Quercus robur L. and Quercus petraea (Matt.) Liebl.: a multivariate approach to the hybrid problem, 1. Data acquisition, analysis and interpretation

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ABSTRACT

A method of examining and evaluating populational variability of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl., using a combination of principal component analysis and cluster analysis, is described. Applying the techniques to 6,673 trees in 135 oak population samples from Wales, the Midlands, and East Anglia, together with pollen viability as supplementary evidence, indicated that seven population types could be recognized: 1. pure *Q. robur*; 2. pure *Q. petraea*; 3. mixed populations; 4. *Q. robur* populations of similar type to 4.; 6. populations with a very high proportion of apparent F_1 hybrid status; 5. *Q. petraea* populations of similar type to 4.; 6. populations which differ from the corresponding pure populations by having significantly smaller leaves. The level of hybridization recorded was between 7.72% (515 trees) and 12.63% (843 trees), depending on the limits set for hybrid definition. Despite the presence of hybrids within the populations, it is argued that the specific rank of these two taxa be maintained.

INTRODUCTION

The taxonomic status of trees morphologically intermediate between Quercus robur L. and Q. petraea (Matt.) Liebl. has been discussed by Carlisle & Brown (1965), Cousens (1962, 1963, 1965), Gardiner (1970), Jones (1959, 1968), Rushton (1974) and Wigston (1971, 1974). Davis & Heywood (1963) have argued that the more closely two species resemble each other, the more difficult hybridization is to detect, but the more likely it may be to occur. This is particularly pertinent to the problem of variation in British oaks. The hybrid oak controversy (Gardiner 1970) appears to centre around the acceptance of morphological data alone as evidence of hybridity, in a genus notorious for variation of morphological characters (Muller 1952, Palmer 1948). Although the sole use of morphology to establish the case for hybridization between these two species cannot be wholly justified, it represents nevertheless the only evidence that can be quickly, cheaply and efficiently assessed. However, the lack of a single diagnostic character (Cousens 1963, Rushton 1974, Wigston 1975) emphasises the need for a multivariate approach if morphological data alone are to be used to assess taxonomic status. There are several multivariate techniques for the analysis of O.T.U.* \times character matrices, the form in which the majority of the data on oak populations are collected. For example, Ledig et al. (1969) and Wigston (1971, 1974, 1975) have used discriminant function analysis of oak populations. The work reported in this paper differs from the approach of Wigston (1971, 1974, 1975) in three important respects:

1. The data have been analysed using a combination of principal component analysis and cluster analysis, following the well-established dual approach of numerical taxonomy (Sneath & Sokal 1973).

2. The area of study encompassed Wales, East Anglia and the Midlands, instead of the south-western peninsula.

3. Pollen viability was assessed to provide supplementary evidence for establishing the case for hybridization between Q. robur and Q. petraea.

This first paper is concerned with the methodology employed in the assessment of the taxonomic status of oak populations; a second paper will consider the results on a geographical scale.

* Operational Taxonomic Unit.

DATA ACQUISITION

SAMPLING: POPULATIONS AND INDIVIDUALS

Since *Q. robur* and *Q. petraea* are anemophilous, any attempt to define an oak population on grounds of reproductive isolation presents severe practical difficulties. For example, Semerikov & Glotov (1971), investigating the degree of genetic isolation shown by populations of *Q. petraea*, determined that trees on opposite sides of a glade full of shrubs and 500m wide effectively belonged to the same population. Under open conditions, pollen may well spread much further. A working definition of an oak population was therefore proposed as a group of trees spatially isolated from other groups of oak trees by at least 1 km. Thus, groups of trees closer than 1 km were treated as sub-populations. This is not an ideal solution since the possibilities of gene flow between sub-populations and populations may be high under conditions favourable to long distance pollen transport.

The choice of populations similarly proved difficult. Some populations were included since they had been used for studies on artificial hybridization (Rushton 1977), but most were included on the recommendation of vice-county recorders. Although the use of a random sample of populations was thought important, the difficulties that this posed for fieldwork proved insurmountable. The main criteria employed in the choice of populations were geographical position and maturity of trees, but this did not preclude some samples from hedgerow trees and comparatively young populations. No attempt was made to confine the samples to 'natural' woodland, since a survey of the variation of Q. robur and Q. petraea within the sampled area was considered the main objective of the investigation.

135 populations (and sub-populations) were chosen for this survey and sampled over three years from 1968 to 1970. Individual trees within populations were sampled using a random walk, but edge trees were eliminated to negate the 'edge-effect' (Finney & Palca 1949; Rushton 1974). Preliminary work (Rushton 1974) had suggested that the character differences between *Q. robur* and *Q. petraea* were enhanced in the lighter parts of the canopy. Since Cousens (1963) had sampled from the southern aspect, this position at a height of 6m was chosen for sampling. Preliminary work indicated that five mid-shoot leaves represented a sufficiently large sample taxonomically to characterize each tree. The majority of the population samples consisted of 50 trees, but in small populations this was reduced to 25. Sampling was usually confined to late August/September. Care was taken not to include samples of lammas growth. No attempt was made to limit the sampling area within the population (cf. Olsson 1975).

TAXONOMIC CHARACTERS

The characters used to distinguish Q. robur and Q. petraea have been well documented, e.g. Carlisle & Brown (1965), Warburg (1962), Cousens (1962, 1963) and Jones (1959, 1968). Rushton (1974) has provided a discussion on how some of these characters vary with respect to canopy position. 17 leaf characters were assessed; these will not be discussed in detail except where they were measured or assessed differently from those in other published accounts.

1. Lamina regularity (LR)

Scored as an index ranging from 0 to 4. A perfectly regular lamina scored 4 and one index unit was deducted for each of the following irregularities:

- a. Presence of subsidiary lobes on the sides of the main lobes;
- b. Lobe depths of opposite sides of the lamina markedly different;
- c. Different number of lobes on opposite sides of the lamina;
- d. Leaf outline on opposite sides markedly different.

Thus a leaf showing all the above traits scored zero.

2. Basal shape of the lamina (BS)

Scored as an index 0 to 4; a cordate base scored 0, a cuneate base 4. A series of type leaves was used for comparison (see Rushton 1974).

3. Auricle development (AU)

Scored as an index 0 to 4; a strong auricle scored 0, no auricle 4. A series of type leaves was used for comparison (see Rushton 1974).

4. & 5. Simple (SPH) and Stellate (STH) abaxial pubescence

Wigston (1975) discussed the different types of abaxial pubescence of Q. robur and Q. petraea. He

recognized five types, but regarded only two as of discriminatory value: type (a), large stellate hairs with ascending rays along the midrib and the axial portion of the large lateral veins, which appear as tufts of simple hairs under a hand lens, are here described as simple abaxial pubescence (SPH); type (b), small stellate hairs with rays appressed to the lamina, here described as stellate abaxial pubescence (STH). Wigston (1975) scored these as present (diagnostic for *Q. petraea*) or absent (*Q. robur*). In this study an abundance scale from 0 to 4 was used, with a series of type leaves for comparison. A glabrous leaf scored zero.

6. Number of lobe pairs (LN)

This count did not include the terminal lobe. Cases where the number of lobes on each side of the lamina varied were usually resolved by reference to lamina venation.

7. Number of intercalary veins (SN)

Decisions as to what constituted an intercalary vein proved 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 in practice proved a useful definition, but it should be noted that Wigston (1971, 1975) found difficulty in scoring this character and later abandoned it. The character was scored as a simple count of the number of intercalary veins per leaf.

8. Percentage venation (VN) A ratio expressed as:

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

9. *Petiole ratio* (*PP*) A ratio defined as:

> Total leaf length Length of petiole

10. Total leaf length (L+P)The total leaf length including both lamina and petiole.

11. Length of petiole (PL)

12. Lamina shape or obversity (OB) A ratio defined as:

Lamina length

Length of lamina from the lamina base to the widest part

13. Lamina length (LL)

14. Length of lamina from the lamina base to the widest part (WP)

15. Lobe depth ratio (LDR)

Lobe depth ratio has been calculated as the ratio of the width of the lobe to the depth of the sinus immediately below, e.g. Maze (1968), Silliman & Leisner (1958), Tucker (1963). This method of assessment was retained for that lobe at, or immediately below, the widest part of the lamina.

16. Lobe width (LW)

Measured from the midrib to the tip of the lobe at, or immediately below, the widest part of the lamina.

17. Depth of sinus (LD)

Computed as follows: Lamina width measured from midrib to the base of the sinus at, or immediately below, the widest part of the lamina =X. Then LD=LW-X

The 17 leaf characters were measured or assessed for the five leaves representative of each tree. For each tree, a mean value for each character was calculated.

In most taxonomic studies, quantitative characters such as petiole length, lobe depth, etc. are corrected for variation in leaf size by expressing them as a ratio of leaf size. However, there are no *a priori* reasons for supposing that the uncorrected characters will give poor taxonomic separation. Indeed, the use of ratios represents 'information' loss. In the present study, single measurements have

been used as well as ratios derived from them. Jeffers & Richens (1970) have used similar arguments against the use of ratios in their study of elm populations and they disregarded ratios for the analysis of their results.

During initial survey work, peduncles and acorns were also collected from trees. The sample sizes varied considerably, but were never greater than 20 and never less than 4 acorns/peduncles per tree. Samples were collected from approximately the same canopy position as the leaf samples.

The following characters were measured on the acorn and peduncle samples:

1. *Acorn shape* Derived as the ratio:

Acorn length Acorn breadth

2. *Peduncle length* Measured from the point of attachment to the first acorn.

3. Peduncle diameter

Measured at the middle of the peduncle with a micrometer.

TABLE 1. CORRELATION COEFFICIENTS BETWEEN VARIOUS LEAF CHARACTERS OF Q. ROBUR AND Q. PETRAEA AND THREE REPRODUCTIVE CHARACTERS

	Repro	Reproductive characters				
Leaf characters	Acorn shape	Peduncle diameter	Peduncle length			
Leaf regularity (LR)	-0.044	0.305	-0.222			
Basal shape of lamina (BS)	-0.305	0.531	-0.339			
Auricle development (AU)	-0.279	0.489	-0.402			
Simple hairs (SPH)	-0.096	0.466	-0.359			
Stellate hairs (STH)	-0.155	0.517	-0.427			
No. of lobe pairs (LN)	0.052	0.310	-0.190			
No. of intercalary veins (SN)	0.038	-0.347	0.339			
Percentage venation (VN)	0.000	-0.387	0.345			
Petiole ratio (PP)	-0.165	0.570	-0.405			
Total leaf length $(L+P)$	-0.163	0.358	-0.129			
Petiole length (PL)	-0.142	0.520	-0.400			
Lamina shape (OB)	-0.093	0.029	0.071			
Lamina length (LL)	-0.148	0.271	-0.045			
Lamina length to the widest part (WP)	-0.081	0.368	-0.187			
Lobe depth ratio (LDR)	0.075	0.036	0.146			
Lobe width (LW)	-0.152	0.211	-0.007			
Depth of sinus (LD)	-0.030	0.083	-0.159			
Acorn shape	1.000	-0.126	0.044			
Peduncle diameter	-0.126	1.000	-0.626			
Peduncle length	0.044	-0.626	1.000			

The correlations are based on raw data.

Levels of significance: P = 0.05, 0.138; P = 0.01, 0.181. N = 205 trees

Mean values for these three characters for each tree were calculated. Correlations established between these three characters and the 17 leaf characters (Table 1) indicated that there was good correlation between the two peduncle characters and the leaf characters (Peduncle length × Leaf characters, 13 characters significant at the 5% level; Peduncle diameter × Leaf characters, 14 characters significant at the 5% level). Acorn shape gave 8 significant correlations at the 5% level. The obvious

restriction of sampling to fruiting trees introduces considerable bias into hybrid studies; the inclusion of both fruiting and non-fruiting trees gives incomplete data sets and thus problems of analysis. The good correlation established between peduncle and leaf characters persuaded the author to use only leaf material for the main analyses. However, fruit material was collected when available, and some analyses were completed on both.

RESCORING POPULATIONS

Since several characters were of a qualitative nature, a system of rescoring was introduced in an attempt to minimise error. The following procedure was adopted:

After every 20 leaves scored, the first leaf was rescored; after every 10 trees, the first tree was rescored; after every eight populations, the first population was rescored.

If minor errors were found during rescoring, these were easily rectified; major errors necessitated rescoring up to eight populations.

HYBRID INDICES

Calculation of hybrid indices has proved useful in hybrid studies (e.g. Woodell 1965; Muller 1961), since they enable inter-populational variation to be compared over a series of characters. This can be performed more efficiently using multivariate analysis, but the hybrid index is a useful initial step in data handling, since it can be quickly and easily calculated. Hybrid scores were derived for each quantitative character, values representative of *Q. robur* being scored as 0, those of *Q. petraea* 4, and intermediate values 1, 2 or 3. The delimitation of the classes of the character hybrid scores was based on a range of reference material of the two species derived from a variety of sources: 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 extensive information on character variation derived from preliminary work. For each of the quantitative characters measured, frequency histograms were constructed. These separated the species well, and each species generally showed a normal distribution for each quantitative character. These were then divided into the following character states:

- 0 A range encompassing the extreme 66% of the normal distribution of the *Q. robur* character histogram
- 4 A range encompassing the extreme 66% of the normal distribution of the Q. petraea character histogram
- 1, 2, 3 The range between the end of state 0 and the beginning of state 4 was divided into 3 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 the 'pure' species.) Qualitative characters were collected on a 0–4 scale and therefore did not require conversion.

Hybrid indices were calculated by the summation of the hybrid scores of each of the 17 characters.

DATA PREPARATION

A matrix of individual tree characters was prepared for each population and this was transferred to computer cards, one card per individual tree. This matrix was incomplete in the sense that characters derived as ratios were not incorporated on the cards, but were calculated during data input to various programs. Similarly, all conversions of raw data to hybrid indices were completed during data input.

DATA ANALYSIS

The traditional approach to population studies of hybrids has followed that of Anderson (1949, 1953), using the pictorialized scatter diagram (PSD). A bivariate scatter diagram is produced using two quantitative characters, and further characters are encoded on to each point to produce a pictorialized dot or metroglyph (Anderson 1957). Although it is useful to summarize the variation within and between populations, interpretation of a PSD may be difficult and misleading. For example, in Figs. 1 and 2, the same two populations have been plotted using different quantitative characters. Position of the individuals in the scatter, rather than ornamentation, is of paramount importance in determining whether a particular individual is critically examined or not. Thus, in Fig. 1, since the scatters for the two populations are restricted, few of the individuals would be closely examined. However, in Fig. 2,



FIGURE 1. Bivariate scatter diagram showing the relationship between petiole ratio (PP) and venation percentage (VN) for two *Quercus* populations: R, tree numbers 1–25; P, tree numbers 26–47. The populational means and standard deviations are also shown.



FIGURE 2. Bivariate scatter diagram showing the relationship between lobe depth ratio (LDR) and venation percentage (VN) for two *Quercus* populations: R, tree numbers 1–25; P, tree numbers 26–47. The populational means and standard deviations are also shown.

the scatters are more diverse and, indeed, some overlap occurs between the two populations. The outliers of the populations would, in this case, be closely examined. Although there is reasonably close agreement between the position of the individuals in Figs. 1 and 2, some major discrepancies do occur. For example, individual 46 in Fig. 1 lies in the zone where it might be critically assessed, whilst, in Fig. 2, it lies well away from the intermediate zone. Similar behaviour is exhibited by individual 36. Individual 26 behaves differently—in the intermediate zone in Fig. 2 and at the extreme in Fig. 1. Consequently, the metroglyphs that are closely examined depend on the initial choice of the quantitative characters for the construction of the PSD. The overall interpretation is thus highly dependent on the choice of character axes. Fig. 1 could be interpreted as consisting of two pure populations; Fig. 2 as consisting of two populations with a range of intermediate (and therefore possibly hybrid) forms. The construction of a PSD is also time-consuming and, unless constructed by a graph plotter, prone to error. The interpretation of the PSD depends largely on positional information, and since the human mind is better able to grasp positional information than a confusing array of metroglyphs, an alternative approach to data analysis has been adopted in this study.

PRINCIPAL COMPONENT ANALYSIS

Principal component analysis (PCA) has been extensively used in taxonomic research, and therefore the mathematical background to the analysis will not be discussed here. Jeffers (1964, 1967) provided an excellent discussion of its use and interpretation, as applied to taxonomic research. Standardization of taxonomic data is normally obligatory, since characters are usually a combination of lengths, indices, etc. Although, in this study, two analyses were always completed, i.e. with each character standardized to zero mean (covariance matrix) and with each character standardized to zero mean and unit variance (a correlation matrix), the results of the latter are more appropriate to the data under discussion.

The analysis of individual populations using PCA is problematical, since the data structure of an individual population is not particularly illuminating. What is important is the data structure of a population compared to that of some reference point. Taxonomists are usually more interested in, for example, the PCA scatter of species within a genus (e.g. Lubke & Phipps 1973) rather than 'testing' a population against a reference point. Unfortunately, this approach leads to the difficulty of defining a suitable reference point. Three approaches appeared available:

1. A 'population' where each individual was represented by a series of characters scored from a herbarium sheet. This approach was rejected on the grounds that herbarium specimens are frequently 'typical' specimens.

2. A spurious 'population' artificially created from published accounts of the diagnostic characters and their ranges for the species. Again, this was rejected as being difficult to produce and prone to bias.

3. The approach finally adopted was to utilize the results from actual populations. These populations should, in all respects, conform to what would be expected of a pure population. All 135 populations were sorted to determine if suitable populations could be found. Two populations were finally chosen, one representing 'pure' *Q. robur* (R) and one representing 'pure' *Q. petraea* (P). Both populations had been sampled on two consecutive years, and both had been used for subsidiary studies, so that the composition of the woods was intimately known. The population sample of 25 trees from the P population showed three trees that did not conform to the *Q. petraea* type, and these were deleted from the analyses leaving 22 trees in the P population. The three non-conformist trees consisted of two *Q. robur* trees and an intermediate. Evidence is presented later that, on the grounds of pollen viability, these two populations, excluding the three abnormal trees, may also be considered to consist of pure individuals.

Data analysis of each population consisted of combining the data for a given population with those of the two reference populations, and completing a single PCA on the combined data set.

CLUSTER ANALYSIS

Cluster analysis (CA), although frequently used for taxonomic studies, is rarely used for hybrid studies. The analysis is applicable to hybrid studies in that the clustering properties of suspected hybrids may be determined. Several clustering techniques have been applied to the data, using options available in CLUSTAN 1A, a suite of FORTRAN IV programs (Wishart 1970). The one discussed here is Ward's Error Sums of Squares method, a polythetic, agglomerative clustering strategy, using squared Euclidean distance as the similarity coefficient (Ward 1963, Wishart 1969). Again, in order to present











FIGURE 5. CA of the two reference *Quercus* populations, R and P, using Ward's error sums of squares method. The membership of each cluster is shown together with the mean hybrid index (\bar{x}) of each cluster.







FIGURE 7. CA of the two reference *Quercus* populations, R and P, and population IR, using Ward's error sums of squares method. The membership of each cluster is shown together with the mean hybrid index (\bar{x}) of each cluster.



FIGURE 8. PCA of the two reference *Quercus* populations, $R(\bullet)$ and $P(\blacksquare)$, and population $IR(\bigcirc)$. The first two components of the correlation matrix are shown together with the percentage of the total variance accounted for by each component.



FIGURE 9. CA of the two reference *Quercus* populations, R and P, and population IP, using Ward's error sums of squares method. The membership of each cluster is shown together with the mean hybrid index (\bar{x}) of each cluster.



FIGURE 10. PCA of the two reference *Quercus* populations, $R(\bullet)$ and $P(\blacksquare)$ and population $IP(\Box)$. The first two components of the correlation matrix are shown together with the percentage of the total variance accounted for by each component.



FIGURE 11. CA of the two reference *Quercus* populations, R and P, and population H, using Ward's error sums of squares method. The membership of each cluster is shown together with the mean hybrid index (\bar{x}) of each cluster.



FIGURE 12. PCA of the two reference *Quercus* populations, $R(\bullet)$ and $P(\blacksquare)$ and population H(X). The first two components of the correlation matrix are shown together with the percentage of the total variance accounted for by each component.



FIGURE 13. CA of the two reference *Quercus* populations, R and P, and population AP, using Ward's error sums of squares method. The membership of each cluster is shown together with the mean hybrid index (\bar{x}) of each cluster.



FIGURE 14. PCA of the two reference *Quercus* populations, $R(\bullet)$ and $P(\blacksquare)$ and population $AP(\Box)$. The first two components of the correlation matrix are shown together with the percentage of the total variance accounted for by each component.



FIGURE 15. PCA of twenty Quercus populations. The first component of the correlation matrix is plotted against the corresponding percentage pollen viability for each individual tree. For clarity only six populations are shown, classified as: R-type population \bullet , IR-type populations \bigcirc and \oslash , IP-type population \square , P-type populations \blacksquare and \blacktriangle .

the analysis with common information, each population was analysed with the two reference populations.

Both PCA and CA were completed on raw data matrices without transformation to hybrid indices.

RESULTS

In order to illustrate the different types of result obtained, four populations have been chosen to cover specific points. Fig. 3 shows the frequency histograms of hybrid indices for these four populations (Fig. 3b, c, d, f), together with the two reference populations, R and P (Fig. 3a, e). Initially, the two reference populations were subjected to PCA and CA without any other population. The results are shown in Figs. 4 and 5. PCA (Fig. 4) separated the two reference populations. There was no overlap of the two reference populations on the first Component which accounted for $54 \cdot 5\%$ of the total variance. The vector loadings for the first two Components are shown in Fig. 6a. All characters, with the exception of LW and WP, showed a high loading on the first Component, suggesting that all but these two characters were responsible for the separation of the two reference populations. Variation within the

populations appeared to be associated with the second Component. As can be seen (Fig. 6a), the characters with the highest loadings on Component 2 are all those associated with leaf size, viz. L + P, WP, LL, OB, LD and LW.

CA of the same two populations (Fig. 5) showed that they clustered discretely. At the 10 cluster level (the lowest level shown in Fig. 5), each population had grouped into five clusters. The R population appeared to be slightly more heterogeneous than the P population, since the ten trees of Cluster 1 did not fuse with the other 15 trees of the population until level $4 \cdot 30$, whilst the clusters of population P had all fused at level $2 \cdot 22$. This greater heterogeneity in the R population can also be seen in the PCA, where the spread of the population over the second Component was much greater than that of the P population.

Both analyses separated the two pure populations. Since PCA is a multivariate technique, positional information alone is critical in evaluation of the component scatter. In the component space produced by a PCA, an individual located towards the intermediate zone between the two populations is positioned there by virtue of a consideration of all its characters. Thus, the position of an individual in component space may be thought of as an amalgam of the bivariate character axes and the metroglyph characters of the PSD.

The frequency histogram for population IR (Fig. 3b) showed a skewed distribution with several trees in the intermediate zone. CA of this population together with P and R is shown in Fig. 7. The R population formed five clusters (Clusters 1, 3, 4, 5 and 6), and the majority of the IR population (35 trees) also grouped with these clusters. 13 trees of the IR population formed a discrete cluster, Cluster 2, which did not contain trees from either the R or P population. The mean hybrid index of this cluster was 133.5, suggesting an intermediate morphology. Cluster 1, with a mean hybrid index of 130.0, contained 16 IR and five R trees; the mean hybrid index of the five R trees being 99.6 and that of the 16 IR trees 139.5. (This cluster was in fact formed by the agglomeration of the R and IR trees at the previous fusion cycle i.e. cluster level 11.) Two IR trees clustered with the P population, although one, with a hybrid index of 206, appeared discrete. PCA of the same populations is shown in Fig. 8. The two P-related trees can be clearly seen. Although the large majority of the IR population occurred at the end of Component 1, within the range of the R population, a proportion of the IR population occurred at the origin or just to the left of the origin. The membership of this latter group corresponded with the membership of Cluster 2 of the CA.

A similar result was obtained for population IP (Fig. 3d). CA yielded three clusters (Fig. 9, Clusters 1, 2 and 3) which consisted exclusively of the R population. 12 of the IP population (Clusters 8, 9 and 10) formed a relatively discrete group (only one alien tree from the P population), whilst the remaining 13 trees clustered with the P population (Clusters 5, 6 and 7). The mean hybrid indices of Clusters 8, 9 and 10 were considerably lower than those of Clusters 4, 5, 6 and 7. PCA (Fig. 10) produced a similar result to that of IR. Part of the IP population grouped with the P population, whilst the rest occupied an intermediate position.

Some populations were similar to the R or P population. For example, in PCA, such populations grouped with population R or P, but did not produce trees in the intermediate zone. Similarly, in CA, they clustered with the R or P population, but did not form discrete clusters like the IR and IP populations above. Such populations accounted for over 50% of the populations studied. A small number of populations proved to be mixed, but with no intermediates.

Two other population types were apparent. The first of these is illustrated in Fig. 3c. CA (Fig. 11) of this population (H) resulted in the population grouping into five clusters. Three of these, Clusters 5, 6 and 7, were discrete with mean hybrid indices of $176 \cdot 6$, $169 \cdot 3$ and $167 \cdot 0$ respectively. One tree fused with the P population (Cluster 9) and the remaining two trees with the R population (Cluster 4). Clusters 5, 6 and 7 finally fused with the clusters of population R, thus showing more affinities with them than with the P population. PCA (Fig. 12) emphasized the highly intermediate nature of this population; the majority of the trees were positioned between the two reference populations, although slightly shifted towards the R population. A small degree of overlap was apparent between population H on the one hand and populations R and P on the other on the first Component.

The final population type (AP) is shown in Fig. 3f. The range of hybrid indices, although marginally greater than the main body of the P population (Fig. 3e), was not sufficiently different to warrant undue attention. However, in CA and PCA, population AP behaved in a totally unexpected fashion. In CA (Fig. 13), the population formed three discrete, non-overlapping clusters (Clusters 8, 9 and 10). The mean hybrid indices of these were 194.5, 212.2 and 200.8 respectively. However, these did not fuse with

the four population P clusters (Clusters 4, 5, 6 and 7) until the comparatively high fusion level of 18.37. This suggested that, although the hybrid indices were not very different from the P population (compare Fig. 3, e and f), the structure of the raw data was sufficiently different for CA to detect differences between the two populations. This difference was reflected in PCA (Fig. 14). Whilst population AP separated from population R on the first Component, it also separated from population P on the second Component. Although well separated from the R population, the centroid of the AP population is closer to the R population than is that of the P population.

The vector loadings diagram (Fig. 6) indicates that, whilst the loadings were substantially the same for PCA using populations R and P alone (Fig. 6a), and R and P together with populations such as IR (Fig. 6b), when using R and P together with AP (Fig. 6c) the vector loadings showed significant differences. The major differences, with the unimportant exception of orientation, was the very high negative loadings on Component 2 given to the leaf-size characters (LW, WP, LL, L + P and PL) and the ratio PP. Both PL and PP had very low loadings on Component 2 in the PCA of R, P and IR (Fig. 6b).

The use of CA and PCA clearly indicated that different population types could be recognised. However, these only have a basis in relation to the two reference populations used. Before generalisations can be made about the nature of these population types, the classification of populations into different types should be rigorous. This was completed by the choice of two further reference populations, and by analysing all the populations with these instead of the originals. The diagnosis of each population did not change significantly. Several further reference populations have been utilised, again with no significant change in the diagnosis of the individual populations.

POLLEN VIABILITY

Hybrid status has been inferred for morphologically intermediate trees, e.g. by Cousens (1963) and Carlisle & Brown (1965). Wigston (1974) has argued that the Theoretical Species Type analysis developed by Cousens (1963, 1965) is a powerful tool for assessment of populational variability. However, no matter how sophisticated the analysis, it relies solely on the original morphological data. Similar criticisms can be expressed about the discriminant function analysis used by Ledig *et al.* (1969) and Wigston (1971, 1974) and, indeed, PCA and CA used in this study. Only the relative ease of interpretation distinguishes the use of discriminant function analysis, PCA and CA from the more cumbersome PSD. Olsson (1975) has recognised the difficulties of interpretation of morphological data alone, and , following the criticisms of Gottlieb (1972), has resorted to the use of pollen stainability as supplementary to morphological data. This present study has used a similar approach.

For a range of populations, showing different morphological patterns, pollen viability was assessed during spring, followed by assessment of leaf morphology later in the year. Pollen viability was determined using nitro-blue tetrazolium (Hauser & Morrison 1964, Rushton 1974) with a minimum count of 200 pollen grains per tree. Twenty populations were assessed for pollen viability and leaf morphology, but, in order to retain clarity, only 6 populations are actually shown in Fig. 15. The total sample size was 960 trees. The morphological data for the 20 populations were subjected to a PCA. Fig. 15 shows the first Component of this analysis plotted against the corresponding percentage pollen viability for each tree. The trees showing morphological intermediacy, towards the centre of the first Component, showed a significant decrease in percentage pollen viability. This general pattern was repeated over 11 of the remaining 14 populations. There were three populations not conforming to this general result. A breakdown of the morphological composition of these three populations is given in Table 2. Despite having a range of intermediate forms, the pollen viabilities of all three populations was generally over 80%. Only six trees (See Table 2) had pollen viabilities below 80%. The status of these three populations is problematical.

INTERPRETATION

It is now generally accepted that low pollen viability is evidence towards the establishment of a case for hybridisation. Thus Woodell (1965) showed that plants regarded on morphological grounds as F_1 hybrids between *Primula veris* and *P. vulgaris* had a mean pollen viability of 43.5% and a range of 9–70%. Similarily, Bradshaw (1958) assigned plants morphologically intermediate between *Agrostis*

В

stolonifera and A. tenuis to F_1 hybrid status on the basis of morphology and low pollen viability. Olsson (1975) has shown that in Sweden oaks intermediate in morphology between Q. robur and Q. petraea had an overall reduced pollen viability. He concluded that such trees were of hybrid status. However, the correlation established was not perfect. Some trees regarded on morphological grounds as being Q. petraea had pollen viabilities as low as 20–30%, some Q. robur trees as low as 40–50%, and some interspecific phenotypes had pollen viabilities as high as 90–100%. Jones (1959) has noted the occurrence of three oak trees (species unspecified) out of 25 that had a low pollen viability (50–75%) and a combination of characters of both species.

The results presented here parallel closely those of Olsson (1975), and the general interpretation of the PCA, CA and pollen viability results is that trees morphologically intermediate between Q. robur and Q. petraea are of hybrid origin. For those populations in which pollen viability was measured, trees regarded on the basis of PCA and CA as being pure types all had pollen viabilities greater than $c \, 80\%$. All trees of the two initial reference populations, R and P (excluding the three non-conformist trees), had pollen viabilities above 85%. This contrasts with some of the results of Olsson (1975), since low pollen viability of morphologically pure types have not been recorded. The high pollen viabilities of some intermediate trees recognised by Olsson (1975) are similar to the results for the three populations detailed in Table 2. These trees may represent F_2 (or F_3 and back-cross) hybrid individuals with restored fertility. Doroszewska (1965) and Woodell (1965) provide comparable examples in other genera.

TABLE 2. DETAILS OF THREE OAK POPULATIONS SHOWING A RANGE OF INTERMEDIATE FORMS, WITH A LARGE MAJORITY OF THE TREES HAVING A HIGH % POLLEN VIABILITY

Classification of trees									Details of trees with <80% pollen viability	
Population code	Sample size	R-type	Inter- mediate	P-type	Mean hybrid index	Hybrid index range	No. trees with <80% pollen viability	Hybrid index	% Pollen viability	
BY	50	31	16	3	131.8	69-262	0	1029a	up solution	
AAD	50	39	10	1	131.1	60–224	3	133 128	67 74	
	Polica v							119	75	
CCE	50	23	16	11	147.5	51–266	3	116 199 154	58 63 74	

The nature of the hybridization between Q. robur and Q. petraea remains an open question, i.e. do the intermediate phenotypes represent only F_1 hybrids, or do they represent a full range of backcross individuals thus indicating introgressed populations? It would be expected that if the intermediates were only of F_1 hybrid status, a third discrete grouping should be apparent in the results of CA and PCA. Consideration of the results displayed in Figs. 8 and 10, typical of a large number of populations, showed a gradation of intermediates, with the majority shifted markedly to one side. The overall view of pollen viability results (Fig. 15) follows the same trend. No identifiable grouping can be seen in Fig. 15; the trees appear to form a complete gradation, from high pollen viability at the end of Component 1 to low pollen viabilities in the middle of the Component. Populations of the IR and IP type probably contain, therefore, not only F_1 hybrids but also a series of backcross hybrids, and may be regarded as introgressed populations.

The results of CA for a small number of populations (e.g. Fig. 11) would suggest a very specific grouping different from either R or P. However, as PCA shows (Fig. 12), the range of variation within

this group is high and overlaps marginally the R and P reference populations. Only one population of this type was examined for pollen viability. The results indicated that the pollen viabilities of the intermediates were below 75%. It was concluded that populations of this type were composed of a large proportion of hybrids, many of which could be regarded as of F_1 status.

The sampling procedures used for the pollen viability investigation require closer scrutiny. Choice of individual trees for sampling within the populations was determined by a random walk. However, since only trees with catkins were chosen, the sample was non-random. Restriction of the sample to flowering trees only serves to decrease the estimate of hybridity. Comparison of the morphology of populations estimated from samples containing both flowering and non-flowering trees with that of the morphology of the same populations estimated from samples containing only flowering trees indicated that the two samples were not significantly different. Within-population morphological comparison of flowering and non-flowering trees showed no significant differences. It is concluded that, although the pollen viability samples were biased, restriction of the samples to flowering trees neither increased nor decreased the estimate of hybridity.

Six populations produced variation patterns similar to population AP (Figs. 13 & 14). Of these, four populations (including AP) clustered with the P population and two clustered with the R population. Arguments were advanced (Rushton 1974) that these populations might represent residual introgressed populations in which assimilation of the alien genes was nearly complete, allowing, therefore, only a minor shift in the populations' centroid along Component 1 in PCA. Inspection of the vector loadings for Component 2 (Fig. 6c) indicated that the leaves of the AP trees were on the whole smaller, both in length and width, than those of the corresponding reference populations. This was true for all the other five populations, nor were pollen viability studies conducted. Nevertheless, the leaf morphology is probably sufficiently different from the reference populations for the AP type populations to be considered for recognition of infraspecific taxa (Valentine 1975, Weimarck 1947a, b). It is interesting to note that no comparable populations were found in which the leaves were larger than those of the reference populations.

It is necessary to present the morphological limits of the parental species together with those of the hybrids. Examination of the results of 6673 trees analysed by PCA and CA, together with the pollen viability results from 960 trees, indicates that those trees within the hybrid index range 150 to 189 can be regarded with a high degree of certainty as being of hybrid origin. 515 trees (7.72% of the total sample) fell into this category. Excluding the three aberrant trees of the P population, the lowest hybrid index was 200 and the highest hybrid index of the R population was 134. Thus, a wider intermediate zone (149–199) might be justified. This would include 843 trees, or up to 12.63% of the total sample. However, extension of the hybrid zone to these limits would include trees with a significantly higher pollen viability (i.e. > 80%). A breakdown of the number of different population types contained in the 135 populations is given in Table 3.

TABLE 3. NUMBER OF POPULATIONS FOUND OF EACH TYPE AMONG THE135 OAK POPULATIONS SAMPLED

Population type	Number of populations	% occurrence	
Pure Q. robur	57	42.2	
Pure \tilde{Q} . robur (but with smaller leaves)	2	1.5	
Q. robur populations with a wide range of intermediates (introgressed)	22	16.3	
H-type populations (i.e. substantially composed of intermediates)	8	5.9	
Mixed populations containing both Q. robur and Q. petraea	7	5.2	
Q. petraea populations with a wide range of intermediates			
(introgressed)	23	17.0	
Pure Q. petraea (but with smaller leaves)	4	3.0	
Pure Q. petraea	12	8.9	

DISCUSSION

The levels of hybridization recorded here are substantially lower than those noted by Cousens (1963, 1965) and Carlisle & Brown (1965), but are in general accord with the conclusion of Jones (1959). He argued that, of populations where both species grew together, it was unusual to consider more than 5% of such populations to be hybrid. Cousens (1963, 1965), for the detailed analysis of his material, restricted his populations to those which contained sufficient fertile material for complete analysis. As argued earlier, such limitation would serve to underestimate the level of hybridization within the populations. Consideration of all the populations sampled by Cousens might therefore enlarge the differences between his results and the present survey. However, the data analyses are substantially different and the surveys completed on different areas.

Olsson (1975) has argued that the occurrence of a large number of hybrids between *Q. robur* and *Q. petraea* under natural conditions indicates that the specific status of these taxa should be questioned. This conclusion cannot be supported. The level of hybridization in oak populations is still a matter for debate, since few studies have used evidence other than morphology. Also there have been few large-scale population studies as compared with casual observation. However, it is important to note that all published accounts of variation in *Q. robur* and *Q. petraea* accept some level of hybridization. Nevertheless, the distinctly unsuccessful attempts to show anything other than minimal interfertility between the species (e.g. Dengler 1941, Rushton 1977) indicate that the interspecific isolating mechanisms are still intact. Until such time as the two morphological types, i.e. *Q. robur* and *Q. petraea*, are shown by crossing experiments to be relatively interfertile, the specific status of *Q. robur* and *Q. petraea* is best maintained.

ACKNOWLEDGMENTS

I should like to extend my gratitude to the following who have contributed to this work: Dr M. C. Lewis for his guidance and supervision; Professor M. H. Williamson for suggesting the use of PCA; the staff of the Drawing Office, New University of Ulster, for preparing the figures; my wife for typing the manuscript of this paper; and the vice-county recorders for sending details of oakwoods in their areas.

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(Accepted January 1978)