for appropriate combinations of degrees of freedom.

LEAF RESISTANCES TO WATER LOSS

GROWTH CONDITIONS

Experimental Conditions	Adult Sun	Adult Shade	Greenhouse	Seedling Leaves 100	75	50	10
R_B total (sec/cm):							
Rsun	0.69	1.15	0.82	0.87	0.89	0.78	1.10
	0.18	0.17	0.19	0.11	0.16	0.11	0.19
Rmedium	0.61	0.99	0.84	0.92	0.82	0.83	0.96
	0.13	0.23	0.23	0.24	0.17	0.17	0.21
Rshade	0.72	1.04	0.87	0.85	0.93	0.84	1.01
	0.21	0.18	0.14	0.17	0.11	0.17	0.13
Psun	0.84	1.19	0.95	0.88	0.92	0.82	0.99
	0.11	0.13	0.14	0.17	0.27	0.18	0.16
Pmedium	0.81	1.09	0.87	0.81	0.84	0.87	1.05
	0.17	0.23	0.17	0.22	0.13	0.17	0.19
Pshade	0.88	1.21	0.98	0.94	0.86	0.97	1.04
	0.19	0.19	0.23	0.12	0.12	0.16	0.27

Least significant difference at:

5%	0.111
1%	0.146
0.1%	0.187

R_C total (sec/cm):							
Rsun	438	311	324	326	287	243	160
	70	89	83	76	83	54	72
Rmedium	463	342	299	318	293	261	203
	85	74	78	84	66	83	84
Rshade	483	381	323	318	313	271	256
	66	95	81	91	72	97	69
Psun	336	283	246	243	248	221	186
	60	74	49	58	71	58	55
Pmedium	325	273	237	260	235	226	191
	71	81	73	55	68	67	69
Pshade	355	323	278	287	263	270	226
	56	57	63	69	74	63	60

Least significant difference at:

5%	45.201
1%	59.500
0.1%	75.873

Experimental Conditions	Adult Sun	Adult Shade	Greenhouse	Seedling Leaves 100	Seedling Leaves 75	Seedling Leaves 50	Seedling Leaves 10
R_S (sec/cm):							
Rsun	9.76	18.67	8.74	10.93	11.21	16.41	19.36
	3.41	2.87	2.71	3.62	2.93	2.81	2.86
Rmedium	10.21	17.43	9.32	9.61	12.43	13.61	17.42
	3.36	3.44	2.69	3.24	3.37	2.72	3.49
Rshade	10.43	15.21	10.69	11.43	10.31	13.46	17.16
	3.52	3.76	2.88	2.67	2.38	2.94	2.81
Psun	6.31	13.71	7.42	8.69	9.42	8.88	10.71
	3.71	3.67	3.11	2.69	2.93	3.31	3.32
Pmedium	5.61	12.82	9.41	8.62	8.71	9.82	12.07
	2.98	3.43	3.14	3.56	3.64	3.26	2.66
Pshade	8.31	15.72	7.17	7.68	9.38	6.42	10.95
	2.83	3.11	3.31	3.23	2.87	2.49	3.47

Least significant difference at:

5%	1.950
1%	2.566
0.1%	3.271

Variance ratios for the different effects and the error variance - Leaf Resistance Data

Effects	Pre-treatment	Experimental conditions	Species	P*EC	EC*Sp	P*Sp	P*EC*Sp	Error Variance
Degrees of Freedom	6	2	1	12	2	6	12	798
R_B	50.705	7.690	24.269	1.313	1.411	4.147	1.532	0.032
R_C	84.205	19.042	109.029	0.864	0.876	7.897	0.782	5318.452
R_S	97.579	1.259	277.937	2.338	2.310	12.322	3.869	9.888

Significance levels:

	1*798	2*798	6*798	12*798
5%	3.84	3.00	2.10	1.75
1%	6.63	4.61	2.80	2.18
0.1%	10.80	6.91	3.74	2.74

APPENDIX 9

Appendix 9 lists the means, standard deviations and analysis of the seedling growth analysis data discussed in Chapter 10.

The first part records the means, standard deviations and least significant difference of means for the growth analysis characters assessed for two harvests t_1 and t_2, for seedlings under different growth chamber light intensities (100, 75, 50, 25 and 10) for the two species. The first figure in each tabular cell records the mean, the second (underneath) the standard deviation. The later assessments of growth performance, eg. relative growth analysis are calculated over the growth period and in consequence are only represented by single results.

The second part records the three-way analysis of variance of the above data, the main effects being harvest times, species and light intensities. The table gives the variance ratios for each effect and interactions between them, together with the error variance and significance levels for appropriate combinations of degrees of freedom. Those parameters recorded over the growth period have been analysed by two-way analyses of variance and these are presented in the same format.

Growth Chamber Light Intensity	Q. robur 100	75	50	25	10	Q. petraea 100	75	50	25	10	
Harvest 1, t_1	574	563	600	581	521	372	354	327	374	336	Cotyledon wt. (mgs)
	74	67	83	54	69	54	47	48	61	53	LSD of means: 0.1% -
Harvest 2, t_2	341	362	444	356	247	272	310	315	270	294	84.4; 1% - 66.2;
	57	46	71	62	53	42	39	45	52	47	5% - 50.3
Harvest 1, t_1	197	183	174	185	181	143	136	128	125	139	Stem wt. (mgs)
	22	19	27	23	31	19	22	18	25	27	LSD of means: 0.1% -
Harvest 2, t_2	290	242	233	255	196	224	199	175	205	214	33.1; 1% - 29.9;
	27	25	33	28	34	28	27	27	22	27	5% - 22.7
Harvest 1, t_1	431	394	427	441	437	347	407	346	327	335	Root wt. (mgs)
	52	49	49	53	47	56	42	48	52	43	LSD of means: 0.1% -
Harvest 2, t_2	1068	939	884	605	389	790	965	759	465	339	118.8; 1% - 93.1;
	102	117	134	98	121	82	79	87	102	94	5% - 70.8
Harvest 1, t_1	351	299	328	354	331	341	325	301	347	306	Leaf wt. (mgs)
	48	57	42	46	43	46	52	39	47	51	LSD of means: 0.1% -
Harvest 2, t_2	501	539	485	379	306	502	472	428	416	329	83.6; 1% - 65.5;
	67	78	52	61	63	61	67	65	72	59	5% - 49.8
Harvest 1, t_1	1553	1439	1529	1561	1470	1203	1222	1102	1173	1116	Total weight (mgs)
	107	122	109	98	127	112	119	124	107	123	LSD of means: 0.1% -
Harvest 2, t_2	2200	2082	2046	1595	1138	1788	1946	1677	1356	1171	183.8; 1% - 144.1;
	136	172	105	121	132	127	134	146	121	135	5% - 109.5
Harvest 1, t_1	57.6	47.3	56.2	54.4	53.9	58.4	57.6	60.2	58.6	58.4	Total leaf area (cm^2)
	9.7	10.3	8.9	8.7	9.3	8.7	7.2	8.3	7.6	8.3	LSD of means: 0.1% -
Harvest 2, t_2	63.9	68.6	74.8	61.8	57.4	65.5	68.8	76.6	73.5	60.2	13.1; 1% - 10.2;
	8.8	9.4	8.6	10.3	9.5	9.4	7.3	6.2	10.3	9.4	5% -7.7

Growth Chamber Light Intensity	Q. robur 100	75	50	25	10	Q. petraea 100	75	50	25	10	
Harvest 1, t_1	5.91	4.96	5.83	5.54	5.53	5.46	5.72	5.06	5.41	5.31	Leaf no.
	1.21	1.12	0.96	1.10	0.76	0.89	0.93	1.32	0.98	1.11	LSD of means: 0.1% -
Harvest 2, t_2	5.85	5.87	5.88	5.63	5.96	5.58	5.62	5.05	5.70	5.70	1.51; 1% - 1.2;
	0.86	1.06	0.83	1.01	1.49	1.31	0.99	0.71	0.68	0.72	5% - 0.90
Harvest 1, t_1	2.18	2.15	2.45	2.38	2.41	2.42	2.99	2.70	2.61	2.41	Root/Stem Ratio
	1.02	0.81	0.71	0.81	0.72	0.94	0.72	0.82	1.06	0.98	LSD of means: 0.1% -
Harvest 2, t_2	3.68	3.88	3.79	2.37	1.98	3.52	4.84	4.33	2.26	1.58	1.24; 1% - 0.97
	0.62	0.74	0.92	0.88	0.73	0.89	0.91	0.71	0.93	0.76	5% - 0.74
Harvest 1, t_1	37.0	32.8	36.7	34.8	36.6	48.5	47.1	54.6	49.9	52.3	Leaf Area Ratio
	7.4	6.0	8.4	7.6	7.8	5.6	9.3	8.7	10.3	9.8	(cms/g): LSD of means:
Harvest 2, t_2	29.0	32.9	36.5	38.7	50.4	36.6	35.3	45.7	54.2	51.1	0.1% - 11.8; 1% -
	7.2	5.6	8.0	6.4	9.3	7.0	8.2	7.7	9.3	8.7	9.3; 5% - 7.1
Harvest 1, t_1	164.2	158.2	171.4	153.6	162.7	171.3	177.2	200.0	168.9	190.8	Specific leaf area
	17.4	18.6	15.4	21.7	22.6	22.1	23.6	19.3	21.4	17.6	(cm^2/g): LSD of
Harvest 2, t_2	127.6	127.2	154.3	187.2	187.6	130.5	145.8	179.2	176.7	183.0	means: 0.1% - 28.8;
	15.3	18.7	19.3	17.4	21.9	19.4	20.7	18.2	17.6	19.3	1% - 22.6; 5% - 17.1
Harvest 1, t_1	9.7	9.5	9.6	9.8	9.7	10.7	10.0	11.8	10.8	10.9	Mean leaf area (cm^2)
	2.6	2.5	2.9	3.0	2.0	2.7	2.5	1.9	2.3	1.8	LSD of means: 0.1% -
Harvest 2, t_2	10.9	11.6	12.7	10.9	9.6	11.7	12.2	15.1	12.8	10.5	3.7; 1% - 2.9;
	1.8	2.5	2.6	2.2	2.7	2.6	1.9	3.2	2.8	2.3	5% - 2.2

	Q. robur					Q. petraea					
Growth Chamber Light Intensiy	100	75	50	25	10	100	75	50	25	10	
$t_2 - t_1$	647 174	643 122	517 98	34 74	-33 27	585 127	724 99	575 73	183 63	60 52	Dry wt. increment (mg): LSD of means: 0.1% - 146.3; 1% - 114.7; 5% - 87.2
$t_2 - t_1$	6.3 3.1	21.3 2.7	18.6 3.5	7.4 4.2	3.5 1.6	7.1 2.3	11.2 4.1	16.5 3.2	14.9 2.1	1.8 0.9	Leaf area increment (cm^2): LSD of means: 0.1% - 4.3; 1% - 3.4; 5% - 2.6
$t_2 - t_1$	35.4 6.3	36.9 5.7	26.2 7.2	19.5 8.1	-19.8 6.4	31.4 6.5	38.1 5.7	28.0 4.0	9.2 3.7	3.3 3.9	Net assimilation rate ($g/m^2/week$): LSD of means: 0.1% - 8.7; 1% - 6.8; 5% - 5.2
$t_2 - t_1$	114.8 20.9	121.6 16.4	96.3 12.7	7.1 9.3	-84.7 10.7	130.3 19.6	152.3 22.2	137.9 14.3	48.1 10.3	17.4 6.7	Relative growth rate (mg/g/week): LSD of means: 0.1% - 22.3; 1% - 17.5; 5% - 13.2

VARIANCE RATIOS FOR THE DIFFERENT EFFECTS AND THE ERROR VARIANCE - GROWTH ANALYSIS DATA

Effects	Light intensities	Harvests	Species	LI*H	H*Sp	LI*Sp	LI*H*Sp	Error Variance
Degrees of Freedom	4	1	1	4	1	4	4	180
Cotyledon weight	8.242	294.180	283.285	3.850	94.169	6.730	1.457	3288.600
Stem weight	11.379	307.012	149.500	4.249	1.862	5.448	3.263	671.250
Root weight	106.542	841.028	67.068	99.780	3.038	8.417	3.376	6517.450
Leaf weight	20.561	178.833	1.742	20.405	0.062	1.449	2.680	3225.000
Total weight	98.019	422.511	261.947	82.760	12.042	8.022	4.430	15602.150
Total leaf area	5.960	74.731	11.145	5.013	0.206	0.830	1.270	78.764
Leaf number	0.320	2.121	2.631	0.350	0.254	1.470	0.590	1.050
Root/Stem ratio	19.391	40.055	4.081	16.527	0.351	2.581	0.380	0.709
Leaf area ratio	12.555	3.078	92.657	7.349	11.702	1.607	1.437	64.986
Specific leaf area	22.590	18.609	21.859	17.150	5.817	2.615	1.433	382.415
Mean leaf area	3.987	19.421	12.835	2.627	0.036	0.764	0.090	6.273
Degrees of Freedom	4		1			4		90
Dry weight increment	193.407		10.302			3.069		9890.100
Leaf area increment	91.245		3.608			23.002		8.691
Net assimilation rate	192.186		3.994			22.406		35.568
Relative growth rate	429.517		120.519			45.533		230.051

Significance levels	1*90	4*90	1*180	4*180
5%	3.60	2.49	3.88	2.10
1%	6.97	3.58	6.74	3.40
0.1%	11.80	5.13	11.10	4.78