Quantitative studies of hybridization in wild plant populations

Clifford, Harold Trevor

Clifford, Harold Trevor (1955) *Quantitative studies of hybridization in wild plant populations*, Durham theses, Durham University. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/9289/

QUANTITATIVE STUDIES OF HYBRIDIZATION IN WILD PLANT POPULATIONS

by

Harold Trevor Clifford

- being a thesis presented in candidature for the Degree of Doctor of Philosophy in the University of Durham, 1955.

QUANTITATIVE STUDIES OF HYBRIDIZATION IN WILD PLANT POPULATIONS

Abstract of the Ph.D. thesis presented by H.T. Clifford, 1955.

The methods for studying populations containing hybrids have been discussed and the quantitative methods for describing and analyzing such populations have been explored. These techniques mostly depend upon the properties of normal univariate or multivariate distributions. If the size of a character is normally distributed in a population free of hybrids, its deviation from normality in other populations may indicate the presence of hybrids. Most methods of analysis require that the characters measured are uninfluenced by the environment. A method of using pairs of correlated measurements when each is influenced by the environment has been proposed.

The techniques described have been applied to the study of introgression in the genera Juncus and Primula.

In the genus Juncus some evidence was found for the introgression of J. effusus (meadow form) into J. inflexus; the data further suggested that J. effusus contained at least two ecotypes, a meadow and a woodland form. Because

of these, evidence of introgression of *J. effusus* by *J. inflexus* was not sought.

In the genus *Primula* the data suggested that slight reciprocal introgression of *P. vulgaris* and *P. veris* had occurred. Plants resembling the artificial F1 hybrid between these species were found in most places where the two grew intermixed; their numbers were always small. Very few putative backcross hybrids were observed. Their scarcity may be real or due to many of them resembling the F1 hybrid or the recurrent parent, as they are known to from artificial backcross families. The absence of hybrids other than the apparent F1 in the field may be due to their inability to compete with their parents. Artificial backcross families are easily secured and they are relatively fertile, so it would appear that external and not internal barriers separate the species.

ACKNOWLEDGEMENTS

The writer wishes to express his thanks to Professor D.H. Valentine who suggested that the genera Juncus and Primula might be suitable subjects for the study of introgression. The writer is further indebted to him for making available some families of artificial Primula hybrids and for offering advice during the course of the work and the preparation of this thesis.

Thanks are due to Professor D.A. Webb, Trinity College, Dublin, for his hospitality which enabled the writer to spend a week in Ireland investigating Primula hybrids.

Dr. E.S. Page has kindly discussed with the writer certain statistical problems that arose in the course of the work.

Finally the writer is indebted to the Australian National University, Canberra for providing an Overseas Scholarship enabling him to study in the Durham Colleges.

CONTENTS

THE QUANTITATIVE STUDY OF NATURALLY OCCURRING HYBRID PLANTS.

THE QUANTITATIVE STUDY OF NATURALLY OCCURRING PLANT HYBRIDS.

1. INTRODUCTION.

In recent years there has been a steadily increasing interest in the study of hybrid plants. There have been many reasons for this increased interest. To the student of evolution hybrids mean new combinations of genetic material; the taxonomist is confronted with the cause of confusion in many groups; the agriculturalist must be careful that his crops do not suffer from breeding with uncultivated plants and so become contaminated.

These are but a few of the reasons for studying hybridization. Here some methods for studying wild populations are considered. Such investigations should always be accompanied by breeding studies for the two are complementary. Sometimes it is from field studies that we know if hybridization is occurring between species because attempts at artificial hybridization may be unsuccessful. At other times hybrids may easily be produced artificially yet under natural conditions they may not occur.

In his book 'Introgressive Hybridization' Anderson (1949) summarizes the methods generally employed in the study of hybrid plant populations. Most of the methods proposed are very useful but suffer from being inherently subjective, though they may appear to be objective. The two principal techniques advocated are the use of indices and of scatter diagrams.

Indices, though they may be treated quantitatively ~~qualitatively~~, have the disadvantage that they are formed by the simple addition of the measurements of several characters. Such arbitrary addition of characters is unsatisfactory in that it does not allow for correlation between characters; furthermore characters such as hairiness are often measured in arbitrary units. It is perhaps for these reasons that these indices or hybrid indices have not often been employed by British workers.

The method of the scatter diagram which has been frequently used by Anderson and other American botanists is highly subjective. In the scatter diagram when complete, each point represents a condensed picture of a particular plant. By a careful examination of the scatter diagram it is sometimes possible to predict something of the characters of the species which gave rise to the hybrid swarm under study. This is done by considering the different characters and the degree of correlation between them. Characters which are closely correlated are regarded as being derived from a common species and so this method of predicting parentage has become known as the method of extrapolated correlates.

As distinct from these methods those which follow are somewhat more objective and are chiefly concerned with quantitative characters. The analysis of field populations will now be considered under two headings.

1. Recognition of Hybrid plants and Hybrid swarms.
2. Recognition of Introgressive Hybridization.

2. RECOGNITION OF HYBRID PLANTS AND HYBRID SWARMS.

Hybrid plants are generally recognized by being intermediate in appearance between their parents. If the F1 generation between a pair of species was quite sterile, the criterion of intermediate morphology might be used to recognize it. Quite probably it would be recognized as a third taxon and would even be granted specific status by taxonomists. Within the genus _Nuphar_ there seems to be an example of such a 'hybrid species' in _N. intermedia_ which has become well established by vegetative reproduction, and which is very probably an F1 hybrid between _N. lutea_ and _N. pumila_ (Y. Heslop-Harrison 1953).

Many interspecific hybrids are however partially if not highly fertile so that they may produce an F2 generation or breed back to one or both of the parents. In this way a very complex series of forms is produced which may form a complete transition from one species to the other. Such a mixture of forms may be very difficult to interpret if the two species have been interbreeding so extensively that the frequencies of the pure species are low. Stands of this type may be conveniently designated as hybrid swarms.

With a knowledge of the distribution of several species of a genus it is sometimes possible to recognize the nature of a hybrid swarm. Especially is this so if the hybrid swarms occur only in those regions where the ranges of the possible parental species overlap. Further support for the nature of a hybrid swarm between two such species may be derived from a statistical investigation of some character of the probable parental species and the putative hybrid swarm.

The application of some statistical methods to the study of hybrid swarms will now be discussed under three headings.

(a) Increased variability
(b) Discordant variation
(c) Progeny testing.

(a) Increased variability.

If the hybrid swarms arose from the pair of species that had been predicted as parents it would be expected that for most characters it would be intermediate between those species. Besides being intermediate, any character of the plants in the hybrid swarm is likely to be more variable from plant to plant than in either of the assigned parents. This point is illustrated by some fruit measurements in the genus Eucalyptus, Table 1. Here the variances of averages of 10 measurements on three characters from each of 50 trees of E. elaeophora, E. goniocalyx and a hybrid swarm are quoted. (Clifford and Binet, 1954, see Appendix A.)

TABLE 1.

Character	E. elaeophora.	Hybrid swarm	E. goniocalyx
Fruit weight (mgm.)	2091	2081	2097
Fruit length (mm.)	0.9	1.6	1.4
Peduncle length (mm.)	3.7	9.5	7.0

TABLE 1: The variances of the means of three fruit characters for a pair of Eucalyptus species and a presumed hybrid swarm between them.

It ought to be noted that the use of the variability of a population to confirm its parentage may not always appear to be reliable. In Table 1 it was shown that although the fruit and peduncle lengths were more variable in the presumed hybrid swarm than in the putative parental species, the reverse held for fruit weight.

To understand why this was so it is necessary to consider the methods for measuring variability; the variance was used in Table 1. Though theoretically the variance is perhaps the best measure of variability it suffers in that it is usually dependent upon the mean, and in general the larger the mean the greater its variance. To eliminate this influence of the mean the coefficient of variation may be employed. This is the standard deviation (the square root of the variance) divided by its mean. Occasionally this value is expressed as a percentage.

When the variability of fruit weight is expressed as the coefficient of variation the presumed hybrid swarm is found to be more variable than either of its assumed parental species, Table 2. This follows from the fact that the average fruit weight of the hybrid swarm is less than that of the parental species, the variance being similar in all three of the groups.

TABLE 2

Character	E. elaeophora	Hybrid swarm	E. goniocalyx
Fruit weight (mgm)	.246	.286	.276
Fruit length (mm)	.113	.143	.120
Peduncle length (mm)	.191	.237	.166

TABLE 2: The coefficients of variation of the mean of three fruit characters for a pair of Eucalyptus species and a presumed hybrid swarm between them.

(b) Discordant variation

An alternative method of confirming putative parentage in hybrid swarms is to compare the pattern of variation within them with that within the presumed parental species. This method is due to Anderson (1951) who has pointed out that within a species there is always a definite association of characters, and if they vary they do so according to a definite pattern. For example seedling and adult leaves may differ in shape but within a given species the transition will always follow the same course. Such patterns of development vary from species to species.

These patterns of variation are described as concordant whereas those for the members of a hybrid swarm may be described as discordant. Segregation and recombination of the original species patterns in the hybrid swarm tend to cause a disjointed pattern of variation. The difference between these two kinds of variation is shown in Table 3, where several qualitative characters are listed for two species of *Iris* and a hybrid swarm between them, (from Anderson, 1949, p.3).

With discordant variation the degree of association between certain pairs of measurements is usually less than the degree of association of similar pairs of measurements showing concordant variation. The measurement of the degree of association in terms of

TABLE 3

Colony		Tube colour	Colour of Sepal Blade	Sepal lgth. (cms)	Petal shape	Stamens	Style Appendages	Crest
Iris hexagona	1	g	Pale violet-blue	9	g	g	g	g
	2	g	Violet-blue	9	g	g	g	g
	3	g	Violet-blue	9	g	g	g	g
Hybrid	1	i	Dark Red-violet	7	f	i	i	g
	2	g	Pale Violet-blue	10	g	g	g	g
	3	i	Red	6	f	i	f	i
	4	g	Pale Blue-violet	10	g	g	g	g
	5	i	Red-violet	7	i	g	i	f
	6	i	Very Dark Violet	8	i	i	g	g
Iris fulva	1	f	Red	5	f	f	f	f
	2	f	Red	6	f	f	f	f
	3	f	Red	6	f	f	f	f

Table 3: A comparison of plants from three separate colonies of *Iris*, twoof which contained only the pure species and one of which was a hybrid swarm. The symbols may be defined as follows: f - resembling *I. fulva*; g - resembling *I. hexagona*; i - intermediate between these species.

the product-moment correlation coefficient (r), is considered later when discussing introgressive hybridization.

(c) Progeny testing

The status of a presumed hybrid plant must always be conjectural until it has been reproduced by deliberate cross-breeding. With certain groups breeding may be difficult so another technique must be developed. Perhaps the most satisfactory technique available is progeny testing.

Seed taken from a single plant presumed to be an F1 hybrid must either give rise to an F2 generation, a series of backcrosses to the parental species, or a mixture of all three. With other hybrids different combinations of offspring would be derived, and amongst them segregation would be expected. Some of the segregates might be expected to resemble or to suggest the parental species. This method of confirming or predicting parentage has often been used, especially since MacDougal (1907) demonstrated its value in the study of hybrid oaks.

The method of progeny testing may be treated quantitatively as has been previously shown by the writer (Clifford, 1954 and Appendix B). Briefly the method consists of growing the progeny from several different plants of the assumed hybrids and putative parental species. Some attribute is measured on the members of each family and its mean and variance are calculated. These two quantities are plotted against each other to obtain a scatter diagram. If this can be enclosed within a triangular outline with the assumed parental species at its basal angles, the parentage of the presumed hybrid swarm is confirmed. This method is illustrated in Figure 1 where data from the genus Eucalyptus are employed.

The triangular relationship follows if the character studied is inherited in an intermediate manner and its size is controlled by several genes. Under these circumstances the F1 and F2 generations have similar means but different variances and families of the parental species have different means and relatively small variances. This is illustrated in Table 4 by data for Maize (East 1950).

TABLE 4

Parentage	Mean Cob Length of Progeny (cms)	Variance of mean
P1 x P1	6.6	0.66
P2 x P2	16.8	3.49
P1 x P2 (F1)	12.1	2.28
F1 x F1 (F2)	12.6	7.89

TABLE 4: The mean and variance for cob length in the parental and two hybrid generations of maize.

Figure 1

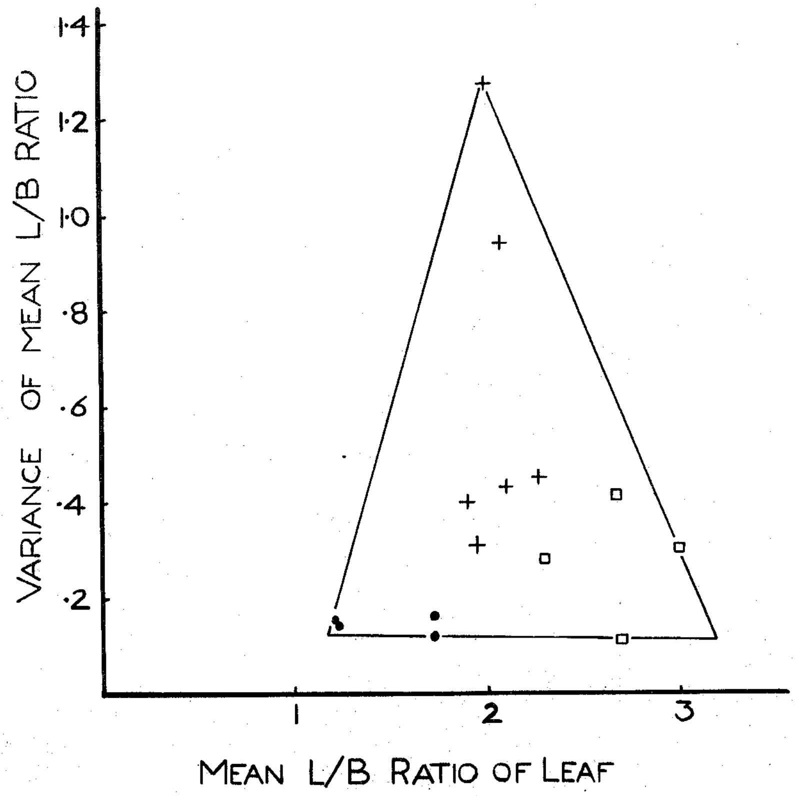


Figure 1 : The relationship between the means and variances of the length-breadth ratio for the leaves of families raised from single trees of two species of *Eucalyptus* and some putative hybrids between them.

● *E. elaeophora* ☐ *E. goniocalyx* + hybrids

Families made up of different proportions of the parental species and hybrid plants have means and variances which fall within the triangle as defined by the means and variances of the parental species and the F2 generation.

Often it is found that the variance increases with the mean and to eliminate this effect it is sometimes necessary to compare the variabilities of different families in terms of the coefficient of variation. Normally this does not materially affect the result but such a measure of variability is theoretically disadvantageous in that the triangle it produces has concave and not straight sides.

The triangle technique for analyzing the variability of a series of families may sometimes be adopted for use in the study of data from natural populations. Consider a series of samples from pure colonies of a pair of species and from colonies where introgression is suspected. The mean size of some measured character of a colony of the pure species is likely to be less variable than the mean size of the character in a hybrid or introgressed colony. By plotting the mean size against its variance for each of several colonies the scatter diagram obtained may be found to fall within a triangular outline. This is well illustrated by the size and variability of tail lengths within different colonies of Red-Eyed Towhees, belonging to two species of birds which appear to hybridize in Mexico (Sibley 1954). These data are shown in Figure 2 which has been prepared from tables quoted by Dr. Sibley.

I have not been able to find any published data for wild plant populations which can be used to illustrate this technique.

Figure 2

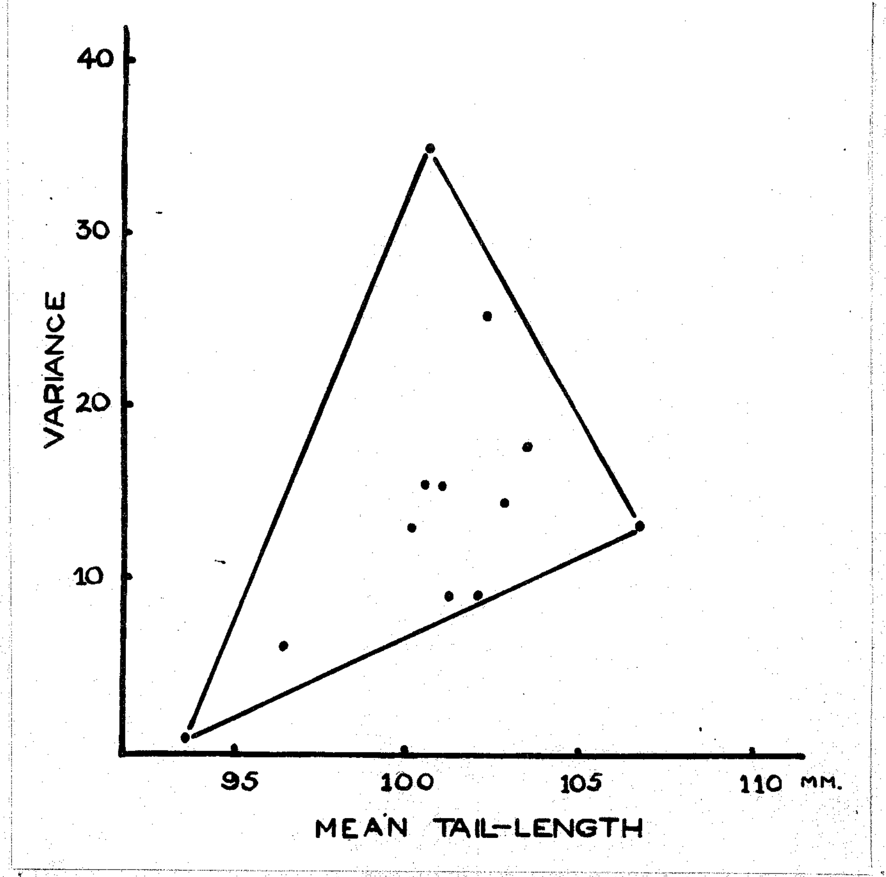


Figure 2 : The relationship between the mean and variance for the tail lengths of the birds in some populations of Red-Eyed Towhees.

3. RECOGNITION OF INTROGRESSIVE HYBRIDIZATION.

With interspecific hybrids that are fertile there is always the possibility of gene exchange occurring between the parental species. In the absence of 'Hybrid Swarms' introgression may not be spectacular, but may nevertheless cause the species to become modified in the region of hybridization. The study of species in a region where their ranges or habitats overlap often suggests that introgression has occurred.

Where the possible hybridizing species are known suitable contrasting characters can be selected for study. Should the species show signs of discordant variation in those areas where they grow intermixed this is good evidence that introgression is taking place.

Where the species differ principally with respect to quantitative characters, resort to biometrical methods is sometimes necessary if it is desired to know whether or not gene flow is occurring between them.

For such a study characters not markedly influenced by the environment must be chosen, or failing this the influence of the environment must be minimized. Some techniques for doing this will now be discussed.

(a) Minimizing Environmental Influences.

Ratios rather than absolute measurements are often employed in an endeavour to minimize the effect of the environment when comparing plant populations. Unless the behaviour of the ratio is known for the pure species grown in several environments this is not a safe procedure. Furthermore, as has been said by Davey and Lang (1939, p. 61) "it seems unsafe to assume that a ratio will be unaffected by growth without some knowledge of these interactions." - where by "these interactions" they mean growth effects.

An alternative means of minimizing environmental influences has been proposed by the writer (Clifford 1955) for use with pairs of correlated measurements. When the measurements for a pair of species are related as in Figure 3, effective elimination of the influence of absolute size may be achieved. Briefly a new set of axes is drawn such that one of them is parallel to the long axes of the scatter diagrams. The other is drawn at right angles to this and is calibrated in an arbitrary scale. Whereas neither measurement alone will discriminate entirely between the two populations, they are completely separated if described in terms of the new scale.

The index values obtained in this way might be conveniently described as 'correlation indices' for their variability depends upon the degree of correlation between the original measurements. With highly correlated measurements the variability of the correlation indices will be less than if the measurements were not highly correlated.

Figure 3

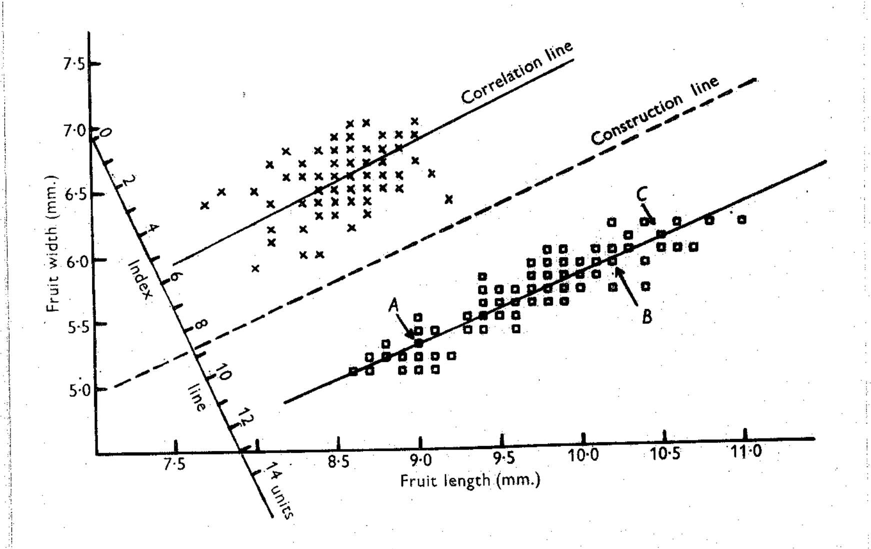


Figure 3 : The relationship between the correlation lines, (lines of " best fit ") and the index scale with reference to the scatter diagrams of two varieties of Camelina, after Clifford (1955) .

The relative degree of separation, for a pair of populations, likely to be achieved by the use of correlation indices and ratios can be illustrated with reference to some data for the genus *Eucalyptus*. A comparison of fruit weights and lengths for *E. elaeophora* and *E. goniocalyx* indicates that although the species differ considerably with respect to these characters neither is in itself satisfactory for distinguishing between the species, Figures 4a and 4b. For many specimens neither the weight nor length of the fruit would indicate to which species it belonged.

The combining of the two measurements into a ratio very much improves the separation of the species, Fig. 4c, but there is still a wide range of values for which the ratio would be of no use for identifying a further specimen. This range of overlap may be further reduced by describing the species in terms of their correlation indices, Fig. 4d.

For this as well as other data it has been found that the correlation indices are often a better means of discriminating between populations than are ratios.

Where the size of the character chosen for study is little influenced by the environment there are several methods available for comparing closely related populations.

These methods will now be discussed under three headings according to the number of measurements involved.

Figure 4

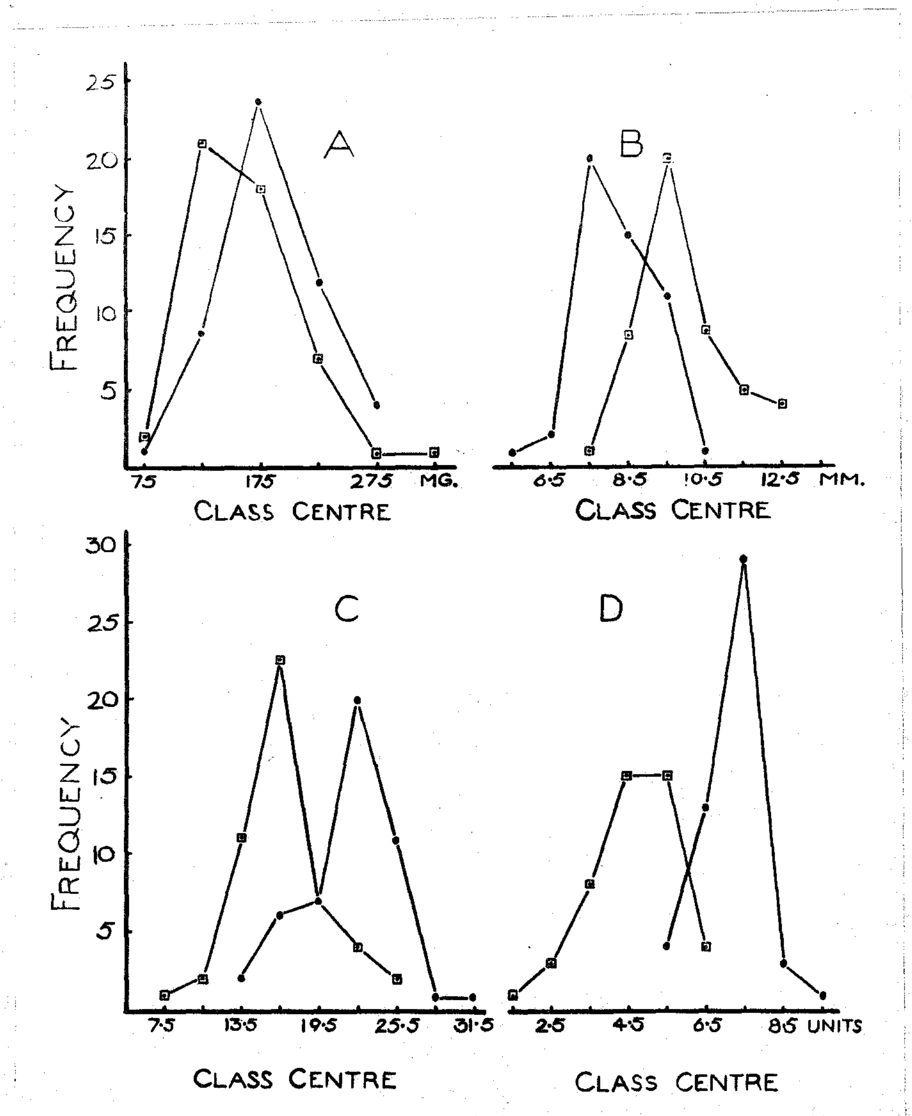


Figure 4 : A comparison of the effectiveness of fruit weight and length , their ratio and the correlation index derived from them , for distinguishing between Eucalyptus elaeophora and E.goniocalyx.

A.Fruit weight B. Fruit length
C. Ratio F.W./F.L. C. Correlation Index

E.elaeophora E.goniocalyx

(b) Single measurements
(c) Pairs of measurements
(d) Several measurements

(b) Single measurements.

With single measurements it is often useful to compare populations with respect to their means, variances and 'skewness.' For various reasons to be given later J. inflexus appears to suffer slight introgression from J. effusus in those areas where the two species grow in mixed stands. The length of the longest perianth segment in J. inflexus does not seem to be much influenced by the environment and so may be used to discuss the application of the statistics mentioned above.

In colonies of J. inflexus which are growing apart from J. effusus, the perianth length of the plants is almost normally distributed, Figure 5, and is not particularly variable. Where J. inflexus is growing intermixed with J. effusus the perianth lengths are more variable, their average size is smaller, and there is a slight negative skewness to the distribution.

These results might have been anticipated from a knowledge of perianth lengths in pure stands of J. inflexus and J. effusus. Introgression of J. inflexus by J. effusus ought to lead to an increased variability of J. inflexus because the two species have different

Figure 5

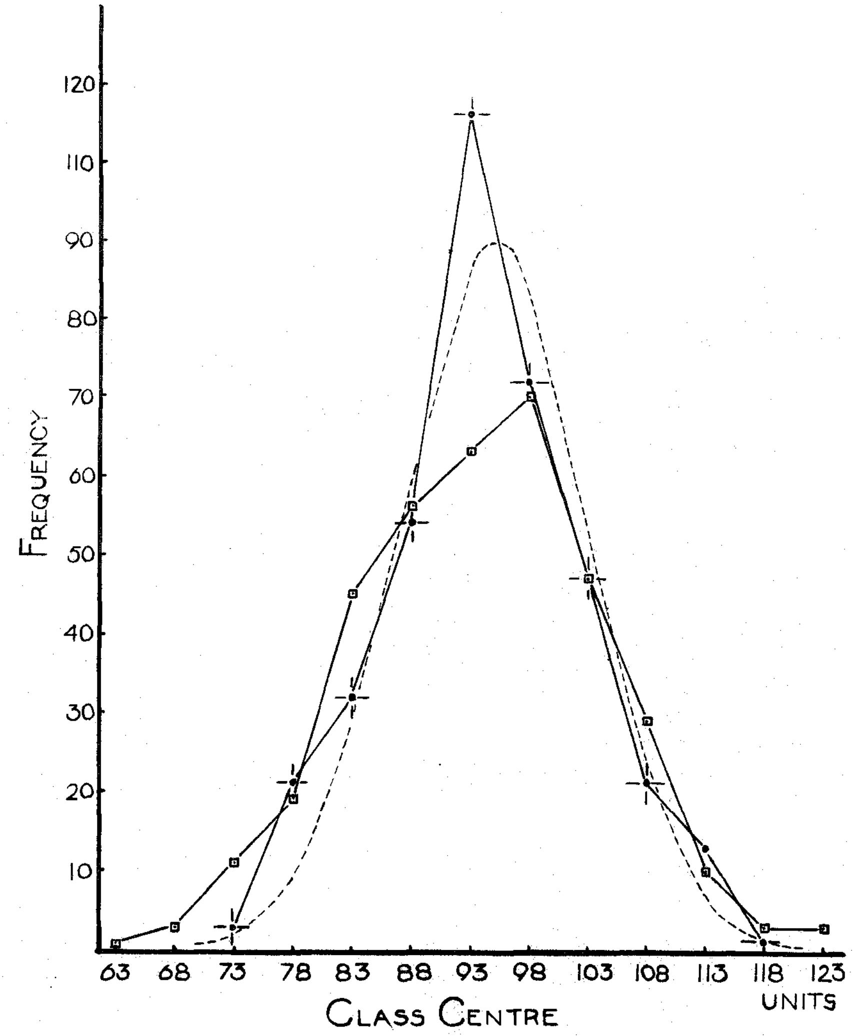


Figure 5 : Frequency polygons for the perianth lengths of J.inflexus growing together with and apart from J.effusus (meadow form) . Each sample contained 360 specimens . The scale is such that 25 units equal 1 mm.

┼──┼ J.inflexus growing apart from J.effusus

□──□ J.inflexus growing together with J.effusus

- - - - Normal distribution curve fitted to the data for J.inflexus growing apart from J.effusus perianth lengths and these appear to be inherited multifactorially. In these circumstances the introgressed plants of *J. inflexus* might be expected to be segregating for factors affecting perianth length; thus variability might be produced which was too slight to detect except biometrically. As *J. effusus* has a smaller perianth than *J. inflexus* introgressed colonies of the latter would be expected to possess on the average shorter perianths than those for the pure species.

Finally if the introgression was only slight, skewness in the direction of the introgressing species might be anticipated. Since the perianth of *J. effusus* is shorter than that of *J. inflexus* a negative skewness would be expected; this has occurred.

As each statistic has behaved as predicted these tests reinforce each other. Unfortunately their use is severely restricted by the condition that the character under study must not be markedly affected by the environment.

(c) Pairs of measurements.

More exacting tests for introgression can be devised using two measurements for each plant. These resolve themselves into a study of the covariances as well as the variances of the measurements. The two general techniques are

(i) Lines of best fit through the data.

(ii) The product-moment correlation coefficient.

(i) Lines of best fit through the data.

This technique may be illustrated by the data of Russell (1954) for leaf measurements made upon a population of white violets. A mass collection of these was made in the field and brought back to the laboratory for study. Here the collection was separated into three categories, *Viola lanceolata*, *V. pallens* and an assumed hybrid group. The length and breadth of the leaf laminas of these groups was measured and the data are presented as scatter diagrams in Figure 6.

From the Figure it is evident that the direction of the line of best fit through each of these sub-populations is different. Furthermore for the presumed hybrid group the slope of the line is intermediate between the slopes for the putative parents.

Such intermediacy of slope for a hybrid population would be expected if the pair of characters making up the scatter diagram were multifactorially inherited. Where the amount of gene flow between the species is only slight, the scatter diagram for an introgressed population will differ only slightly from that for the pure species; its displacement will be in the direction of the introgressing species.

To determine accurately the direction of the line of best fit the slope must be calculated. The

Figure 6

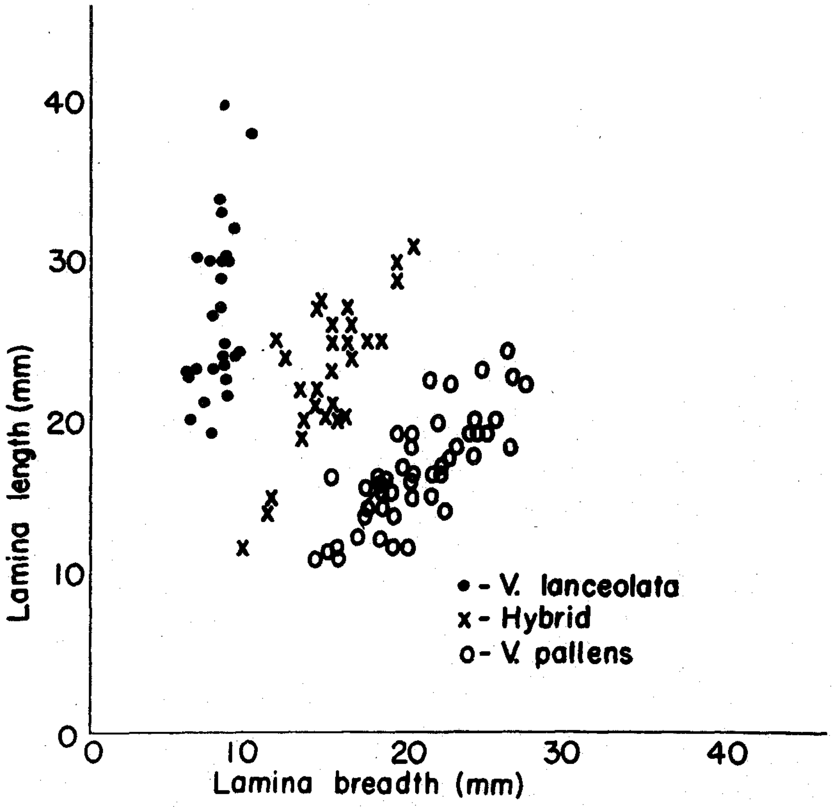


Figure 6 : Scatter diagrams of the lamina length and breadth for two species of Viola and a putative hybrid population between them , (from Russell 1954) .

necessary formulae are

$$(Y - \bar{y}) = (X - \bar{x}) \tan \alpha$$

$$\text{where } \tan 2\alpha = \frac{2\left(\sum_1^n xy - \frac{\sum_1^n x \sum_1^n y}{n}\right)}{\left(\sum_1^n x^2 - \frac{\left(\sum_1^n x\right)^2}{n}\right) - \left(\sum_1^n y^2 - \frac{\left(\sum_1^n y\right)^2}{n}\right)}$$

and x and y are the pair of correlated characters.

This line is one such that the sum of the squares of the perpendicular distances of the sample co-ordinates from the line is a minimum and it thus differs considerably from a normal regression line.

Unfortunately I have been unable to find suitable data to test the merit of this method for detecting or confirming introgression. The data quoted for Viola are not really suitable in that the mass collection was subjectively divided into three groups, had it been possible to do this objectively the method would have been applicable.

(ii) The Product-moment Correlation Coefficient.

The use of this, the commonest means of measuring correlation as an indicator of introgression, may also be illustrated by reference to Figure 6. If it is assumed that in a colony of Viola pallens there are a few plants that have suffered slight introgression from *V. lanceolata* it is improbable that they would be conspicuous.

Nevertheless a biometrical study of the colony containing such introgressed plants would when plotted as a scatter diagram reveal the existence of a few individuals situated between *V. pallens* and the hybrid plants in Fig. 6. The extension of the scatter diagram in this direction would indicate a reduction in the degree of correlation between lamina length and breadth in the introgressed population.

The application of this method may be conveniently illustrated with reference to data collected for the genus *Primula*. If it is assumed that colonies of Cowslips and Primroses growing intermixed represent introgressed colonies and that the same pair of species when growing apart represent pure colonies, the data of Table 5 well illustrate the decrease of correlation between suitable pairs of measurements in introgressed colonies.

TABLE 5

Colony Type	n	Primrose	n	Cowslip
Intermixed	12	.327	9	.380
Separate	12	.336	8	.414

Table 5: The weighted average correlation coefficients for length of calyx tooth and circumference of calyx for several colonies of cowslips and primroses growing intermixed and separately; pin flowers only. For further details of Table see p.

When growing in mixed communities both Cowslip and Primrose show a decrease in the degree of correlation between the length of the calyx teeth and the circumference of the calyx, though more samples would be needed to determine if the difference were statistically significant. In the Primrose the calyx has long teeth and a narrow diameter whereas in the Cowslip the teeth are short and the calyx is broad.

It is unfortunate that the use of the correlation coefficient must be restricted to such pairs of contrasting measurements. Furthermore it must be used cautiously for sometimes the degree of correlation between quantitative characters may be greater in the hybrid colonies than in the pure colonies of the parental species. This was found to be so in the study of a hybrid swarm in the genus Eucalyptus (Clifford and Binet l.c.) Here it was discovered that both fruit weight and peduncle length and fruit weight and fruit length were more strongly correlated in the hybrid swarm than in the parental species. These data are shown in Table 6.

TABLE 6

Population	Fruit weight Peduncle length	Fruit weight Fruit length
E. elaeophora	.739	.287
Hybrid Swarm	.797	.494
E. goniocalyx	.684	.427

TABLE 6: The degree of correlation between two pairs of characters for a hybrid swarm and two parental species, in the genus Eucalyptus.

The reason for the increase in magnitude of the correlation coefficient in the hybrid swarm is puzzling but may be related to its peculiar origin. It is thought to be growing in a situation where selection is favouring the F1 - like hybrids at the expense of the other segregates and even the parental species.

(d) Several measurements.

To demonstrate small differences between populations it is often necessary to employ several measurements. The combining of these into a single value raises several difficult problems. As groups of measurements made upon most biological material are correlated allowances must be made for the correlation. It is often found that an organ large with respect to one measurement is also large with respect to a second. Because of this the arbitrary addition or subtraction of measurements may be regarded as of little value.

Perhaps the most satisfactory way of combining several measurements is to employ the methods of multivariate analysis, and in particular discriminant analysis. Though this technique was devised especially for dealing with taxonomic problems (Fisher 1936) and was first illustrated with data for the genus Iris it does not appear to have found favour with botanists until recently. Later, when discussing the genus Juncus a discriminant analysis is undertaken and in Appendix A the method has been employed in the study of a hybrid swarm in the genus *Eucalyptus*.

In a recent paper intended to demonstrate the advantages of discriminant analysis Whitehead (1954) illustrated the method with reference to the genus *Cerastium*. A more detailed account of the methods of multivariate analysis is given by Rao (1952).

An ingenious method of combining any number of measurements was proposed by Anderson and Whitaker (1934) when studying the genus *Uvularia*. The method has been discussed more recently by Melville (1951) who suggested that the value it yielded might be called a 'Pythagorean Index.'

As the name suggests the method is based upon the properties of a right angled triangle. For a pair of measurements the index is derived by regarding them as the sides of a triangle enclosing the right angle and accepting the Pythagorean Index as the hypotenuse of the triangle. The method as applied to several measurements is further illustrated by Figure 7 which comes from Melville's paper.

Though the Pythagorean Index is fully objective it is of little practical use. No effort is made in the method to allow for correlations between the measurements, and furthermore in combining a series of them in this way differences between groups are sometimes disguised rather than accentuated.

Figure 7

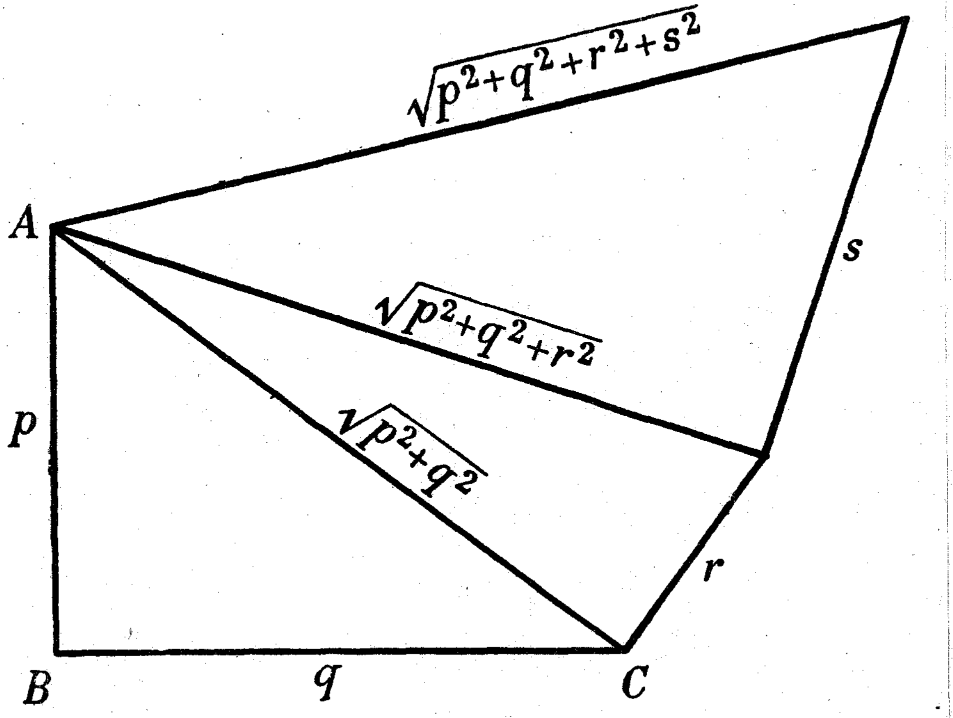


Figure 7 : The method for constructing the Pythagorean Index. The succesive indices combine p+q , p+q+r and p+q+r+s into a single figure equivalent to the square root of the sum of their squares .

This may be conveniently shown by data due to Tedin (1925) for the genus Camelina. In Table 7 the mean measurement of three capsule characters are quoted and also the Pythagorean Index derived from them. The Index is little better than the single measurements as a means of discriminating between the two varieties.

TABLE 7

Character	Var. C1	Var C2
Capsule length	9.7 mm.	8.6 mm.
Capsule width	5.7 mm.	6.6 mm.
Capsule breadth	3.7 mm.	5.4 mm.
Pythagorean Index	11.8	12.0

TABLE 7: A comparison of two varieties of Camelina microcarpa (L) Crantz, in terms of three capsule measurements and the Pythagorean Index derived from them.

4. DISCUSSION.

The principal biometrical techniques available for comparing populations have been discussed and several of them will be employed below for the study of introgression in the genera Primula and Juncus.

Before leaving this subject it is necessary to discuss some of the limitations to the statistical analysis of field data. The chief of these is that plants are usually highly plastic and may be considerably modified by the environment. In a biometric sense any species may be regarded as a series of populations each corresponding with a different micro-habitat. Sampling of only certain of these habitats may give a biased picture of the species. If the habitats are not themselves normally distributed in the mathematical sense, a random sample of them will not be normal. Plants collected from such a group of habitats may show an apparently non-normal distribution for any measured character that is influenced by the environment.

In any study every attempt must be made to eliminate the influence of the environment on the plant. Unless this is possible, and the data are available in great quantity great care must be exercised in the analysis and interpretation of measurements taken from field samples

Finally it would seem appropriate to comment upon the statistical significance of any differences observed between populations. For most of the techniques described it is possible to test the significance of the differences observed and the methods for doing so are described in most standard texts and in particular that of Rao (l.c.) It is frequently found that the difference observed is not significant at the probability level desired. When this is so increase of the sample size may raise the difference to the level of significance. Where the labour to do this may be prohibitive it may sometimes be more convenient to compare the populations by several of the methods described. If for most comparisons the differences between the populations are in the senses predicted this strongly suggests their reality, though no single difference may appear to be significant.

AN INVESTIGATION OF POSSIBLE INTROGRESSION BETWEEN *JUNCUS EFFUSUS* L. AND *JUNCUS INFLEXUS* L.

1. INTRODUCTION.

In the vicinity of Durham there are several large swamps and areas of moorland where members of the genus *Juncus* grow in abundance. Rarely do they grow in separate stands, more often than not several species grow intermixed. These mixed populations seemed particularly suitable for the study of introgression, especially as hybrids between certain of the species had been recorded. Furthermore material could be collected in the winter as the fruits and inflorescences of the plants remained undamaged until as late as January, a consideration of importance to a visiting botanist arriving in early October.

Attention was focussed on the Genuini section of the genus and in particular upon *J. inflexus* and *J. effusus*, for the hybrid between these has been stated to be relatively common (Salmon, 1931). The only other member of this section of the genus encountered was *J. conglomeratus*. The principal differences between the species are listed in Table 8.

Whether hybridization between *J. effusus* and *J. inflexus* was leading to introgression could be determined only by a series of field studies and so field populations were sampled and brought into the laboratory for analysis.

TABLE 8

Character		Species		
		J. inflexus	*J. effusus*.	*J. conglomeratus*
Pith		Chambered	Continuous	Continuous
Stem	(a) sculpture	ribbed	not ribbed	ribbed
	(b) surface	smooth	smooth	rough
	(c) colour	bluish-green	green to yellow-green	yellow-green to grey-green
	(d) appearance	not glossy	glossy	not glossy
Sheathing leaves	(a)	glossy	not glossy	not glossy
	(b)	brown to black	red-brown to pale brown	pale brown
Spathe base		not inflated	not inflated	inflated
Infloresceneee		lax	lax	congested

Table 8: The principal morphological characters of *J. inflexus*, *J. effusus* and *J. conglomeratus*. Floral and fruit characters have been omitted as they were seldom employed for identifying species.

The first step of the analysis was to measure, for each specimen, the length of the longest perianth segment, the spathe and the inflorescence. A survey of the field and herbarium specimens showed that the two species differed quantitatively in these characters. The lengths of the stem and of the longest sheathing leaf were also observed to differ but these characters were neglected, partly because thay were not always available for the herbarium specimens, and partly because suitable material was difficult to collect during the winter. The number of ribs on the stem, although a recognized criterion for separating J. effusus from J. inflexus, was not recorded. It was regarded as unsatisfactory for this study because the number of ribs is proportional to the diameter of the stem and so decreases along its length. Furthermore it is a discontinuous variable which would not be well suited to the discriminant analysis that was envisaged to be ultimately necessary.

Early in the investigation it became evident that although J. inflexus was apparently a simple species in the area studied J. effusus appeared to include two forms that were apparently ecotypes. Accordingly, a short account of the taxonomy of J. effusus will be given next, in order to clear the way for a discussion of hybridization.

2. TAXONOMY.

(a) Ecotypes of *J. effusus*.

As has been stated, the recognition and description of *J. inflexus* was quite straightforward, but the same could not be said for *J. effusus*. As suggested by Richards and Clapham (1941) this species appears to posses several varieties or ecotypes, though these do not appear to be adequately described in any of the floras consulted.

From field studies mostly conducted in the north of England it has become evident that in the areas investigated *J. effusus* consists of at least two taxa. They occupy different habitats, appear to flower at different times and differ in respect to several quantitative characters.

One of them occurs principally in woods or recently cleared woodlands and the other usually occurs on the edges of swamps or on meadows, moorlands and other non-shaded habitats. Their principal morphological differences are shown in Fig. 8; it can be seen that the woodland from has a laxer inflorescence and smaller flowers than what might conveniently be called the 'meadow' form. The extra laxness of the inflorescence of the woodland form is due to the pedicels of the ultimate flowers of the inflorescence being longer than those of the meadow form (Fig. 8).

Figure 8

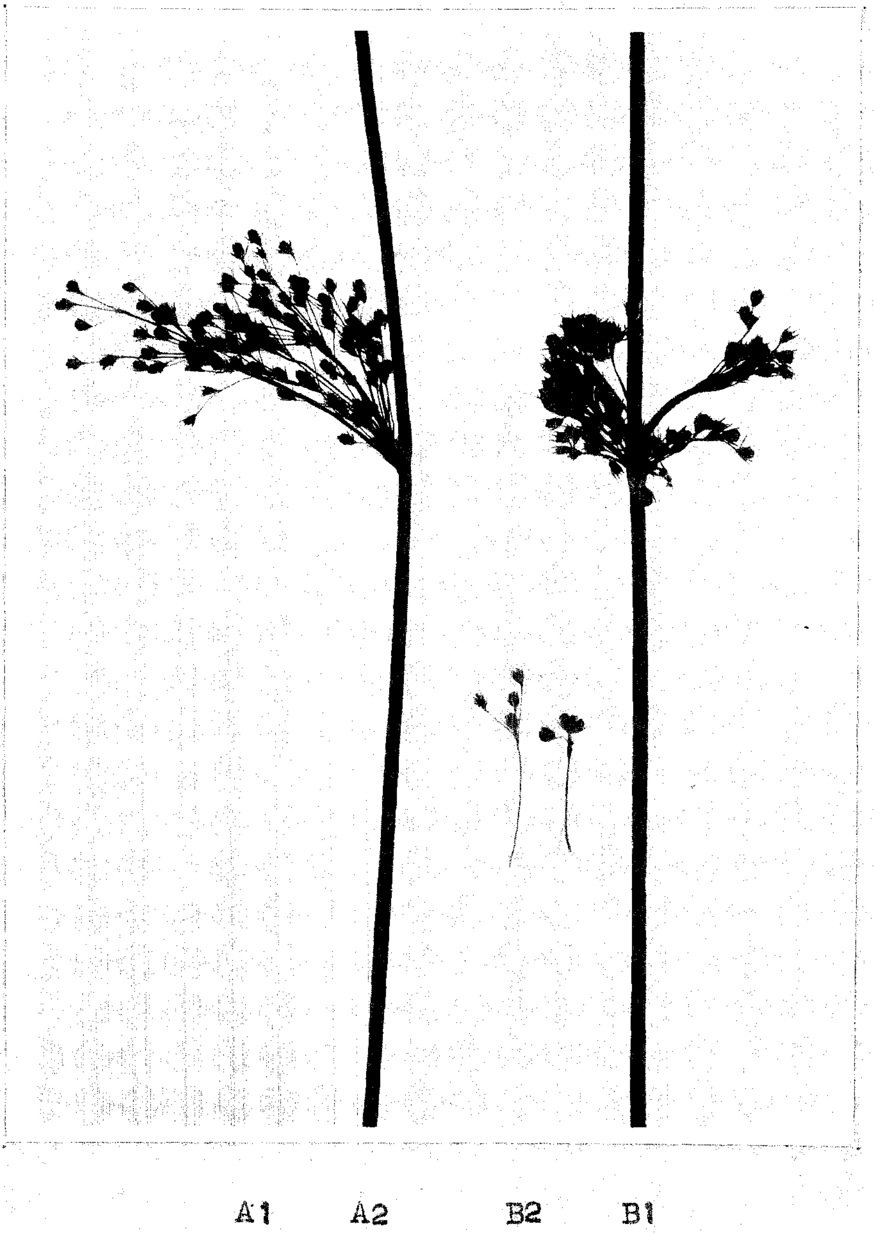


Figure 8 : A comparison of the inflorescences of the meadow and woodland forms of J.effusus growing near Durham, natural size.

A1 and A2	Woodland form
B1 and B2	Meadow form

From their very brief description it would seem that the woodland form is what Richards and Clapham (l.c.) describe as an effuse form growing in the woods, fens and swamps of southern England; they apparently regard the meadow form as typical of the species. They describe a third taxon within *J. effusus*, the var. *congestus* Lej. et Court. but this has not been encountered in the present study.

The absence of definite qualitative differences between the meadow and woodland forms of *J. effusus* makes it difficult to describe them objectively for there is a constant danger of choosing only those plants which stress their differences. Furthermore the two forms intergrade in some localities, so that some plants cannot be placed in either form with certainty. Nevertheless an attempt has been made to describe them quantitatively by comparing some woodland populations near Durham with several meadow populations.

The results are shown in Table 9, from which it can be seen that the two forms differ considerably for the characters measured, and especially in the length of the longest perianth segment.

The woodland populations sampled were chosen because they appeared to contain no plants of the meadow form. With the meadow populations no effort was made to select only the meadow form and a few woodland forms may have been included in the samples. The absolute difference between the two forms is therefore greater than that shown in Table 9, which may be taken to indicate the way in which the forms differ, rather than the magnitude of their difference.

TABLE 9

Habitat.	n	Perianth	Inflorescence	Spathe
Meadow	360	73 units	18 mms.	18 cms.
Woodland	40	62 units	30 mms.	18 cms.

Table 9: The mean inflorescence, perianth and spathe lengths for J. effusus collected from two different habitats. (25 units = 1 mm.)

(b). The relationships of J. diffusus Hoppe

The putative hybrid between J. effusus and and J. inflexus is often regarded as an equivalent to the plant which Hoppe (1819) described as J. diffusus. This plant came from a meadow where it was growing intermixed with both J. effusus and J. inflexus. It was described as a very beautiful plant and worthy of the epithet floribundus, and its name was selected to indicate its affinities with J. effusus with which it agreed in possessing a continuous pith. This is a point of considerable interest as will be seen when considering this character as represented on specimens labelled J. diffusus in two of the herbaria studied.

The plant generally recognized as *J. diffusus* is described in the latest British Flora (Clapham, Tutin and Warburg, 1952) as intermediate between *J. inflexus* and *J. effusus*. As two forms of *J. effusus* appear to exist it is clearly necessary in such a description to define which form of *J. effusus* is meant, and this has apparently not been done. With a view to investigating this problem a comparison of the herbarium material of *J. inflexus*, *J. diffusus* and *J. effusus* was undertaken

Specimens from the herbaria of the Universities of Cambridge and Oxford, the British Museum and Edinburgh have been examined. Where possible the length of the longest perianth segment, spathe and inflorescence were measured. No attempt was made to identify or correct any determination, every specimen being accepted as it was named on the sheet. These results are shown in Table 10, and the pooled data are presented graphically in Figs 9, 10, and 11.

Several of the results call for further comment, particularly those for *J. effusus* and *J. diffusus*. The herbarium collectors have not distinguished the two forms of *J. effusus*. Indeed the collections show little evidence for the existence of two forms. Except for the Edinburgh collection, which contained a fair proportion of both forms, the herbarium material consisted mainly of the woodland form.

Table 10

Species		Character Measured								
		Perianth			Inflorescence			Spathe		
		n	m	v	n	m	v	n	m	v
J.inflexus	O.	24	89·5	96·4	24	52·4	552·4	19	18·1	79·1
	C.	19	88·3	82·0	19	47·2	432·9	18	12·6	28·0
	E.	16	81·3	43·1	16	52·6	289·0	17	15·8	48·1
	B.M.	21	85·5	34·1	21	47·1	460·2	20	15·8	23·5
J.diffusus	O.	22	73·9	47·5	23	47·0	233·5	20	15·0	32·8
	C.	21	71·9	139·5	21	39·4	236·8	14	16·2	62·4
	E.	9	71·1	208·6	9	40·2	674·3	9	17·4	10·7
	B.M.	11	75·3	129·8	11	35·5	178·8	9	14·3	14·9
J.effusus	O.	22	58·3	44·0	22	34·5	285·9	13	20·1	59·6
	C.	26	55·0	66·6	26	29·8	339·4	19	19·7	56·9
	E.	11	60·2	87·4	11	20·6	171·1	11	17·5	58·7
	B.M.	21	61·1	107·2	21	34·1	401·2	18	18·5	36·2

Table 10: A comparison of the mean (m) and variance (v) for three characters of *J.inflexus*, *J.diffusus* and *J.effusus*. The measurements were made upon material from the herbaria of The Oxford (O.) and Cambridge (C.) Universities, the British Museum (B.M.) and Edinburgh (E.). The number of plants used to determine each mean and variance is also shown (n).

Figure 9

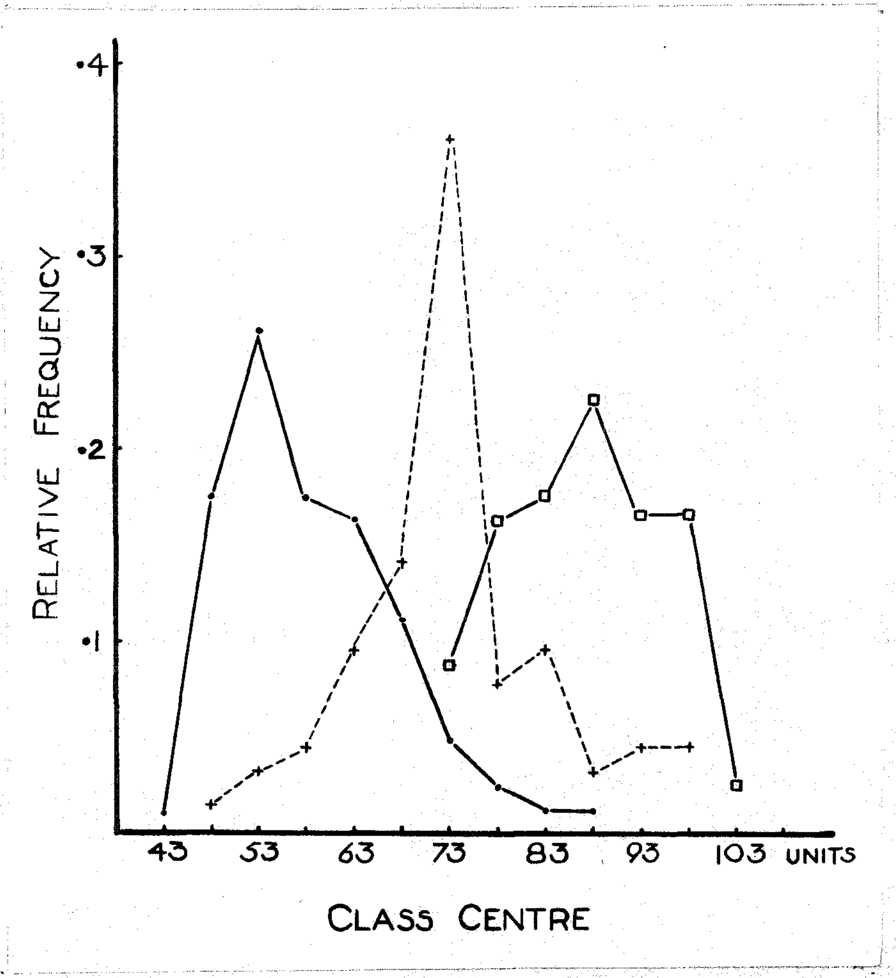


Figure 9 : Frequency polygons for the perianth lengths of J.effusus , J.inflexus and J.diffusus (or J.effusus x J.inflexus) as measured upon herbarium material. The scale units are such that 25 equal 1 mm.

□ ——— □ J.inflexus

+ - - - - + J.diffusus or J.effusus x J.inflexus

● ——— ● J.effusus

Figure 10

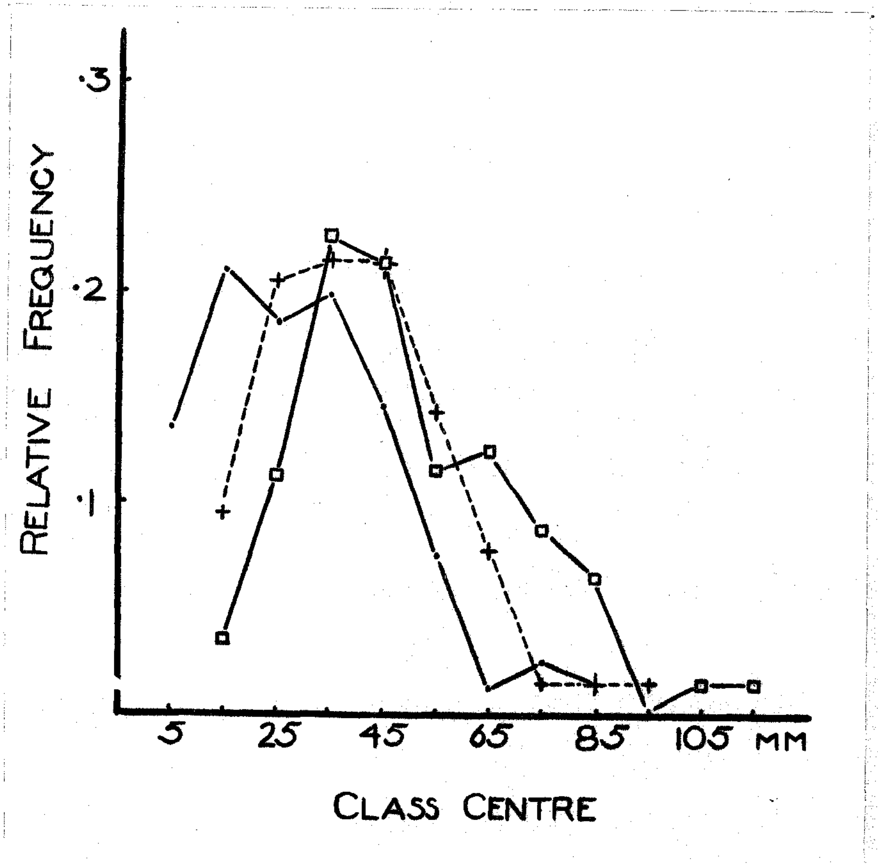


Figure 10 : Frequency polygons for the inflorescence lengths of J.effusus , J.inflexus and J.diffusus (or J.effusus x J.inflexus) as measured upon herbarium material.

□——□ J.inflexus

+-----+ J.diffusus or J.effusus x J.inflexus

●——● J.effusus

Figure 11

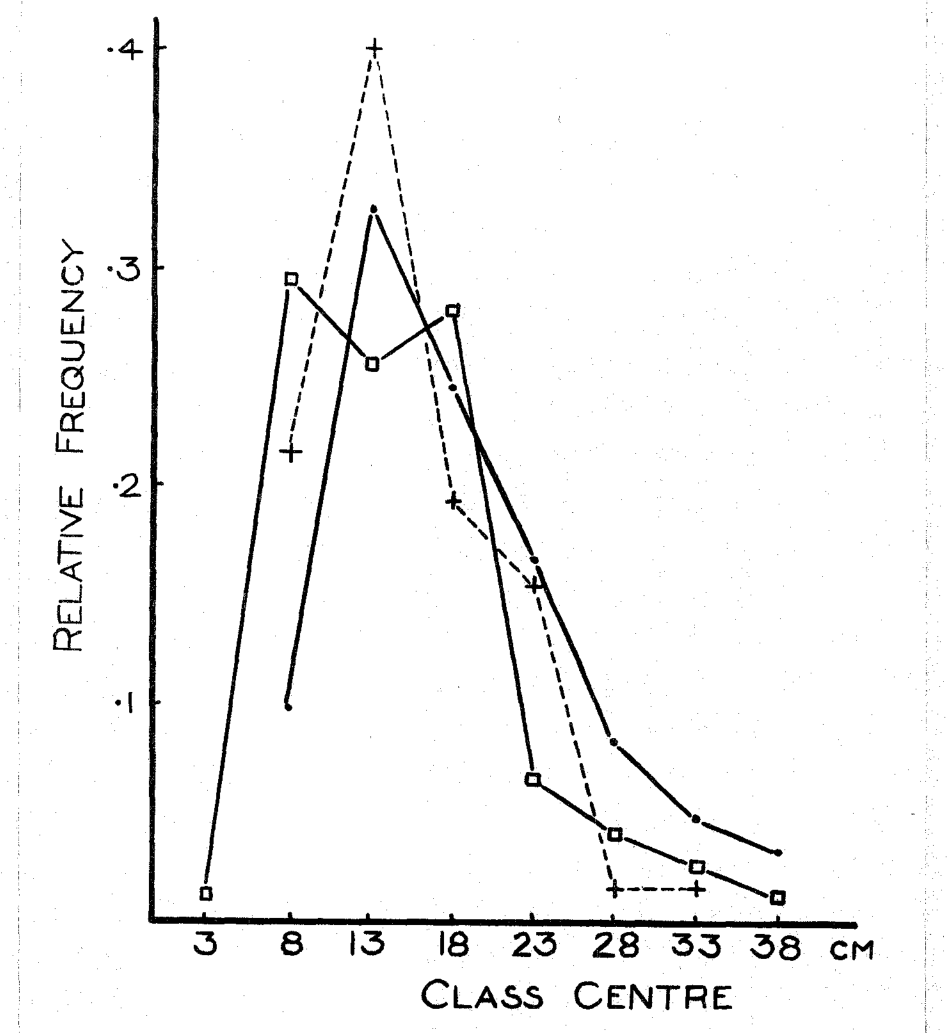


Figure 11 : Frequency polygons for the spathe lengths of J.effusus , J.inflexus and J.diffusus (or J.effusus x J.inflexus) as measured upon herbarium material.

□———□ J.inflexus

+- - - - -+ J.diffusus or J.effusus x J.inflexus

●———● J.effusus

Why the three southern herbaria possess mostly the woodland form is difficult to decide. That the meadow form is fairly common in the south of England is quite definite. A comparison of J. effusus from a meadow habitat in Surrey with that from similar habitats in Durham and Northumberland shows that there is little difference between the plants collected in the two areas, Table 11.

TABLE 11.

Counties	n	Perianth	Inflorescence	Spathe
Northern	300	72 units	17 mms.	18 cms.
Southern	30	69 "	16 "	15 "

Table 11: A comparison of the meadow form of J. effusus from two northern counties and a southern county of England.

Whatever the reason there is little doubt that the herbarium material of J. effusus consists mainly of the woodland forms. Considering Table 10 again, it can be seen that J. diffusus is indeed intermediate between J. inflexus and J. effusus, which in the herbarium sense means the woodland form of J. effusus. The striking similarity of much of the material labelled J. diffusus and the meadow form of J. effusus is evident from Tables 9 and 10.

It is doubtful whether the material labelled J. diffusus represents a simple Fl hybrid; indeed it is difficult sometimes to decide what it represents. Many of the specimens appear to be aberrant forms of either J. effusus (meadow form) or J. inflexus. Several of them seem to be what might be expected of backcross hybrids to these species. As previously mentioned the type plant of J. diffusus is described as having a continuous pith. An examination of the herbarium material shows that continuity of pith is not a characteristic of all the material labelled J. diffusus. Thus the specimens from Oxford and Cambridge may be divided into two groups, according to whether the pith is chambered or continuous.

A comparison of these two groups suggests that the plants identified as J. diffusus have not been drawn from a homogeneous population, (Table 12). The two groups differ especially with respect to spathe and

TABLE 12

Pith	Perianth	Inflorescence	Spathe
continuous	71 units	42 mms.	17 cms.
Chambered	81 "	43 "	11 "

Table 12: A comparison of plants labelled J. diffusus but which possess either a continuous or chambered pith.

perianth lengths, though none of the differences are significant at the 5% level. Those plants possessing a continuous pith closely resemble the meadow form of *J. effusus* except for possessing longer inflorescences, (cf. Table 9). The plants with a chambered pith, a character typical of *J. inflexus*, very much resemble that species except for possessing spathes and perianths slightly smaller than usual (c.f. Table 10).

It would seem that though *J. diffusus* is defined as intermediate between *J. inflexus* and *J. effusus* it is probably a mixture of plants with different histories. Possibly several of the plants labelled *J. diffusus* are introgressed forms of *J. effusus* or *J. inflexus*. Something like this may have been what J.H. Little had in mind when he wrote in a letter, preserved at the British Museum, that he had some plants which tended towards *J. effusus* but which were not that species. He had, it seems, begun a paper on this subject but then fell ill and it was apparently never finished.

Whatever the nature of the plants labelled *J. diffusus* it is evident that they include some plants intermediate between *J. inflexus* and *J. effusus*, even when both forms of *J. effusus* are recognized. However, many of these intermediates between *J. inflexus* and the woodland form of *J. effusus* are only distinguishable with difficulty if at all from the meadow form of *J. effusus*.

Such a series of intermediate forms suggests that some hybridization may be occurring between the species and field evidence can be utilised to test this view.

3. FIELD STUDIES.

(a) Evidence for Hybridization.

The examination of many stands of the meadow form of *J. effusus* and *J. inflexus* growing both separately and intermixed failed to reveal any plants which appeared to be approximately intermediate between them. Nevertheless there were signs that hybridization between them might be occurring.

Several plants strongly resembling the meadow form of *J. effusus* but possessing inflorescences longer than usual for that species were observed to be partially sterile. They failed to set seed and had anthers which were distorted and contained only small amounts of viable pollen, as determined by staining in acetocarmine. The failure of plants resembling the meadow form of *J. effusus* to produce capsules was particularly striking as the meadow form commonly produced abundant fruits. Such infertile plants were rare but a few have been collected from Butterby Marsh near Durham and were observed in cultivation for two seasons. Their behaviour was similar in both seasons though other plants of the meadow form of *J. effusus* growing with them set abundant capsules.

At Waldridge Fell near Chester-le-Street some sterile plants resembling *J. inflexus* were observed. They set no capsules and produced only small amounts of apparently viable pollen. In this instance failure to set capsules was not remarkable as J. inflexus does so only rarely in the open in Durham County, though it does so readily if grown in a greenhouse. The occurrence of distorted anthers and an almost continuous pith (Fig. 12) in a species normally possessing chambered pith suggested a hybrid origin for these plants, and the presence of the meadow form of J. effusus in the same swamp led to its being suspected of being responsible for the hybridization, especially as this species has a continuous pith. Plants from the presumed hybrid were then compared with plants from colonies of the meadow form of J. effusus and J. inflexus growing separately. These further confirmed the belief that the sterile plants resembling J. inflexus had been derived from hybridization from these two species. Not only did the presumed hybrids look intermediate in general stem morphology, (Fig. 12) but they were also somewhat intermediate with

TABLE 13

Species	n	Perianth	Inflorescence	Spathe
J. inflexus	360	95 units	50 mms.	17 cms.
Presumed hybrid	30	78 units	39 mms.	25 cms.
J. effusus	360	73 units	18 mms.	18 cms.

Table 13: A comparison of a presumed hybrid population of J. inflexus with colonies of J. inflexus and J. effusus growing separately.

Figure 12

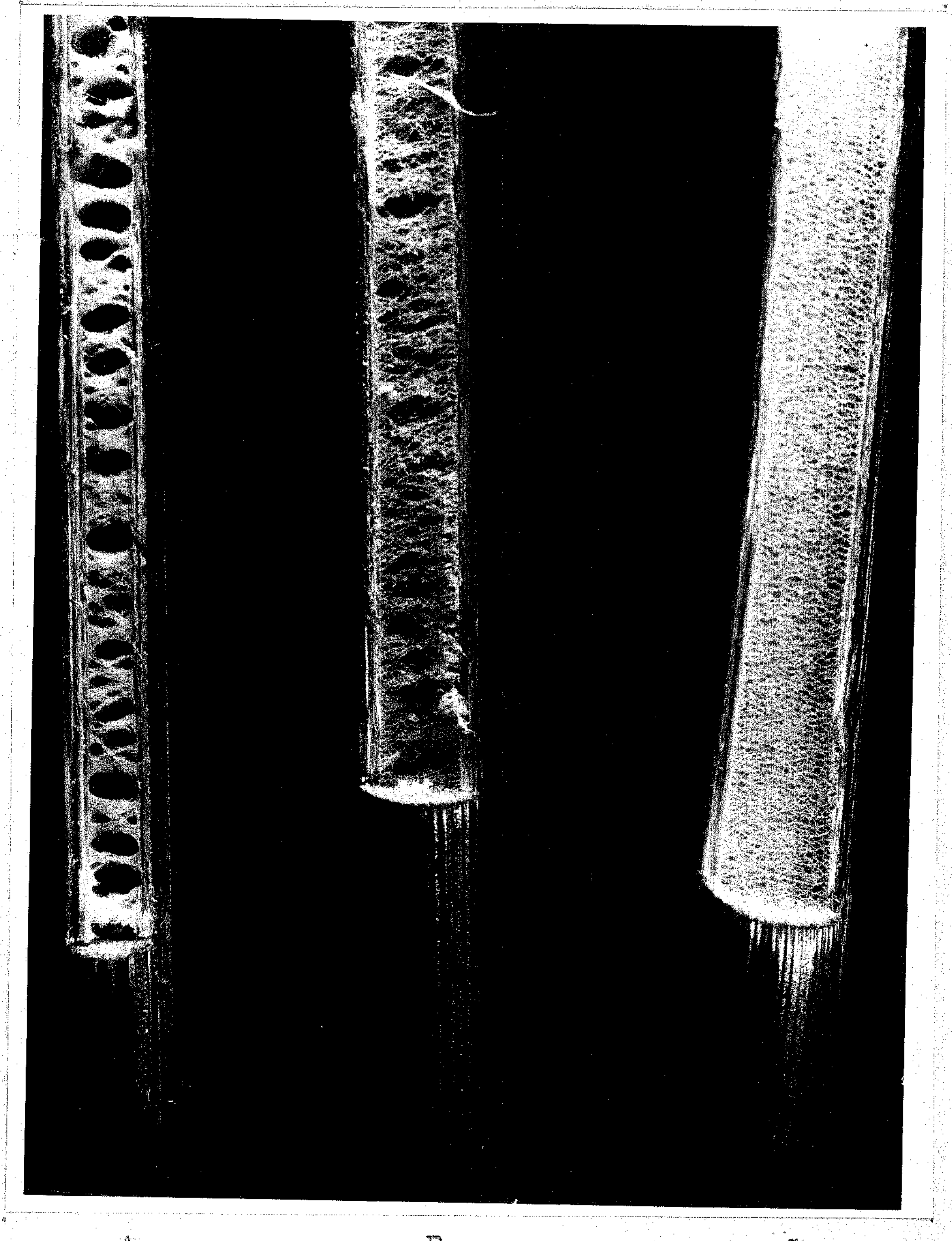


Figure 12 : A comparison of the pith in 3 specimens of *Juncus*; magnification about 20 diameters.

A *J.inflexus*

B Putative hybrid between *J.inflexus* and *J.effusus*

C *J.effusus*

respect to perianth and inflorescence measurements, though not with respect to spathe length, Table 13.

These were the only apparent hybrids observed. Though they were seed sterile, they did produce varying amounts of apparently viable pollen so introgression between the meadow form of J. effusus and J. inflexus seemed possible.

(b) Evidence for introgression.

Hybrids of any kind were rare and difficult to recognize so it was found impossible to compare directly hybrid swarms or colonies containing hybrids, with pure colonies of either species. It has been assumed that hybrids or backcrosses are more likely to occur in stands containing both J. inflexus and J. effusus (meadow form), than in stands of either species growing alone. Though individual introgressed plants may be overlooked through resembling the recurrent parent, in population studies their presence might be expected to be reflected in the statistics measured.

To investigate this problem a single stem from each of 30 plants was collected from each of 12 colonies of J. inflexus growing in localities from which J. effusus was absent. A similar number of stems was collected from colonies in which J. inflexus and J. effusus grew intermixed. As before for each stem, the spathe, inflorescence and perianth length was measured, and for each colony the mean and variance for each of these measurements was calculated. The results are presented in Tables 14 and 15, and the pooled data are shown in Figures 13, 14, and 15.

Similar data have been gathered for meadow populations of _J. effusus_ growing intermixed with or apart from _J. inflexus_. The results, which will be referred to below, are presented in Tables 16 and 17 and the pooled data are presented together with those for _J. inflexus_ in Figures 13, 14, and 15.

Table 14

Locality	Perianth m	Perianth v	Inflorescence m	Inflorescence v	Spathe m	Spathe v
Cassop: Bowburn Rd.	99	59·1	53	276·9	23	47·0
: Cassop Vale	93	53·6	43	167·3	14	30·2
: New Cassop	95	74·0	41	62·4	17	29·5
Crimdon: Dene to South	98	69·7	51	156·9	14	27·1
:Cliff-face	93	37·6	55	151·5	17	33·1
:Road Cutting	93	58·5	64	190·4	12	16·7
Bedford: Colony 1	99	43·0	53	257·7	21	20·0
Colony 2	95	25·3	61	288·1	15	15·9
Colony 3	93	86·7	45	110·4	19	45·9
Wicken Fen	94	67·0	46	172·2	13	20·1
Cambridge	94	66·9	56	229·7	16	21·8
Mitcham Common	90	61·2	34	81·3	17	20·8
Pooled Data	95	63·7	50	245·7	17	38·4

Table 14: The means and variances for three characters of *J.inflexus*, no *J.effusus* was observed growing in any of these colonies. Each colony is represented by a sample of 30 plants.

Table 15

Locality	Perianth		Inflorescence		Spathe	
	m	v	m	v	m	v
Waldridge Fell:1	78	40·8	39	137·4	25	32·6
:2	97	52·5	48	126·9	17	41·6
:3	94	68·4	51	210·9	14	19·6
:4	85	76·4	52	161·1	23	46·1
Old Cassop	95	72·0	43	173·5	22	59·6
Bowburn	95	65·2	51	90·7	17	16·8
Croxdale:1	104	61·2	58	205·2	19	28·4
:2	99	46·4	55	192·1	17	23·7
New Cassop	99	58·2	48	101·6	17	24·4
Brasside	93	114·0	53	249·8	22	80·3
Epsom Common	93	33·6	34	80·3	13	21·2
Wimbledon Common	91	44·3	54	113·2	20	34·4
Pooled Data	93	99·0	49	193·3	19	44·6

Table 15: The means and variances for three characters of *J.inflexus*, from colonies also containing *J.effusus*. Each colony is represented by a sample of 30 plants.

Table 16

Locality	Perianth		Inflorescence		Spathe	
	m	v	m	v	m	v
Waldridge Fell:1	79	62·0	18	36·4	18	27·6
:2	75	40·0	16	25·8	19	17·3
:3	76	48·5	19	31·9	19	17·8
:4	70	19·5	15	32·3	23	19·7
Browney Valley:1	68	22·8	20	33·6	18	10·9
:2	72	22·4	20	26·5	16	24·9
:3	71	35·0	17	23·2	18	12·0
Alnwick	74	40·4	18	97·0	16	18·5
Shropshire	78	51·0	28	71·9	13	6·0
Muckle Moss	71	21·6	12	17·6	19	19·0
Durham City	68	42·9	17	46·1	18	34·2
Epsom Common	69	27·4	16	19·1	15	34·7
Pooled Data	73	48·3	18	50·7	18	28·0

Table 16: The means and variances for three characters of J.effusus, from colonies which contained no plants of J.inflexus. Each colony is represented by a sample of 30 plants.

Table 17

Locality	Perianth		Inflorescence		Spathe	
	m	v	m	v	m	v
Old Cassop	71	58·0	13	12·6	21	33·2
Brasside:1	72	56·3	26	148·1	19	27·1
:2	69	25·2	16	62·2	19	27·2
Croxdale:1	67	38·1	34	70·1	24	40·8
:2	71	59·8	15	22·2	22	43·6
:3	73	52·6	23	63·4	21	32·6
Waldridge Fell	74	16·1	17	35·5	21	16·1
Wimbledon Common	76	41·8	20	52·0	15	19·4
Mitcham Common	70	49·2	22	78·2	14	8·6
Cheam, Surrey	65	80·4	34	150·6	20	21·3
Blade's Wood	73	26·1	19	41·5	19	27·0
Durham City	73	39·9	29	111·3	19	24·7
Pooled Data	71	51·7	22	66·2	20	33·1

Table 17: The means and variances for three characters of *J.effusus*, from colonies which also contained plants of *J.inflexus*. Each colony is represented by a sample of 30 plants.

Figure 13

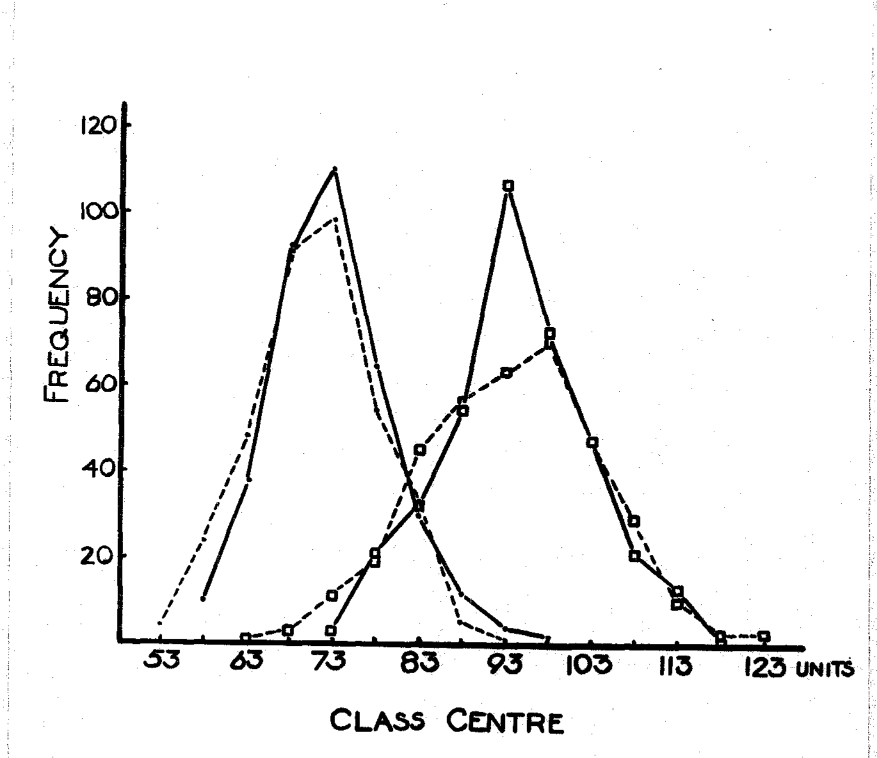


Figure 13 : Frequency polygons for the perianth lengths of **J.effusus** (meadow form) and **J.inflexus** as measured on plants growing together or separately. Each sample contained 360 specimens.
The scale is such that 25 units equal 1 mm.

□ ——— □ **J.inflexus** growing apart from **J.effusus**

□ - - - - □ **J.inflexus** growing together with **J.effusus**

● ——— ● **J.effusus** growing apart from **J.inflexus**

● - - - - ● **J.effusus** growing together with **J.inflexus**

Figure 14

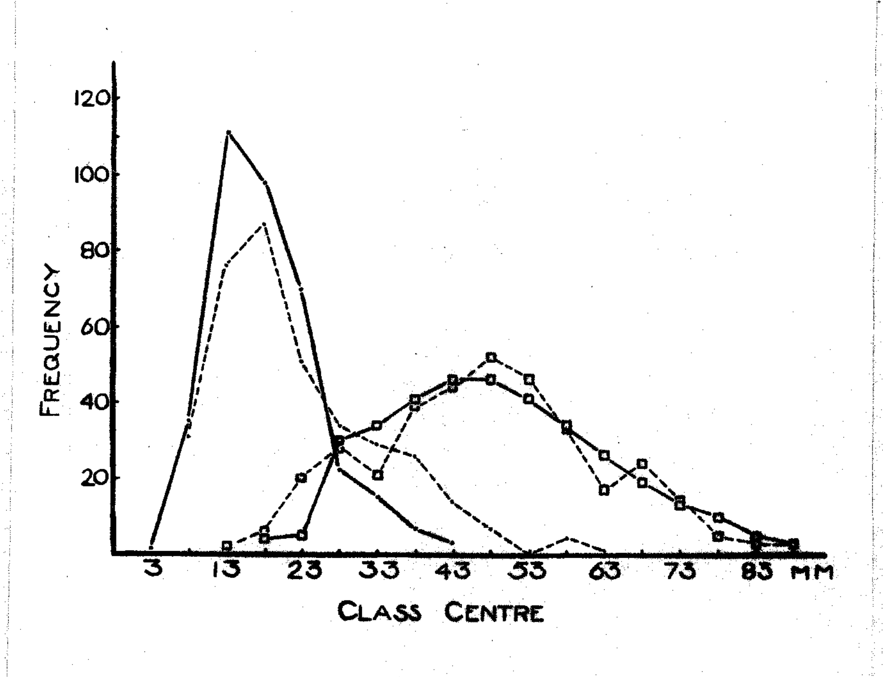


Figure 14 : Frequency polygons for the inflorescence lengths of J.effusus (meadow form) and J.inflexus as measured on plants growing together or separately. Each sample contained 360 specimens.

□———□ J.inflexus growing apart from J.effusus

□- - - -□ J.inflexus growing together with J.effusus

●———● J.effusus growing apart from J.inflexus

●- - - -● J.effusus growing together with J.inflexus

Figure 15

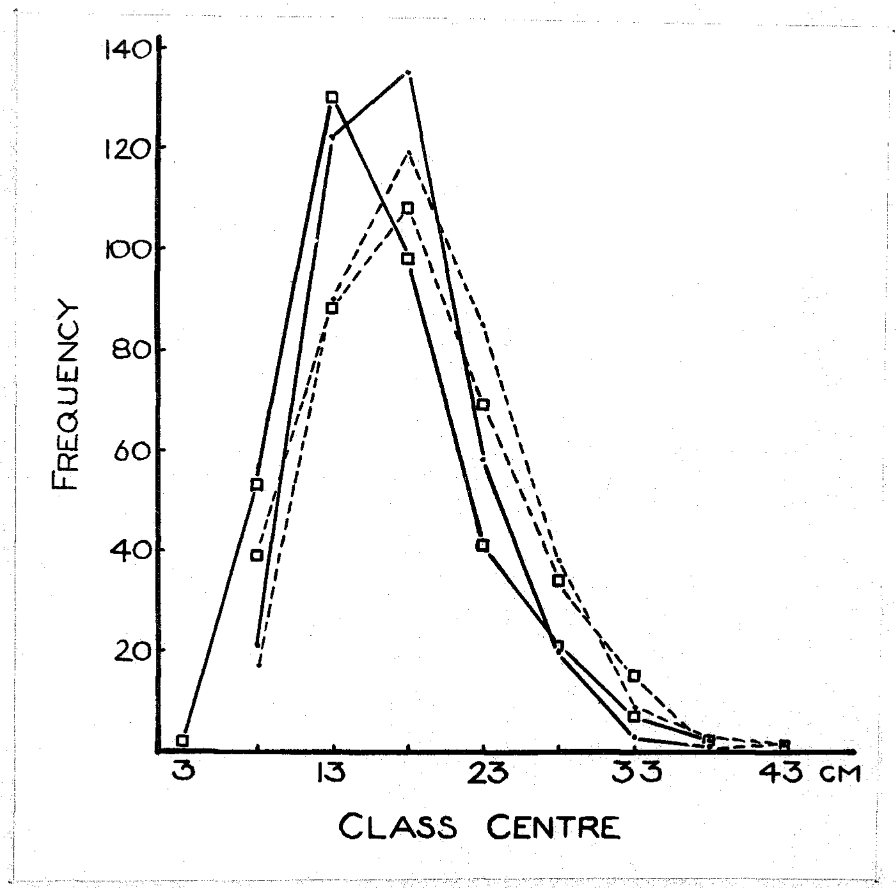


Figure 15 : Frequency polygons for the spathe lengths of *J.effusus* (meadow form) and *J.inflexus* as measured on plants growing together or separately. Each sample contained 360 specimens.

□——□ *J.inflexus* growing apart from *J.effusus*

□- - - -□ *J.inflexus* growing together with *J.effusus*

●——● *J.effusus* growing apart from *J.inflexus*

●- - - -● *J.effusus* growing together with *J.inflexus*

4. ANALYSIS OF RESULTS.

From the Tables and Figures presented it is evident that neither the meadow form of J. effusus nor J. inflexus differ markedly when collected from colonies in which they both occur or from colonies in which only one of them occurs. There are differences within either species dependent on whether it is collected from unmixed or mixed stands of the species but these differences though small, appear to be real, and further analysis is possible.

As J. effusus possesses at least two intergrading forms, and the relationship of these to each other has not been fully investigated, the data for this species will not be further analyzed. On the other hand J. inflexus is a very uniform species and some evidence has been presented to suggest that it may occasionally hybridize with J. effusus. It seemed worthwhile in consequence to analyze more carefully the differences observed when J. inflexus was collected from colonies in which J. effusus was either present or absent. The pooled data for J. inflexus from these two types of colony are presented in Table 18.

The differences in this Table are such as to be compatible with the suggestion of J. inflexus mixed with J. effusus (meadow form) may have contained some plants that had undergone hybridization with the meadow

TABLE 18

J. effusus	n	Perianth	Inflorescence	Spathe
Absent	360	95 units	50 mms.	17 cms.
Present	360	93 "	49 "	19 "

Table 18: A comparison of J. inflexus from colonies in which J. effusus was either present or absent. None of the differences in the Table are significant at the 5% level.

form of J. effusus. If it is assumed that the characters measured are inherited multifactorially, hybrids would be expected to be intermediate and backcrosses to be intermediate between these and the recurrent parent. In this way a plant of J. inflexus that contained some genes of J. effusus would be expected to possess slightly shorter perianths and inflorescences and slightly longer spathes than a pure plant of J. inflexus. This would be expected because it is in these ways that the meadow form of J. effusus differs from J. inflexus.

None of the differences observed between J. inflexus from the two types of colony studied was significant statistically, but as the differences were consistently in the direction of J. effusus it was thought that a discriminant analysis of the data might improve the significance of the difference between the colonies. This was undertaken and yielded a discriminant function of the form:

$$Y = .044x - .033z - .0068y$$

Where x = spathe length in cms.
y = Inflorescence length in mms.
z = Perianth length in arbitrary units (25 units = 1 mm.)

On testing the significance of this function it was found to be highly significant with a probability of arising by chance less than once in a thousand times. The values of Y for *J. inflexus* from each of the two types of colony in which it was collected are summarized as frequency polygons in Figure 16.

From this Figure it is evident that although the discriminant function may achieve significant discrimination, the overlap between the two types of colony is too great for the function to be of any real use as a means of deciding from which colony type a given plant has come.

The small differences shown to exist between *J. inflexus* growing in pure colonies and the same species growing intermixed with *J. effusus* suggests that introgression of *J. inflexus* by *J. effusus* may be occurring. Further support for this view is obtained from a comparison of intercolony variation of *J. inflexus* in pure stands and intermixed with *J. effusus*. In the latter situations the *J. inflexus* tends to be more variable *inter se* than when growing apart from *J. effusus*.

Figure 16

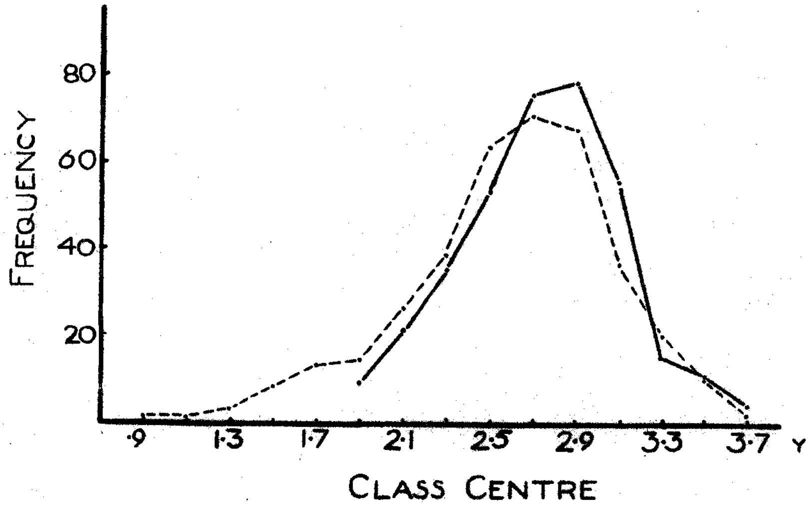


Figure 16 : Frequency polygons for the discriminator derived from certain measurements of Juncus inflexus growing together with or apart from J.effusus (meadow form) .

———	J.inflexus growing apart from J.effusus
------	J.inflexus growing together with J.effusus

This is shown in Table 19 where the pooled data from the two types of colony are compared.

TABLE 19

J. effusus	n	Perianth	Inflorescence	Spathe
Absent	360	63.7 units	245.7 mms.	38.4 cms.
Present	360	99.0 "	193.0 "	44.6 "

Table 19: A comparison of the variances of the mean values of three characters of *J. inflexus* collected from colonies in which *J. effusus* was either present or absent. Only the difference between the variabilities for mean perianth length is significant at the 5% level.

The variance ratio for the perianth length is the only one to attain statistical significance; of the other two characters the spathe length is more variable for *J. inflexus* growing together with *J. effusus* than when growing apart from that species, but the reverse appears to be so for the variability of the inflorescence length. That two of the three characters investigated should be more variable in colonies of *J. inflexus* intermixed with *J. effusus* than in colonies growing apart from that species suggests that some gene flow may be occurring between them.

When the correlation coefficients between the characters were considered they were found to be similar in each of the two colony types for *J. inflexus*. Likewise the slopes of the lines of best fit through the data when plotted together in pairs were also similar for *J. inflexus* growing together with or apart from *J. effusus*. So these two tests do not suggest any difference between the *J. inflexus* collected from stands in which *J. effusus* was either present or absent.

5. DISCUSSION.

Before discussing the data just presented it is perhaps desirable to mention that a hybrid between J. inflexus and J. conglomeratus has also been reported (Clapham, Tutin and Warburg, l.c.). Whether introgression between these species has occurred or is possible has not been investigated. If introgression has taken place it is unlikely to have disguised the effects of gene flow from J. effusus into J. inflexus, because for the characters studied J. conglomeratus differs from the latter species in much the same way as does J. effusus.

It is evident that though some evidence for the introgression of J. inflexus by J. effusus has been presented, the amount of gene flow between these species is very slight.

It would appear that the isolating factors are probably internal; for over much of their ranges the species often grow intermixed and their flowering times overlap considerably. Both species are self compatible and may be largely self fertilized. Plants of each species isolated in separate greenhouses and not artificially pollinated set good capsules which contained seed that has subsequently germinated. The extent of inbreeding in natural populations as compared with outbreeding could not be ascertained; but if inbreeding is frequent, the chances of interspecific pollination would be diminished.

A further restriction upon gene flow into J. inflexus is the frequent failure of that species to set capsules. This was observed particularly in the north of England where it was sometimes impossible to find a mature capsule on a single plant, though it possessed a fully developed inflorescence. The observed sterility of J. inflexus in the field is not necessarily genotypic. A plant transplanted into the experimental garden as a clonal series failed to set capsules except for one of the clonal plants which had been covered with a glass dome. It would seem therefore that it may be an environmental influence which prevents seed set.

Two flowers of J. inflexus were hand pollinated with pollen of J. effusus. These flowers developed ripe fruits but all the seedlings obtained from these resembled J. inflexus, the female parent. This suggests that genetic incompatibility either at the pollination or post-fertilization stage may be largely responsible for failure to hybridize.

Finally the fact that the putative hybrids observed are highly infertile indicates that this too provides another barrier to gene flow. It may be noted that the two species have $2n = 40$ (Confirmed for British material).

We may summarize the position as follows:-

Within J. effusus there appear to be at least two ecotypes whose status and ecology are in need of further study.

There is some evidence to suggest that hybrids may occasionally be produced between J. inflexus and J. effusus, ~~but~~ and it would appear that ~~[illegible]~~ slight introgression between these species has occurred in the areas studied.

AN INVESTIGATION OF POSSIBLE INTROGRESSION BETWEEN PRIMULA VULGARIS Huds. AND PRIMULA VERIS L.

1. INTRODUCTION

The primrose (*P. vulgaris* Huds.) and the cowslip (*P. veris* L.) have overlapping geographical distributions. Their habitat preferences are different but not sufficiently so to prevent them frequently growing together, especially in disturbed localities. Furthermore their flowering periods overlap. Thus ecological and geographical isolation between them is not complete.

Experimental investigations have shown that there are internal isolating barriers between the species (Valentine 1955). Reciprocal crossing experiments have indicated that a first generation hybrid is apparently only possible when the cowslip is the seed parent. Even then the seed produced is variable in quality and only about a third of it at most may be expected to germinate. Nevertheless, apparent F1 hybrids are found in the field.

Controlled experiments have indicated that an F2 generation can be raised from the F1 hybrids and that these also backcross successfully to both the parent species. Hence, it would seem quite likely that some introgression between the primrose and the cowslip might occur. Field evidence has been sought to test this view.

2. ARTIFICIAL HYBRIDS

It has been possible to examine families of primrose, cowslip, the F1 hybrid generation and both the backcross generations as these had been raised for seed compatibility studies which have already been reported upon (Valentine l.c.) With the exception of the F1 hybrids, the families were grown in essentially the same environment, and so provided excellent material for studying the inheritance of certain quantitative characters. The F1 generation was grown in the experimental garden and the other families were grown in boxes placed in cool frames. These were closed during periods of snow and at night in frosty weather so the plants in them were grown in a milder environment than those in the experimental garden.

The characters chosen for special study were those that were expected to be useful for studying introgression in field populations. The species differ markedly in several respects but only a few could be readily measured. These were the dimensions of floral structures that had also been found useful by Fey (1929) when studying the F1 and F2 generations derived from primrose and cowslip. The form of the inflorescence, pedunculate or otherwise, was also noted as every plant could be decisively scored for this character. The leaves, though differing in shape in the two species, were not regarded as suitable for studying introgression as they are somewhat variable within the species and because they do not as a rule attain their maximum size until after flowering.

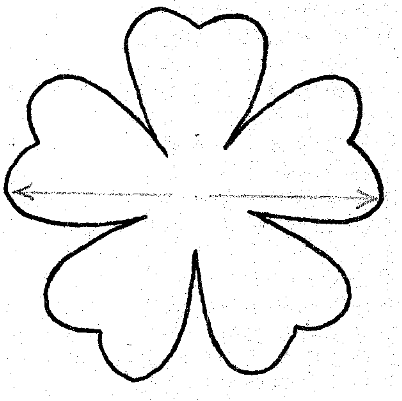
The floral dimensions chose for measurement were so selected as to include two pairs of contrasting characters (p. 29) It was anticipated that a correlation index analysis of the kind already discussed would be necessary. In most respects primrose flowers are larger than those of cowslip, but the circumference of the calyx is an exception. The calyces of primrose flowers usually have a small diameter than those of cowslips. This dimension is more conveniently expressed in terms of the circumference of the calyx at the base of the calyx teeth.

The data obtained from the artificial hybrids will be considered below.

(a) Floral dimensions.

From each plant a fully expanded flower was taken and preserved in 70% alcohol. This meant that the flowers could be measured at any convenient time and also that data from them would comparable with those from the field collections which had also been preserved in 70% alcohol. For each flower the diameter of the corolla, the length of a calyx tooth, and the circumference of the calyx at the base of the teeth was measured. To determine the calyx circumference the calyx tube was split down one side and the upper portion cut off just

Figure 17



Diameter of Corolla

Calyx

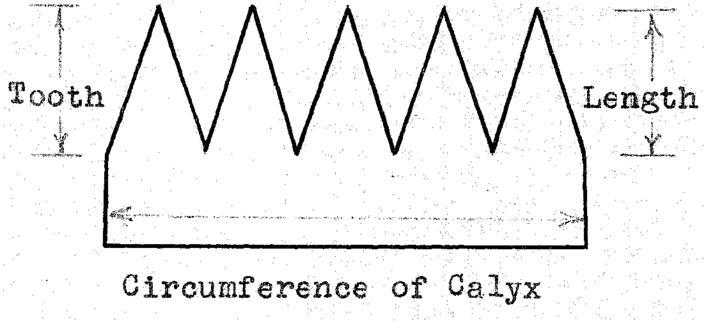


Figure 17 : Scheme of Floral dimensions.

below the base of the teeth; this was then flattened between a pair of slides and its length measured at the base of the teeth. At the same time tooth length was also measured, Fig. 17.

These measurements are summarized in Tables 20 and 21. Data from both field and cultivated plants indicate that pin and thrum flowers have slightly different dimensions and so throughout the results for each flower type are presented separately.

(b) Peduncle.

The cowslip inflorescence is always pedunculate whereas that of the primrose is usually basal; that is in primrose the flowers are borne singly and spring directly from the rosette. The results presented below refer to a series of hybrid generations derived from a typical primrose with such a basal inflorescence. The frequencies with which plants bearing basal flowers occurred under the conditions of the experiments are given in Table 22.

The frequencies with which plants bearing at least a single pedunculate inflorescence occurred under the the conditions of the experiments are given in Table 23.

To obtain the frequencies with which plants bearing both pedunculate and basal flowers occurred it is necessary to consider jointly Tables 22 and 23.

TABLE 20

Family	Code	n	D. of C. (mms)		L. of C.T. (mms)		C. of C. (mms)	
			m	v	m	v	m	v
Primrose	B91	10	28.2	8.28	6.43	.73	14.2	2.57
	B88	4	28.9	10.38	6.28	.38	13.1	.72
Primrose backcross	B41	17	29.6	13.34	6.40	1.14	14.2	3.51
	B45	13	25.3	5.61	5.93	.91	14.3	4.83
F_1	A	10	21.5	2.22	4.51	.42	14.6	5.84
	B	3	21.8	-	4.67	-	13.8	-
Cowslip backcross	B80	17	17.2	8.81	4.21	.53	18.1	6.53
	B72	15	19.0	10.29	4.31	.55	15.1	4.83
Cowslip	C14	13	14.0	3.14	2.97	.17	17.6	6.64

Table 20: The means and variances for some dimensions of the flowers from families of pin plants of cowslip, primrose and certain hybrids between them. The abbreviations when expanded read as follows: D of C, the diameter of the corolla; L of C.T. length of the calyx teeth; C of C circumference of the calyx. These abbreviations are used with the same meanings in subsequent Tables.

TABLE 21

Family	Code	n	D. of C. (mms)		L. of C.T. (mms)		C. of C. (mms)	
			m	v	m	v	m	v
Primrose	B91	13	27.7	4.85	6.42	.57	14.8	1.68
	B88	6	29.7	4.07	6.56	.88	13.8	.85
Primrose backcross	B41	13	27.3	16.79	5.89	.89	14.5	3.45
	B45	6	23.8	10.37	5.63	.32	13.6	1.08
F_1	A	2	23.8	-	4.35	-	15.5	-
	B	10	22.3	3.07	4.41	.25	13.6	.78
Cowslip backcross	B80	28	17.6	8.72	4.17	1.10	16.8	6.42
	B72	9	17.2	7.20	3.27	.78	15.1	6.71
Cowslip	C14	10	13.7	3.72	3.00	.29	15.8	6.52

Table 21: The means and variances for some dimensions of the flowers from families of thrum plants of cowslip, primrose and certain hybrids between them. Abbreviations as in Table 20.

TABLE 22

Family	Percentage of plants with basal flowers.
Primrose	100
Backcross Primrose	100
F1	61
Backcross Cowslip	12
Cowslip	0

Table 22: The frequencies with which basal flowers occurred in several families of primrose, cowslip and certain hybrids between them.

TABLE 23

Family	Percentage of plants with pedunculate inflorescences.
Primrose	0
Backcross Primrose	10
F1	100
Backcross Cowslips	100
Cowslip	100

Table 23: The frequencies with which pedunculate inflorescences occurred in several families of primrose, cowslip and certain hybrids between them.

(c) Discussion Of Artificial Hybrids.

The data represented in Tables 20 and 21 reflect the segregation for flower size which occurred in the backcross generations. This might have been anticipated from the intermediacy of the F1 generation. The average diameter of the flowers of the backcross families was approximately midway between that of the F1 generation and the recurrent species, suggesting that this character is controlled by several genes. The segregation for size is expressed by the much greater variability of flower size in the backcross families than within either the F1 families or those of the cowslips and primroses.

The results for the calyx measurements also suggest that this character is controlled by several genes which segregated in the backcross families.

That some segregation for the presence of a peduncle or solitary flowers occurred is shown in Tables 22 and 23 but this character was of little use for field studies. It would have been very useful for detecting backcross primroses but no plants which could be definitely classified as such were observed.

Segregation of flower colour occurred in both backcross generations but no effort was made to score this as no suitable scale was devised. Furthermore, the colour varied with the age of the flower and so was of little use for field studies. Slides showing the segregation for colour are preserved in the Botany Department of the Durham Colleges.

As segregation in the backcross families led to the production of a complete series of forms intermediate between the F1 generation and the recurrent parents, it would have been impossible to recognize the status of some individuals, without a prior knowledge of their ancestry. This, coupled with a certain amount of apparently inherent variability in the cowslip and primrose made it impossible to recognize with certainty backcrosses in the field. With very few exceptions all the plants collected in the field resembled F1 hybrids, cowslips or primroses.

Evidence for possible introgression between cowslip and primrose had to be sought in population studies. Because the metrical characters studied were apparently multifactorially inherited it was expected that the presence of backcross or introgressed individuals in a colony might be detected by suitable statistical techniques. For example, the mean size and variability of a suitable character is likely to be different in introgressed and non-introgressed colonies.

3. FIELD METHODS.

Collections were made of apparent cowslips and primroses both where they were growing together and where they were growing apart. The qualifying term 'apparent' has been used, (and is therefore to be inferred where appropriate), because it is known for artificial hybrids that some backcross individuals may closely resemble, and so be confused with their recurrent parent. When sampling populations that contained apparent F1 hybrids these flowers were kept separate; any other putative hybrid individuals collected were grouped with one or other probable parent. This method of sampling populations is similar to that used in the study of *Juncus* (p. 56). Here as in that study it was assumed that backcross hybrids would be more likely to occur in colonies where the species grew intermixed than in colonies where they were separate.

Evidence for introgression was sought by examining numerous colonies of cowslip and primrose in the north of England and in Ireland. The areas investigated in England were included within the rectangle whose corners are Scarborough, Grange-over-Sands, Newcastle-upon-Tyne and Carlisle. In Ireland a narrow zone from Dublin to Limerick was explored. If possible, at each locality visited about 100 fully expanded flowers of cowslip, primrose or both were collected. No conscious selection of the plants was exercised except to avoid those that may have been ~~closely~~ clonally related.

Of the samples collected 41 have been studied in detail. This involved the measurement of the corolla diameter, calyx tooth length and calyx circumference for each of 25 pin and thrum flowers for most colonies. For a few colonies there were not 25 of each flower type available, and this has been indicated in the appropriate places in the tables.

The results of these measurements are presented in the forms of Tables 24, 25, 26, 27, 28, 29, 30, and 31.

TABLE 24

Locality	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Farndale	25	27.2	6.61	7.1	1.37	14.5	2.88
Stanhope	25	26.7	13.00	6.1	.97	12.5	2.14
Nr. Stokesley	25	29.4	9.23	6.2	.59	14.4	3.07
Whale	25	29.7	7.58	6.3	.88	14.4	3.12
Melmerby	25	30.7	10.02	7.2	.71	15.4	2.69
Ullswater	25	29.8	13.56	6.7	1.70	14.5	2.82
Ravenbeck	25	30.0	10.46	6.5	.73	15.3	4.35
Shincliffe	25	28.3	6.29	6.7	.60	14.9	1.92
Cassop	25	25.4	4.00	6.2	.33	14.7	2.66
Brancepeth	25	30.8	8.95	6.8	.73	15.2	2.80
Brittas I	25	31.7	11.39	6.4	.82	16.3	4.09
Corregin I	25	28.9	4.80	6.3	.45	14.2	1.28

Table 24: The means and variances for certain dimensions of the flowers of pin primroses collected from colonies that were free of cowslips or apparent F1 hybrids. I indicates an Irish locality.

TABLE 25

Locality	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Blackhall 1	25	28.6	6.53	6.2	.41	15.2	2.28
Blackhall 2	25	26.9	4.98	5.8	.56	15.1	1.87
Rievaulx	25	28.6	6.69	6.9	.99	14.2	2.71
Grange-over-Sands	25	30.9	8.89	6.1	.67	15.0	2.38
Kettlewell	25	26.1	8.84	5.6	.82	12.5	2.76
Penrith	25	31.7	3.68	7.4	.80	14.3	1.85
Burnsall	25	25.9	6.56	6.2	.84	12.3	1.41
Wolsingham	25	23.9	8.73	6.7	.70	12.5	1.93
Rookdale	25	23.0	10.90	6.4	.77	13.0	2.26
Nr. Hawnby	25	28.5	10.70	6.9	1.13	13.7	3.01
Dalgany I	25	33.3	6.56	7.7	.72	15.4	3.50
Corrogin I	25	34.0	12.68	7.2	1.50	15.2	3.26

Table 25: The means and variances for certain dimensions of the flowers of pin primroses collected from colonies that included either cowslips or apparent F1 hybrids. I indicates an Irish locality.

TABLE 26

Locality	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Farndale	25	26.9	4.27	7.6	.69	14.0	2.94
Stanhope	25	24.4	5,74	6.0	.97	12.3	2.69
Nr. Stokesley	25	28.3	9.74	6.2	.98	13.6	1.97
Whale	25	29.1	9.80	6.8	1.86	14.4	2.24
Melmerby	25	30.1	12.12	7.7	1.26	15.2	3.78
Ullswater	25	30.2	11.75	7.1	2.59	14.9	5.21
Ravenbeck	25	31.2	9.41	6.8	.97	15.6	4.51
Shincliffe	25	26.9	9.84	6.8	1.06	15.0	3.10
Cassop	25	25.5	6.28	6.6	.47	15.7	3.64
Brancepeth	25	29.0	11.88	6.8	.60	14.8	1.04
Brittas I	25	29.9	11.00	6.5	1.18	15.7	3.14
Corrogin I	25	29.9	7.59	6.5	.73	14.8	2.02

Table 26: The means and variances for certain dimensions of the flowers of thrum primroses collected from colonies that were free of cowslips or apparent F 1 hybrids.

TABLE 27

Locality	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Blackhall 1	25	28.1	5.27	6.4	.99	15.0	2.49
Blackhall 2	25	27.8	6.04	6.1	1.17	16.4	2.24
Rievaulx	25	27.8	4.50	6.4	.92	13.5	3.41
Grange-over-Sands	25	28.3	8.73	5.9	.72	14.4	3.02
Kettlewell	25	26.5	6.32	6.0	.59	13.1	1.39
Penrith	25	31.2	6.56	7.3	.98	14.5	4.25
Burnsall	25	25.6	10.05	6.4	.55	12.4	2.66
Wolsingham	25	25.1	6.13	6.7	.89	12.8	2.70
Rookdale	25	22.9	4.94	6.4	.52	13.5	3.86
Nr. Hawnby	25	28.5	7.83	6.6	.41	14.2	1.89
Dalgany I	25	32.7	9.44	7.4	1.05	16.1	2.19
Corrogin I	25	32.9	9.31	7.1	.98	15.5	2.44

Table 27: The means and variances for certain dimensions of the flowers of thrum primroses collected from colonies that included either cowslips or apparent F1 hybrids. I indicates an Irish locality.

TABLE 28

Locality	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Cassop	25	12.4	2.39	3.5	.54	16.2	8.91
Quarrington	25	11.9	2.62	3.2	.25	17.1	5.13
Elemore	25	12.9	2.23	3.1	.24	15.4	4.58
Witton	25	12.6	1.51	3.3	.23	16.8	3.86
Cayton Bay	25	12.5	2.41	3.0	.38	16.0	5.23
Blackhall	25	11.8	3.46	3.1	.38	16.3	4.86
Brittas I	25	12.6	1.78	3.6	.20	17.4	2.11
Tullamore I	25	13.8	3.60	4.2	.40	18.3	2.47

Table 28: The means and variances for certain dimensions of the flowers of pin cowslips collected from colonies that were free of primroses or apparent F 1 hybrids. I indicates an Irish locality.

TABLE 29

Locality	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Nr. Hawksley	25	13.4	2.79	3.6	.24	17.6	6.72
Nr. Helmsley	25	13.8	2.54	3.9	.33	18.3	4.89
Rievaulx Abbey	25	12.9	2.44	3.4	.28	16.0	4.01
Nr. Rievaulx 1	25	11.3	2.06	3.6	.33	16.8	2.70
Nr. Rievaulx 2	18	12.1	3.25	3.6	.36	18.0	5.88
Penrith	25	12.3	2,58	3.6	.14	17.1	3.29
Nr. Cawsley	24	12.3	2.72	3.6	.29	18.2	4.37
Brittas I	25	11.4	1.80	3.5	.18	15.4	2.12
Dalgany I	25	11.6	1.97	3.9	.31	18.6	6.26

Table 29: The means and variances for certain dimensions of the flowers of pin cowslips collected from colonies that included either primroses or apparent F 1 hybrids. I indicates an Irish locality.

TABLE 30

Locality	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Cassop	25	11.9	1.99	3.2	.30	16.2	4.16
Quarrington	25	11.6	1.35	3.5	.33	16.6	3.78
Elemore	25	12.4	2.32	3.1	.13	16.1	6.69
Witton	25	11.8	1.93	3.1	.41	15.8	4.51
Cayton Bay	25	12.5	3.12	3.1	.33	16.5	7.52
Blackhall	25	10.7	1.65	3.2	.21	16.3	3.31
Brittas I	25	12.5	2.79	3.7	.30	16.5	3.22
Tullamore I	25	13.7	2.64	4.0	.18	18.0	5.55

Table 30: The means and variances for certain dimensions of the flowers of thrum cowslips collected from colonies that were free of primroses or apparent F1 hybrids. I indicates an Irish locality.

TABLE 31.

Locality	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Nr. Hawksley	25	12.7	4.32	3.5	.51	15.9	4.28
Nr. Helmsley	25	12.6	2.22	3.7	.32	17.0	5.54
Rievaulx Abbey	25	12,5	3.92	3.6	.23	16.4	7.89
Nr. Rievaulx 1	25	11.9	2.32	3.6	.29	17.3	4.94
Nr. Rievaulx 2	13	12.2	4.46	3.8	.12	16.3	2.68
Nr. Penrith	25	12.2	1.53	3.7	.34	17.4	4.74
Nr. Cawsley	22	12.1	1.54	3.3	.36	18.4	5.19
Brittas I	25	10.6	2.16	3.5	.31	15.0	3.86
Dalgany I	25	11.7	2.17	3.5	.34	17.7	3.35

Table 31: The means and variances for certain dimensions of the flowers of thrum cowslips collected from colonies that included either primroses or apparent F1 hybrids. I indicates an Irish locality.

4. ANALYSIS OF FIELD DATA.

The field data have been analyzed by several methods each of which will be considered separately below. The methods employed were as follows:

- (a) Comparison of means
    - (i) Control Experiments
    - (ii) Primrose
    - (iii) Cowslip
- (b) Correlation Index Analysis
- (c) Correlation Coefficient Analysis
- (d) Analysis of Apparent F1 Hybrids.

(a) Comparison of Means.

(i) Control Experiments

The dimensions of characters not markedly influenced by the environment are useful for comparing groups recognized by criteria other than those dimensions. Cowslips and primroses are readily identified by qualities other than the sizes of the floral structures measured. Though it has not been possible to study the plasticity of the structures in detail some results are available that allow comparisons of small samples of backcross cowslips and of primroses grown in separate environments.

In the summer of 1954 two samples of sibling backcross cowslips were grown, one in an experimental plot and the other in boxes sheltered in cool frames against a north facing wall. The results for the three floral dimensions measured are summarized in Tables 32 and 33. From the tables it can be seen that from the small samples available the environment does not appear to affect materially flower size. With the exception of that for calyx circumference of pin flowers none of the differences observed are significant at the 5% level. Pin flowers from the experimental plot had more inflated calyces than those grown in the cool frames.

Two experiments have provided data on the plasticity of primroses. From some sibling primrose plants whose flowers were measured in the early spring of 1954 some flowers were taken and measured in the spring of 1955. These results are summarized in Tables 34 and 35. None of the differences in size observed between the floral characters as measured in successive years was significant at the 5% level.

The flowers from two samples of sibling primroses flowering for the first time in the spring of 1955 have been compared and the results are summarized in Tables 36 and 37. One sample was grown in cool frames against a north facing wall and the other was grown nearby in a less sheltered position. Except for the calyx circumference of the pin flowers none of the differences between the mean values of comparable floral dimensions were significant at the 5% level. The calyces of flowers from

TABLE 32

Habitat	n	D. of C. (mms)		L. of C.T. (mms)		C. of C. (mms)	
		m	v	m	v	m	v
Cool Frames	15	19.0	10.29	4.3	.55	15.1	4.83
Garden	12	20.9	9.72	3.7	.89	17.3	3.52

Table 32: The means and variances for certain dimensions of the flowers of sibling pin cowslip back-crosses samples of which were grown in two separate environments. The only difference to be significant at the 5% level was that for the calyx circumference.

TABLE 33

Habitat	n	D. of C. (mms)		L. of C.T. (mms)		C. of C. (mms)	
		m	v	m	v	m	v
Cool Frames	9	17.2	7.20	3.3	.78	15.1	6.71
Garden	7	19.7	11.82	3.7	.89	15.1	3.69

Table 33: The means and variances for certain dimensions of the flowers of sibling thrum cowslip back-crosses samples of which were grown in two separate environments. None of the differences was significant at the 5% level.

TABLE 34

Season	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
1954	10	28.2	8.28	6.4	.73	14.2	2.57
1955	5	27.8	1.97	6.0	.81	15.2	.93

Table 34: The means and variances for certain dimensions of the flowers of sibling pin primroses, samples of which were collected in successive years. None of the differences was significant at the 5% level.

TABLE 35

Season	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
1954	13	27.7	4.85	6.4	.57	14.8	1.68
1955	12	27.5	3.93	6.1	.72	14.9	.75

Table 35: The means and variances for certain dimensions of the flowers of sibling thrum primroses, samples of which were collected in successive years. None of the differences was significant at the 5% level.

TABLE 36

Habitat	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Cool Frames	7	28.9	2.77	6.0	1.81	13.2	.42
Garden	9	28.4	4.59	6.4	.72	11.6	.65

Table 36: The means and variances for certain dimensions of the flowers of sibling pin primroses samples of which were grown in two separate environments. The only difference significant at the 5% level was that for the calyx circumferences.

TABLE 37

Habitat	n	D of C (mms)		L of C.T. (mms)		C of C (mms)	
		m	v	m	v	m	v
Cool Frames	5	26.8	19.4	5.7	.16	12.8	1.08
Garden	7	27.9	2.64	6.4	.75	12.6	.30

Table 37: The means and variances for certain dimensions of the flowers of sibling primroses samples of which were grown in two separate environments. None of the differences was significant at the 5% level.

plants grown in the less sheltered environment had greater circumferences than those of the plants grown in the cool frames.

Though the samples were small it would seem that of the floral dimensions measured corolla diameter is the least influenced by the environment. For none of the comparisons made was the difference between mean corolla diameters significant even at the 10% level. On the other hand two of the differences between mean calyx circumferences were significant at the 5% level and one of the differences for mean calyx tooth length almost attained significance with a probability 5 - 10% on the null hypophesis (t. test).

Of the dimensions measured diameter of corolla would therefore appear to be the best single criterion for comparing colonies. The data from the various colonies could be pooled as this dimension is relatively free of environmental influence. The results obtained for field samples of cowslip and primrose when the data are pooled will be considered below.

Some of the size differences observed between the Irish and the English samples were highly significant statistically, so the data for the two countries has been kept separate.

(ii) Primrose

The results for pooling all the comparable data for the English samples are presented in Tables 38 and 39. From the Tables it can be seen that primroses growing together with cowslips have smaller corolla diameters than those growing apart from them. The differences are highly significant statistically being many times larger than their standard errors. Such a reduction in mean size of corolla diameter would be expected if primrose were introgressed by cowslip.

Introgression by cowslip might also be expected to increase the variability of the corolla diameter of the introgressed primroses, but the evidence for this occurring is not conclusive. The pin flowered primroses growing apart from cowslip are less variable than those growing intermixed with cowslip, but the thrum flowered primroses are about equally variable in the two types of colony.

In Ireland the primroses growing together with cowslip appear to have larger flowers than those growing apart form them. To confirm this observation more data are required for only 200 Irish flowers were measured compared with 1000 from England.

TABLE 38

Colony Type	n	Diameter of Corolla		
		Mean (mms)	Variance	Coeff. of Var.
Cowslips absent	250	28.81	11.73	.118
Cowslips present	250	27.41	14.79	.140
		Diff. = 1.40	S.E. of Diff = .23	

Table 38: A comparison of the means and variabilities of the corolla diameters of the flowers of pin primrose plants growing with or apart from cowslips.

TABLE 39

Colony Type	n	Diameter of Corolla		
		Mean (mms)	Variance	Coeff. of Var.
Cowslips absent	250	28.16	13.04	.128
Cowslips present	250	27.18	11.04	.122
		Diff. = .98	S.E. of Diff. = .22	

Table 39: A comparison of the means and variabilities of the corolla diameters of the flowers from thrum primrose plants growing with or apart from cowslips.

(iii) Cowslip.

The results of pooling all the comparable data for the English samples of cowslip are presented in Tables 40 and 41. From the Tables it can be seen that cowslips growing together with primroses are larger than those growing apart from them. The increase in size is statistically significant for flowers of both pin and thrum plants. Such an increase in size of the cowslips growing together with primroses could result from introgression of cowslip by primrose. That this may be the correct explanation is suggested by the greater variability of corolla size for cowslips growing together with primroses compared with the variability of those growing apart from primroses.

In Ireland the cowslips growing apart from primroses appear to have larger corolla diameters than those growing together with them. This is the opposite relationship to that observed with the English cowslips, but the result must be regarded as tentative until more Irish data are available.

TABLE 40

Colony Type	n	Diameter of Corolla		
		Mean (mms)	Variance	Coefi. of Var.
Primrose absent	150	12.34	3.23	.128
Primrose present	150	12.60	2.51	.143

Diff. = .26 S.E. of Diff. = .19

Table 40: A comparison of the means and variabilities of the corolla diameters of the flowers from pin cowslip plants growing with or apart from primroses.

TABLE 41

Colony Type	n	Diameter of Corolla		
		Mean (mms)	Variance	Coeff. of Var.
Primrose absent	150	11.82	2.31	.129
Primrose present	167	12.33	3.11	.143

Diff. = .51 S.E. of Diff. = .18

Table 41: A comparison of the means and variabilities of the corolla diameters of the flowers from thrum cowslip plants growing with and apart from primroses.

(b) Correlation Index Analysis.

Evidence has already been presented which suggests that the calyx dimensions of primroses and backcross cowslips are dependent upon the environment. The field data indicate that the calyx dimensions of cowslips are also influenced by the environment. Because of this it is impossible to pool the data for either calyx tooth length or circumference to make comparisons as was done with the data for corolla diameter. Nevertheless, it is possible to combine the calyx measurements into a simple index in such a way that the influence of the environment is minimized.

The calyx dimensions in primrose and cowslip form a pair of contrasting characters and so may be combined into a correlation index (p. 27). Primrose calyces have long teeth and a relatively small circumference, whereas cowslips have calyces with relatively short teeth and relatively large circumferences. The average slopes of the lines of best fit through the scatter diagram resulting from plotting the calyx tooth length against the calyx circumference has been calculated from the formula previously quoted (p. 27); The results are presented in Table 42.

From the Table it can be seen that it is possible to compare the calyces of primroses growing together with and apart from cowslips as the average slopes of the lines of best fit through the scatter diagrams for the primroses for the two types of community are similar. Likewise the calyces of cowslips growing together with and apart from primroses are comparable. It is impossible though to compare the calyces of cowslips and primroses directly as the lines of best fit through their scatter diagrams are not even approximately parallel.

TABLE 42.

Colony	Average slope of the line of best fit (Tan $\measuredangle$)
Primrose alone	.2560
Primrose - cowslips or F1 hybrids	.2627
Cowslips - primroses or F1 hybrids	.1206
Cowslips alone	.0959

Table 42: The average slopes of the lines of best fit through the scatter diagrams for tooth length and calyx circumference for each of the English colonies measured.

However, this is not a serious difficulty as it is by comparing cowslips growing together with and apart from primroses and *vice versa* that evidence of introgression between the species will be detected. The appropriate comparisons were made by obtaining the index values for the calyces of all the English pin flowers that had been measured. The index values were determined from a diagram similar to Figure 18 in which the directions and scales of the index axes are shown relative to the mean slopes of the lines of best fit through the scatter diagrams. Not all the points of the scatter diagrams could have been shown because they were too numerous; instead the mean values have been plotted of the calyx measurements for the colonies whose index values were determined. For comparison the results for the Irish populations have been added to the Figure.

The index values which were derived are summarized in Figures 19 and 20.

From Fig. 19 it can be seen that there is little difference between the calyces of primroses that were collected from colonies growing together with or apart from cowslips. This analysis therefore provides little evidence for the introgression of primrose by cowslip. Had introgression occurred it might have been expected that primroses growing amongst cowslips would have had an average index value greater than those growing apart

Figure 18

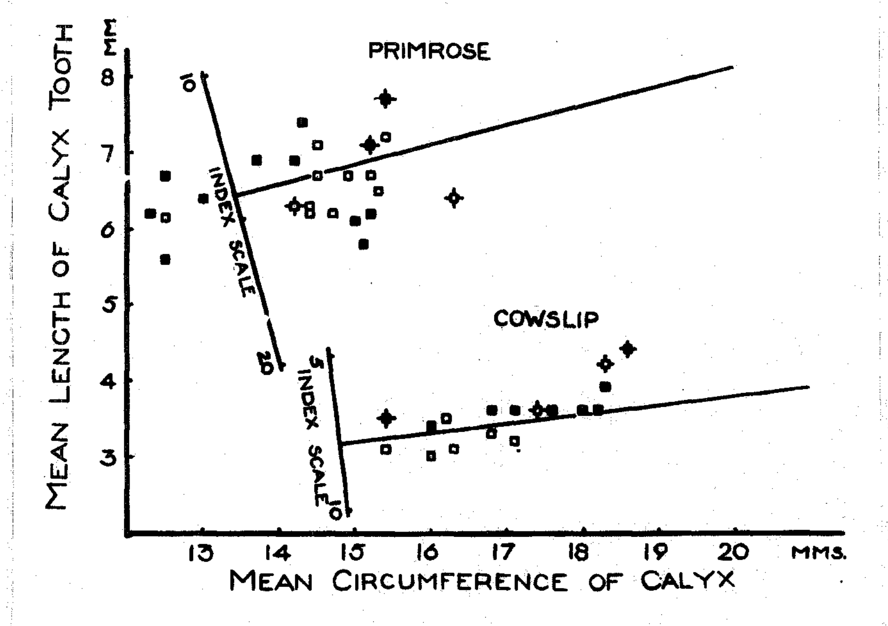


Figure 18 : The mean calyx circumference and calyx tooth length for the pin flowers of the various colonies of primrose and cowslip measured.

□	Hollow squares represent colonies of cowslips or primroses each growing to the exclusion of the other.
■	Solid squares represent colonies which contained both cowslips and primroses or either species plus apparent F1 hybrids.
✛	Squares with radiate arms attached represent Irish colonies.

Figure 19

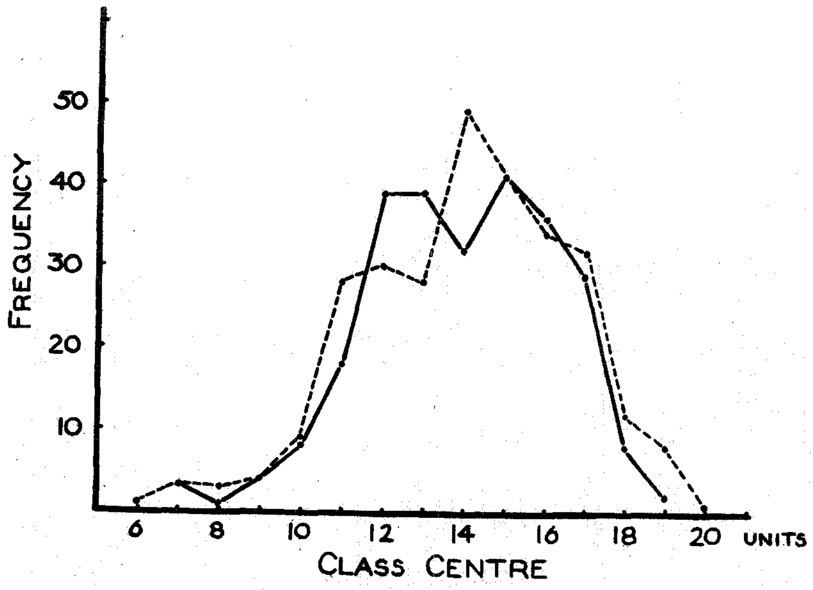


Figure 19 : Frequency polygons for the correlation indices of primroses growing together with or apart from cowslips or apparent F1 hybrids.

•———• Primroses growing apart from cowslips or apparent F1 hybrids.

•- - - -• Primroses growing together with cowslips or apparent F1 hybrids.

Figure 20

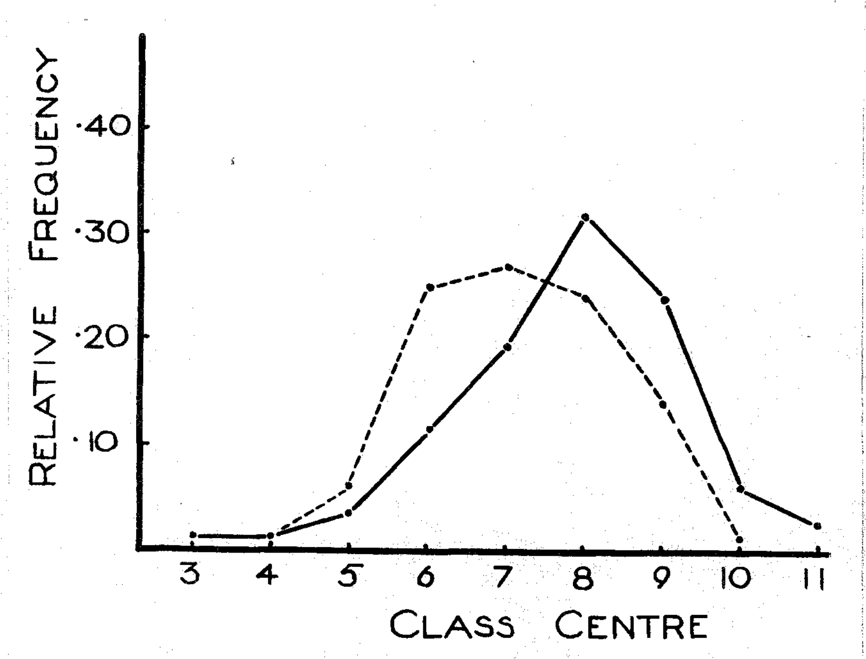


Figure 20 : Frequency polygons for the correlation indices of cowslips growing together with or apart from primroses or apparent F1 hybrids.

●——● Cowslips growing apart from primroses or apparent F1 hybrids.

●- - -● Cowslips growing together with primroses or apparent F1 hybrids.

from them, as can be seen from Figure 18.

From Figure 20 it can be seen that cowslips from colonies growing intermixed with primroses have an average index value which is less than that for cowslips growing apart. This would follow if introgression of cowslip by primrose had occurred, as can be seen from Figure 18.

The measurements for the English thrum flowers have not been analyzed by the correlation index method because a survey of the data revealed that the results would probably be similar to those obtained for the pin flowers.

The measurements for the calyces of the Irish pin flowers were not included in the analysis along with the English plants. For similar calyx circumferences the Irish Cowslip flowers appear to possess longer calyx teeth than the English flowers, Figure 18. More data are needed to confirm this observation.

(c) Correlation Coefficient Analysis.

The calyx dimensions of primroses and cowslips growing separately are positively correlated and in general an increase in calyx tooth length is accompanied by a proportional increase in calyx circumference. Since the calyx dimensions of the species are different, introgressed individuals of either are likely to differ from average in such a way as to be somewhat intermediate between the introgressed and the introgressing species.

With pairs of contrasting characters (p. 29) such as those of calyx tooth length and circumference of calyx the presence of introgressed individuals in a colony will result, if plotted, in a scatter diagram which extends more towards the introgressing species than usual, Figure 18. The degree of correlation between characters in such a diagram will be less than usual.

The results of calculating the correlation coefficient between the calyx dimensions for all the colonies measured are presented in Tables 43, 44, 45, 46, 47, 48, 49 and 50. The correlation coefficient between the diameter of the corolla and the circumference of the calyx has also been calculated as these form another pair of contrasting characters. Both sets of data are summarized in Tables 51 and 52. These Tables include the results from both the English and Irish samples as they appear to differ little in this respect.

TABLE 43

Locality	Correlation Coefficient	
	D. of C. – C. of C.	L. of C.T. – C. of C.
Farndale	.3258	.3502
Stanhope	.5546	.4547
Nr. Stokesley	.5132	.3978
Whale	.6146	.1669
Melmerby	.5349	.3914
Ullswater	.3645	.5111
Ravenbeck	.7500	.2198
Shincliffe	.5662	.1552
Cassop	.2305	.3852
Brancepeth	.7903	.2381
Brittas I	.5867	.2083
Corrofin I	.3825	.4467

Table 43: The correlation coefficients for two pairs of dimensions measured upon the flowers of pin primroses from colonies which included neither cowslips nor apparent F1 hybrids. I indicates an Irish locality.

TABLE 44

Locality	Correlation Coefficient	
	D.of C. – C.of C.	L.of C.T. – C.of C.
Blackhall i	.6509	.4423
Blackhall 2	.4565	.0822
Rievaulx	.5088	.3468
Grange-over Sands	.4585	.0109
Kettlewell	.4818	.5615
Penrith	.3654	.2689
Burnsall	.4112	.2989
Wolsingham	.3250	.2801
Rookdale	.5218	.4975
Nr. Hawnbey	.6443	.3846
Dalgany I	.6086	.0843
Corrofin I	.5898	.5615

Table 44: The correlation coefficients for two pairs of dimensions measured upon the flowers of pin primroses from colonies which contained either cowslips or apparent F1 hybrids. I indicates an Irish locality.

TABLE 45

Locality	Correlation Coefficient	
	D of C - C of C	L of C.T. - C of C
Farndale	.6796	.5158
Stanhope	.2973	.0170
Nr. Stokesley	.6491	.3779
Whale	.6305	.5061
Melmerby	.4694	.1671
Ullswater	.5519	.6358
Ravenbeck	.4665	.1616
Shincliffe	.4495	.4972
Cassop	.4047	.4645
Brancepeth	.3732	.3845
Brittas I	.3825	.4467
Corrofin I	.1468	.4176

Table 45: The correlation coefficients for two pairs of dimensions measured upon the flowers of thrum primroses from colonies which included neither cowslips nor apparent F1 hybrids. I indicates andIrish locality.

TABLE 46

Locality	Correlation Coefficient	
	D of C – C of C	L of C.T. – C of C
Blackhall i	.0697	.8172
Blackhall 2	.3857	.0939
Rievaulx	.1189	.0761
Grange-over-Sands	.0323	.4776
Kettlewell	.1757	.5043
Penrith	.4928	.4257
Burnsall	.2315	.5681
Wolsingham	.0191	.3414
Rookdale	.3659	.3011
Nr. Hawnbey	.3424	.0441
Dalgany I	.5044	.1282
Corrofin I	.5927	.1190

Table 46: The correlation coefficients for two pairs of dimensions measured upon the flowers of thrum primroses from colonies which contained either cowslips or apparent F1 hybrids.
I indicates an Irish locality.

TABLE 47

Locality	Correlation Coefficient	
	D of C - C of C	L of C.T. - C of C
Cassop	.8733	.4753
Quarrington	.7965	.3670
Elemore	.5410	.3268
Witton	.3353	.2067
Cayton Bay	.4690	.3041
Blackhall	.5249	.4736
Brittas I	.4185	.6840
Tullamore I	.5527	.3754

Table 47: The correlation coefficients between the dimensions of two pairs of characters measured upon the flowers of pin cowslips collected from colonies that contained neither primroses nor apparent F1 hybrids. I indicates an Irish locality.

TABLE 48

Locality	Correlation Coefficient	
	D of C - C of C	L of C.T. - C of C
Nr. Hawksley	.5531	.2390
Nr. Helmsley	.4718	.3418
Rievaulx Abbey	.3569	.4811
Nr. Rievaulx i	.6410	.4894
Nr. Rievaulx 2	.5431	.4113
Nr. Penrith	.4393	.3963
Nr. Cowsley	.4724	.6445
Brittas I	.4504	.0959
Dalgany I	.7266	.2390

Table 48: The correlation coefficients between the dimensions of two pairs of characters measured upon the flowers of pin cowslips collected from colonies that included either primroses or apparent F1 hybrids. I indicates an Irish locality.

TABLE 49

Locality	Correlation Coefficient	
	D of C	L of C.T.
	–	–
	C of C	C of C
Cassop	.2211	.5082
Quarrington	.3231	.2169
Elemore	.4746	.6859
Witton	.7169	.3294
Cayton Bay	.8256	.3801
Blackhall	.6246	.4626
Brittas I	.2442	.0728
Dalgany I	.5097	.3063

Table 49: The correlation coefficients between the dimensions of two pairs of characters measured upon the flowers of thrum cowslips collected from colonies that contained neither primroses nor apparent F1 hybrids. I indicates an Irish locality.

TABLE 50

Locality	Correlation Coefficient	
	D of C - C of C	L of C.T. - C of C
Nr. Hawksley	.6076	.2675
Nr. Helmsley	.7395	.5017
Rievaulx Abbey	.6909	.4007
Nr. Rievaulx i	.6238	.4237
Nr. Rievaulx 2	.4069	.2564
Nr. Penrith	.5580	.5572
Nr. Cowsley	.5139	.4367
Brittas I	.5668	.2764
Dalgany I	.4056	.6841

Table 50: The correlation coefficients between the dimensions of two pairs of characters measured upon the flowers of thrum cowslips from colonies that contained either primroses or apparent Fl hybrids. I indicates an Irish locality.

TABLE 51

Colony	No. of Colonies	Weighted Average Correlation Coefficient	
		D. of C. – C. of C.	L. of C.T. – C. of C.
Pure Primrose	12	.537	.336
Mixed Primrose	12	.508	.327
Pure Cowslip	8	.598	.414
Mixed Cowslip	9	.523	.380

Table 51: The weighted average correlation coefficients for two pairs of dimensions of the pin flowers of primrose and cowslip from two types of colony. Pure colonies are those where each species grew to the exclusion of the other; in mixed colonies the species grew intermixed or together with apparent FI hybrids.

TABLE 52

Colony	No. of Colonies	Weighted Average Correlation Coefficient	
		D. of C. – C. of C.	L. of C.T. – C. of C.
Pure Primrose	12	.470	.398
Mixed Primrose	12	.282	.388
Pure Cowslip	8	.530	.388
Mixed Cowslip	9	.591	.454

Table 52: The weighted average correlation coefficients for two pairs of dimensions of the thrum flowers of primrose and cowslip from two types of colony. Pure colonies are those where each species grew to the exclusion of the other; in mixed colonies the species grew intermixed or together with apparent FI hybrids.

It can be seen from Table 51 that for the pin flowers of both cowslip and primrose, the weighted average correlation coefficient between calyx circumference and either calyx tooth length or corolla diameter, was less for either of the species growing intermixed than it was for them growing separately.

The results are however not so consistent for the data from thrum flowers, Table 52. With these, primroses growing intermixed with cowslips had lesser weighted average correlation coefficients than those for primroses growing separately. The results for cowslips behave in the reverse way and those growing together with primroses have weighted average correlation coefficients greater than those for cowslips growing separately.

The correlation coefficients have been averaged after transforming to z and weighting where necessary. The z values have been reconverted to r for presentation in Tables 51 and 52. None of the differences observed between the weighted average correlation coefficients for the pure and mixed colonies are significant at the 5% level except that for the difference in correlation between diameter of corolla and circumference of calyx for thrum primrose flowers.

Considering both pin and thrum flowers the correlation coefficient analysis suggests that primrose is being introgressed by cowslip where they grow intermixed. Introgression of cowslips by primrose is suggested by the data from the pin but not by those from the thrum flowers.

(d) Analysis of apparent F1 hybrids.

The variability of the corolla diameter of plants collected as apparent F1 hybrids tends to be greater than would have been expected from a knowledge of the variability of the known F1 hybrids grown in the experimental garden, Table 53

TABLE 53

Habitat	Coefficient of Variation					
	Primrose		F1		Cowslip	
	Pin	Thrum	Pin	Thrum	Pin	Thrum
Field	.12	.13	.15	.13	.13	.13
Garden	.10	.08	.07	.08	.13	.14

Table 53: The variability of the corolla diameter of flowers collected in the field and grown in the experimental garden.

The cowslips, primroses and F1 hybrids from the experimental garden each belonged to one or two families so it would be expected that the corolla diameters of these would be less variable than those of field populations. The differences however between the variabilities of the corolla diameters of the flowers of cowslip and primrose from the field and garden are relatively slight. On the other hand the apparent F1 hybrids from the field are twice as variable as their garden counterparts.

This extra variability of the apparent F1 hybrids suggests that they may have included some backcross individuals. Such an interpretation must be treated cautiously for nothing is known of the relative plasticity of the hybrid compared with its parents.

5. DISCUSSION

(a) Results

The various analyses of the data indicate that some reciprocal though slight introgression of cowslip and primrose has occurred. The introgression has been slight at least in terms of the characters studied but a priori these appear to be satisfactory. It would have been surprising had no introgression been detected because the field evidence for the occurrence of hybrids other than the F1 appears to be quite definite.

When discussing the cowslips and primroses of Northumberland and Durham Harrison (1931) stated that he had occasionally seen hybrids other than the F1 but went on to say "it must be confessed that ninety per cent of the hybrid plants observed result from first crosses." Near Lake Como in Italy, the apparent F1 hybrid is reported to be very common and in some places may be as abundant as its parents (Pugsley 1927). There the hybrids are very variable suggesting that some backcrossing may have occurred, but it does not appear to have been frequent enough to influence the appearance of the adjacent cowslips and primroses.

From Ireland Webb (1951) reported the existence of apparent backcrosses and stated that the cowslip was more frequently the recurrent parent. The writer has visited the areas studied by Webb and here too the commonest hybrids apparently belonged to the F1 generation.

These accounts suggest that though the backcross hybrids are infrequent they do occur in some localities. The only undoubted backcross seen by the writer in this present study was a backcross cowslip growing near Rush about 12 miles north of Dublin. As some backcross hybrids closely resemble their recurrent parent they may be more common than has been suspected. Nevertheless, they cannot be abundant because in those areas where both species and the apparent F1 hybrids are growing, backcross individuals are seldom recognizable.

The failure of the species to introgress extensively does not appear to be determined by their ecological requirements for they often grow intermixed and have overlapping flowering periods. It is possible that backcross and other hybrids are unable to compete successfully with their parents, but there is little evidence of this in their behaviour under cultivation.

It is possible that introgression has been relatively limited because of the infrequency and infertility of the hybrids. Though the apparent F1 hybrids are well known in nature they are seldom abundant in any locality, especially in the south of England (Christy 1923). At most, a few only are found in the company of a large number of the parental species. The apparent F1 hybrids are usually found where both the cowslip and primrose are growing intermixed or adjacent, but in several localities they have been found growing amongst cowslips only. In some of these places it has not always been possible to find primroses growing anywhere in the vicinity.

The artificial F1 hybrids are relatively infertile even when grown under experimental conditions. Nothing is yet known of the seed fertility of the apparent F1 hybrids observed in the field, but it has been determined that their pollen viability is similar to that of the artificial F1 hybrids. The percentage of viable pollen (tested by staining in acetocarmine), produced by apparent Fi hybrids in the field is about a third that of the cowslip and the primrose. Whether this hybrid pollen can compete successfully with that of the parent species is not known, but by virtue of its lesser viability it may be less effective.

From the analyses it would appear that introgression has been reciprocal. The results of the analyses are summarized in Table 54 from which it can be seen that although not all the analyses yielded evidence of introgression most did. Webb (l.c.) has suggested that in Ireland the introgression occurs in one direction only, from primrose into cowslip. This may be so, but from a study of several Irish populations the writer is of the opinion that the situation there is similar to that in England. However, more quantitative data are required to test this view.

TABLE 54

Analysis		Evidence of Introgression			
		Primrose into Cowslip		Cowslip into Primrose	
		Pin	Thrum	Pin	Thrum
Comparison of Means		−	+	+	+
Correlation Index		+	×	−	×
Correlation Coefficient	$\frac{\text{D. of C.}}{\text{C. of C.}}$	+	−	+	+
	$\frac{\text{L. of C.T.}}{\text{C. of C.}}$	+	−	+	+

Table 55: A summary of the results of the various analyses to test for the occurrence of introgression. Test positive = +; test negative = −; test not undertaken = ×.

Finally it must be said that introgression may have been greater than that observed. A colony of either species was judged to be non-introgressed if the other species or the apparent F1 hybrid was absent. Whether this is true it is impossible to know, just as it cannot be said that mixed stands of the species were necessarily introgressed. It is possible that the method employed of sampling the species was unsatisfactory, but there appeared to be no alternative.

It may be noted that the primrose and cowslip from Ireland possess flowers whose dimensions appear to be different from those growing in the north of England.

(b) Comparison with primrose-oxlip hybrids.

It is interesting to compare the results quoted here for primrose-cowslip hybrids with those presented by Valentine (1948) when discussing hybridization between the oxlip and the primrose. These two species hybridize more readily in controlled experiments than do the primrose and cowslip and the F1 hybrid produced is almost as fertile as the parent species. Under these circumstances it might be expected that hybridization between the oxlip and primrose would be extensive where their habitats coincided. Yet this was not so, for in such areas the apparent F1 hybrids were frequently common and plants resembling backcross and other hybrids were only occasionally found. Although a biometrical study of the populations was not made, it did not appear that introgression was extensive.

In those areas where hybrids between the oxlip and primrose were found it is surprizing that hybridization between them has not proceeded farther. The internal barriers separating the species are apparently overcome with the production of the F1 hybrid. The seeds that give rise to the F1 plants are smaller than those of the oxlip or primrose but the F1 plants show a marked degree of hybrid vigour, and from the number of apparent F1 hybrids found in the field it would appear that this hybrid vigour may be responsible for their success. A further factor contributing to their frequency may be their relative longevity for under garden conditions the Fi hybrids live longer than either the primrose or the oxlip.

The failure of the F1 hybrid to give rise to extensive hybrid swarms in the field may be due to the loss of hybrid vigour in the backcross and F2 generations. Experiments to test this view are proceeding but few data are yet available. Still it is already evident that the F2 generation is much less vigorous than either of the parent species or the F1 generation. This may be conveniently expressed in terms of the percentage of plants which flowered in their first season, Table 55.

TABLE 55

Family	Percentage of plants flowering	
	Series A	Series B
Primrose	100	86
Backcross Primrose	94	89
F1	92	100
Backcross Oxlip	100	83
Oxlip	100	83
F2	72	56

Table 55: The percentage of plants flowering in their first season for primrose, oxlip and a series of hybrids between them. Series A was grown in cool frames, Series B was grown in the open.

It may be tentatively suggested that the failure of the oxlip and primrose to hybridize extensively may be due to the differences in their ecological requirements and the inability of the backcross and F2 hybrids to compete successfully with their parents. The apparent F1 hybrids may be reasonably abundant because they are more vigorous and longer lived than the oxlip and primrose.

It is possible that a similar situation obtains with the primrose-cowslip hybrids. The artificial F1 hybrid between them appears to possess some hybrid vigour and field evidence suggests that the apparent F1 hybrids may be long-lived. Clusters of apparent F1 hybrids are not infrequently found, the members of which are all pin or thrum plants. Though they are quite separate it is usually evident that they have arisen by cloning.

Thus F1 hybrids though only rarely formed because of low seed compatibility may slowly accumulate in certain populations, though never reaching a very great abundance. As already suggested the low density of the apparent F1 hybrids and their relative infertility will very much lessen the chances of introgression.

6. SUMMARY.

Evidence for the occurrence of introgression between primrose and cowslip has been sought by the biometrical study of the species collected from many localities in the north of England and in Ireland.

The dimensions of certain floral characters of the species growing separately and together were measured. These were then subjected to various analyses to determine whether they provided evidence of introgression. The inheritance of these dimensions had been previously investigated by studying families of primrose, cowslip and certain artificial hybrids between them.

The analyses of the data indicated that there was apparently a slight amount of reciprocal introgression between the species but not enough to alter appreciably the floral morphology of either.

It has been suggested that the failure of the species to introgress appreciably probably results both from the relative rarity and infertility of the first generation hybrid, and possibly from the inability of hybrids other than the first generation to compete successfully with the primrose and cowslip.

There was some indication that the cowslips and primroses in Ireland have somewhat different floral dimensions from those in the north of England.

SUMMARY AND CONCLUSION.

In this thesis the writer's primary interest has been in the study of hybrid populations and introgression. The main difficulty in such problems is that of devising suitable methods for dealing objectively with the data from field populations. Hence much time was devoted to the exploration of existing methods and to attempts to devise new ones. It was found that new methods are in fact needed where the hybrids are not very conspicuous or extensive.

These methods have been described in the first part of the thesis: They can be briefly summarized as follows.

When the flora is well known and the species have been defined and their ranges delimited hybrid plants may often be recognized from their morphology. With such plants further support for their hybrid origin may be obtained from a comparison of their ranges and habitats with those of the named parent species. Hybrid swarms in such circumstances may be further analyzed by comparing the variabilities of the species where they are believed to be hybridizing with the same species in areas where they grow separately. Whether the variation between plants in a putative hybrid swarm is concordant or discordant may also provide corroborative evidence of its parentage. Another indication of the hybrid status of a plant is to study the variability of its offspring. This may be particularly useful if the variabilities of the progeny of some members of th putative parent species are also available for comparison.

When the hybrids are rare or inconspicuous resort must be had to other methods. These are somewhat refined techniques based in general upon the assumption that the dimensions of the properties investigated are normally distributed or that normality can be achieved by a suitable transformation. Unfortunately such techniques may only be applied satisfactorily to characters whose dimensions are not markedly influenced by the environment. Unless care is taken to establish this, differences observed between populations may merely reflect the influence of the differences between the environments from which the populations were taken.

A method of minimizing the influence of the environment when dealing with pairs of correlated characters has been proposed.

Populations regarded as introgressed may be compared with those which are taken as non-introgressed in terms of the differences between the means, variances and degrees of skewness for particular characters. With pairs of characters the slopes of the lines of 'best fit' through their scatter diagrams may be compared. The strength of correlation between pairs of contrasting characters may also be compared. Where several characters have been measured apparently introgressed and non-introgressed populations may be compared in terms of a discriminator, which combines the measurements in a linear function in such a way as to reduce the differences within populations to a minimum compared with that within populations.

The remainder of the thesis is concerned with the investigation of two situations in which the above methods were employed. Not all the techniques were suitable to each problem, but all have been illustrated in one or other situation except that of comparing populations in terms of their skewness.

For *Juncus* the following techniques were employed:

(a) Comparison of means.
(b) Comparison of variances.
(c) Comparison of the slopes of the lines of 'best fit'
(d) Comparison of Correlation coefficients.
(e) Discriminant analysis

For *Primula* the following techniques were employed.

(a) Comparison of means.
(b) Comparison of variabilities (Coefficient of Variation)
(c) Comparison of the slopes of the lines of 'best fit'
(d) Correlation index analysis.
(e) Correlation coefficient analysis.

Within each of these genera it appeared that, although slight introgression had occurred between the species investigated the effects were very subtle. Not all the analyses yielded results that were statistically significant, though most of them gave answers that were consistent with the view that introgression had occurred. These answers might have been raised to the level of significance with larger samples but it was not possible in the time available to test this view. Larger samples would also assist in evaluating the importance of the results from the analyses which did not yield evidence of introgression.

The situation in Juncus was complicated by the discovery of two possibly ecotypes, a meadow and a woodland form, within J. effusus. Evidence for the introgression of the woodland form of J. effusus into J. inflexus was sought by comparing the dimensions of certain inflorescence and floral dimensions for J. inflexus growing together with and apart from J. effusus. Though it was found that the average dimensions of J. inflexus differed somewhat in the two types of colony studied, the differences were very slight and were only satisfactorily demonstrated by a discriminant analysis of the data. Very few putative hybrids were found and the introgression of J. inflexus by the meadow form of J. effusus did not appear to have proceeded very far. This is probably due to the sterility of the hybrids and the difficulty of making the interspecific cross.

Further work on this pair of species might be profitably directed towards a more detailed study of the possible ecotypes within *J. effusus* and further attempts should be made to artificially cross these species.

The situation in *Primula* was much clearer than that in *Juncus* because apparent Fl hybrids were found in many localities. The great scarcity of putative backcross plants made it necessary to compare populations for evidence of introgression. Comparisons were made of the floral dimensions of both cowslips and primroses growing together and in separate colonies. The evidence for reciprocal introgression was fairly definite but as with *Juncus* the amount was slight. The reasons here seemed to be connected again with the difficulty of making the interspecific cross and also its infertility. In addition, it was suspected that hybrids other than the Fl are unable to compete successfully with their parents and that this accounted for the absence in the field of hybrids other than the apparent Fl generation.

It would be interesting to test this view experimentally. This might be done by experiments similar to those already referred to, in which the relative vigour of the primrose, oxlip and certain hybrids between them is being studied.

Few studies have been based on species whose breeding behaviour and artificial hybrids are as well known as those of *Primula vulgaris* and *P. veris*. Much, especially of the American literature on introgression refers to examples in which the introgression has been inferred, and usually there has been no attempt to confirm the hybrid origin of the putative hybrids. There are exceptions however, two of which will be considered.

Heiser (1951) reported introgression between two species of *Helianthus* whose Fl hybrid was highly sterile producing only small amounts of viable pollen and a few seeds when backcrossed to one of the parent species. From breeding studies in the genus *Aster* Avers (1953) knew that several species were capable of hybridizing and backcrossing to produce fertile offspring, yet she found only occasional hybrids in the wild. She furthermore observed that even where the species ranges overlapped "No extreme modification of the species pattern as a result of hybridization was evident."

From these two examples and the *Primula* study reported above it is evident that even though the artificial Fl hybrid between species may be partially or fully fertile this is not sufficient data from which to predict whether introgression between them will have occurred. This may be determined only from field studies of the species.

LITERATURE CITED.

ANDERSON, E.A. and WHITAKER, T.W. 1934 Speciation in Uvularia. Journ. Arnold Arboret. 15, 28

ANDERSON, E.A. 1949 Introgressive Hybridization. John Wiley and Sons.

ANDERSON, E.A. 1951 Concordant versus discordant variation in relation to introgression Evolution 5, 133.

AVERS, C.J. 1953 Biosystematic studies in Aster. 1 Crossing relationships in the Heterophylli. Am. Jour. Bot. 40, 669.

CHRISTY, M. 1923 Primula vulgaris var. caulescens. New Phyt. 22, 233.

CLAPHAM, A.R., TUTIN, T.G., and WARBURG, E.F. 1952 Flora of the British Isles, Cambridge University Press.

CLIFFORD, H.T. 1954 Analysis of suspected hybrid swarms in the genus Eucalyptus. Heredity 8, 259.

CLIFFORD, H.T. and BINET, F.E. 1954 A quantitative study of a presumed hybrid swarm between Eucalyptus elaeophora and E. goniocalyx. Aust. Journ. Bot. 2, 325.

CLIFFORD, H.T. 1955 An index for use in quantitative taxonomic problems. New Phyt. 54, 132.

DAVEY, V. M^{c}M and LANG, J.M.S. 1939 Experimental taxonomy III Correlation of characters within a population. New. Phyt. 38, 32.

EAST, E.M. 1950 As quoted in Principles of Genetics by E.W. Sinnott et al., 1950 M^{c}Graw Hill Book Co.

FEY, L. 1929 Untersuchungen zur Phänanalyse des Artbastardes Primula variabilis Goupil (P. veris L. em Hudson x P. vulgaris Hudson), der Elternarten und von Primula elatior (l.) Schreber. Institut für Allgemeine Botanik der Universität Zürich. II. Serie No. 10.

FISHER, R.A. 1936 The use of multiple measurements in taxonomic problems. Ann. Eugen. Lond. 7, 179.

HARRISON, J.W.H. 1951 The Northumberland and Durham Primulas of the section Vernales. Trans. North. Nat. Union 1, 48.

HEISER, C.B. 1951 Hybridization in the annual sunflowers Helianthus annuus x H. debilis var. cucumerifolius. Evolution 5, 42.

HESLOP-HARRISON, Y. 1953 Nuphar intermedia Ledel. A presumed relict hybrid, in Britain. Watsonia 3, 7.

HOPPE, D.H. 1819 Botanische Notizer Flora 1, 182.

MacDOUGAL, D.T. 1907 Hybridization in wild plants. Bot. Gaz. 43, 45.

MELVILLE, R. 1951 On the application of biometrical methods in plant taxonomy. Proc. Linn. Soc. 162, 153.

PUGSLEY, H.W. 1927 Primula hybrids. Journ. Bot., Lond., 65, 351.

RICHARDS, P.W. and CLAPHAM, A.R. 1941 Biological Flora of the British Isles, *J. effusus* L. Journ. Ecol. 29, 375.

RAO, C.R. 1952 Advanced statistical methods in Biometric Research. John Wiley and Sons. Inc.

RUSSELL, N.H. 1954 Three field studies of hybridization in the stemless white violets. Am. Journ. Bot. 41, 679.

SALMON, C.E. 1931 Flora of Surrey. G. Bell and Sons. Ltd.

SIBLEY, C.G. 1954 Hybridization in the Red-Eyed Towhees of Mexico. Evolution 8, 252.

TEDIN, O. 1925 Vererbung, Variation und Systematik in der Gattung *Camelina*. Hereditas 6, 275.

VALENTINE, D.H. 1948 Studies in British Primulas 2. Ecology and taxonomy of primrose and oxlip (*Primula vulgaris* Huds. and *P. elatior* Schreb.) New Phyt. 47, 111

VALENTINE, D.H. 1955 Studies in British Primulas 4. Hybridization between *Primula vulgaris* Huds. and *P. veris* L. New Phyt. *54*, 70.

WEBB, D.A. 1951 Hybrid plants in Ireland. Irish Nat. Journ. *10*, 201.

WHITEHEAD, F.H. 1954 An example of taxonomic discrimination by biometric methods. New Phyt. *53*, 496.

APPENDIX A

Reprinted from the
AUSTRALIAN JOURNAL OF BOTANY
VOLUME 2, NUMBER 3, PAGES 325-336, 1954

A QUANTITATIVE STUDY OF A PRESUMED HYBRID SWARM BETWEEN *EUCALYPTUS ELAEOPHORA* AND *E. GONIOCALYX*

By H. T. CLIFFORD and F. E. BINET

A QUANTITATIVE STUDY OF A PRESUMED HYBRID SWARM BETWEEN *EUCALYPTUS ELAEOPHORA* AND *E. GONIOCALYX*

By H. T. CLIFFORD* and F. E. BINET†

(*Manuscript received March 5, 1954*)

Summary

From measurements of fruit lengths, fruit weights, and lengths of fruiting umbels of trees from the species *Eucalyptus elaeophora* and *E. goniocalyx* and their presumed hybrid swarm, a formula is calculated for taxonomic classification using statistical discriminant theory. The rates of misclassification expected with the use of this formula are estimated. A simple geometrical model of the relationship of the species and the hybrid is investigated.

I. INTRODUCTION

(*a*) *Preliminary Remarks*

In many localities in south-central Victoria there are stands of trees whose taxonomic position appears intermediate between *Eucalyptus elaeophora* F. Muell. and *E. goniocalyx* F. Muell. One of the authors (Clifford 1953, 1954) suggested previously that these intermediate trees are hybrids between *E. elaeophora* and *E. goniocalyx*. Although not yet confirmed by experiment, this relationship is assumed hereafter to save qualifying the terms "hybrid" and "parent" each time they occur.

The usual taxonomic classification of trees involves an unavoidable subjective element; although experience reduces the errors committed in this procedure, there is no easy method of assessing the extent of erroneous classification.

(*b*) *On Discriminant Functions*

In the statistical technique of discriminant analysis (Fisher 1936) we find a taxonomic tool almost entirely free from subjective impressions. By the use of the discriminant function, a tree is assignable to one of the two species or the swarm under consideration on the basis of numerical measurements alone. There is no doubt that misclassifications may occur in this process, but reliable estimates of the expected percentage rates of misclassification are easy to calculate.

Furthermore, a generalization of discriminant analysis (Fisher 1938) yields a geometrical picture of the interrelations of the species studied. A genetic model to account for these interrelations is under consideration.

Discriminant functions are linear combinations (that is, weighted sums and/or differences) of the numerical values of the measurements. In some cases, it might be convenient to use for these functions a scale

---

* Botany Department, University of Melbourne; present address: Botany Department, University of Durham.

† Department of Statistics, University of Melbourne.

different from the one in which the actual measurements were carried out (cf. Mather 1938); this is achieved by the use of certain functions (e.g. logarithms) of the measurements, rather than of the measurements themselves.

The coefficients in the linear combinations are chosen so as to maximize the ratio of the variance between the means of samples from different species to the variance within the samples. In other words the weights (which may be positive or negative) are adjusted so that interspecies differences are emphasized at the expense of intraspecies, individual differences.

This paper describes such an analysis of the measurements of properties made upon samples from each of 50 trees from a hybrid swarm and the same number of each of the parental species.

(c) *Collection of Material*

The parental species were sampled only in localities where interbreeding with the other parent or with a hybrid swarm could be reasonably excluded; in areas with a low rainfall gradient no collections were made unless the nearest hybrid or second parent was several miles away; in areas with steep rainfall gradient this minimum distance was reduced to 2 miles. These localities spread from the Southern Flinders Range, the Mount Lofty Range, and the Grampians Range through the Eastern Highlands as far north as Canberra for *E. elaeophora*; and from the Grampians Range along the Eastern Highlands to Mt. Wilson (N.S.W.) for *E. goniocalyx*.

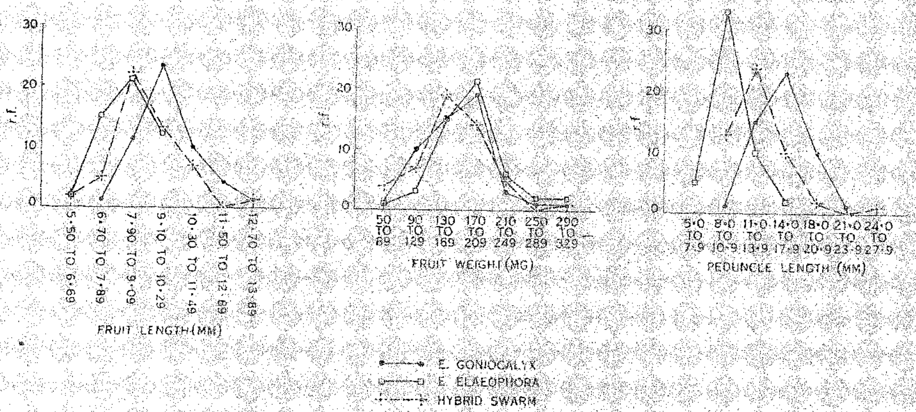


Fig. 1.—Frequency polygons for fruit length, fruit weight, and peduncle length in the three samples.

The hybrid swarm sampled was in a forest on a hillside between Upper Ferntree Gully and Tremont (two townships in the Dandenong Range about 20 miles east of Melbourne). In this forest samples were collected from the first 50 accessible trees apparently belonging to the group

E. elaeophora-E. goniocalyx. No difficulty was encountered in deciding whether a tree belonged to this group or not, because all the other *Eucalyptus* species present were very different from those being studied. (They were *E. radiata, E. obliqua, E. viminalis,* and *E. macrorrhyncha.*)

TABLE 1

SAMPLE MEANS AND VARIANCES

		E. elaeophora	Hybrid Swarm	*E. goniocalyx*
Fruit weight (mg)	Mean	186.02	159.78	166.20
	Variance	2090.9	2080.91	2097.31
Fruit length (mm)	Mean	8.226	8.928	9.920
	Variance	0.8665	1.616	1.413
Peduncle length (mm)	Mean	10.058	13.012	15.864
	Variance	3.685	9.529	6.963

From each of the 150 trees sampled, 10 fruits were taken at random and their weights and lengths measured. A similar procedure was adopted for peduncle lengths of fruiting umbels. These organs were chosen because they were mature and growth in them had ceased. The weight of each fruit as well as its length was measured because, for a given length, weight was very dependent on shape, and this had been shown to be characteristically different in the two species.

II. STATISTICAL ANALYSIS

(*a*) *Processing the Measurements*

The measurements are presented as frequency polygons in Figure 1, from which it can be seen that for each set of measurements the values for the parents and the hybrids overlap considerably. If, however, the characters are considered in pairs and their measurements plotted in two-dimensional scatter diagrams, it can be seen from Figure 2 that any of the three pairs divides the parents reasonably sharply. The hybrids

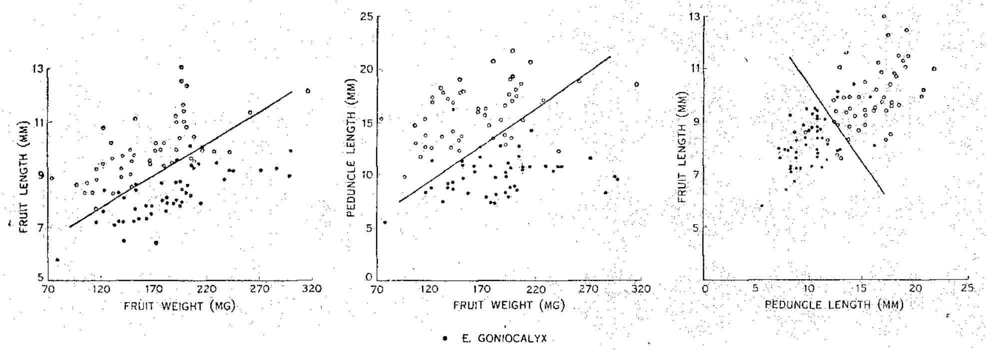


Fig. 2.—Scatter diagrams for the three pairs formed from three measurements in the parental samples.

were omitted here as they would have obscured the dividing line between the parental species. The straight lines were drawn by eye and represent crude approximations to standard linear discriminant functions.

Table 2

TRANSFORMED SAMPLE MEANS

	W	L	P
E. elaeophora	122.10	91.20	99.78
Hybrid swarm	115.74	96.62	113.34
E. goniocalyx	117.42	99.36	125.50

The first step towards statistical analysis was the computation of the sample means and variances of the original measurements. These are shown in Table 1.

The measurements were transformed so as to satisfy approximately an assumption underlying the standard technique of discriminant analysis, namely equality of population variances. The transformations follow:

$$\begin{aligned} W &= 10 \times (\text{fruit weight in mg})^{\frac{1}{3}} \\ L &= 100 \times \log_{10} (\text{fruit length in mm}) \\ P &= 10 \times (\text{peduncle length in mm})^{\frac{1}{2}}. \end{aligned}$$

Tests on the transformed measurements showed no evidence of non-normality.

Table 3

TRANSFORMED SAMPLE VARIANCES AND COVARIANCES

		W	L	P
E. elaeophora	W	103.68	37.45	27.90
	L	37.45	24.73	25.47
	P	27.90	25.47	91.27
Hybrid swarm	W	138.11	57.18	75.11
	L	57.18	37.26	49.66
	P	75.11	49.66	167.49
E. goniocalyx	W	117.72	37.78	49.26
	L	37.78	25.95	29.04
	P	49.26	29.04	112.91

Tables 2 and 3 summarize the means, variances, and covariances of the transformed measurements. These tables contain all the information needed for discriminant analysis.

(b) *Comparison of the Populations*

(i) *Comparison of Variances.*—The corresponding sample variances for the parental species and the hybrid swarm were compared by means of the F-test. The differences between the parental species were not significant at the upper $2\frac{1}{2}$ per cent. point (corresponding to a 5 per cent. two-tailed level), thus indicating that the transformations set out in the section above were successful. The appropriate parental sample variances were pooled and compared with the corresponding sample variances in the hybrid swarm (see Table 4). All the characters appear more variable in the hybrids than in the samples from the parental species, but only for P is this difference significant.

TABLE 4

COMPARISON OF SAMPLE VARIANCES

	W	L	P
Parents pooled	110.70	25.34	102.09
Hybrid swarm	138.11	37.26	167.49
Ratio H.S./P.P.	1.248	1.470	1.641*

* Significant at the 5 per cent. level.

(ii) *Comparison of Correlations.*—Using Fisher's method (see e.g. Snedecor 1946, p. 151) the corresponding correlation coefficients in the samples from the parental species and in the sample from the hybrid swarm were compared. All three correlation coefficients in the hybrid sample were larger than the corresponding parental ones, but none significantly (Table 5).

TABLE 5

SAMPLE CORRELATIONS

	W and L	W and P	L and P
E. elaeophora	0.739	0.287	0.536
Hybrid swarm	0.797	0.494	0.629
E. goniocalyx	0.684	0.427	0.537
Pooled, within parent species	0.710	0.363	0.536
Pooled, within all three groups	0.745	0.417	0.576

(iii) *Comparison of Dispersions.*—The tests described in the above two sections do not supply evidence for discrepancy between the correlation structures of the three populations. Nevertheless a trend seems to appear pointing towards larger variances and covariances in the hybrid swarm than in the parental species. As the tests indicating this trend are not independent, their results cannot be pooled. However, a blanket-test exists to reveal overall differences between correlation structures; this test consists of the comparisons of the determinants of the dispersion matrices (Box 1949). This test appears particularly appropriate to our data as all the apparent discrepancies are of a comparable order and in the same direction.

Two tests were carried out: (1) comparison of the three determinants; (2) comparison of the determinant of the pooled parental sample dispersion matrix with that of the sample from the hybrid swarm. Neither test turned out to be significant.

(iv) *Comparison of Means*.—A study of the variances, correlations, and dispersions within the three samples revealed no evidence in favour of any discrepancy between the populations. It may be, however, that there are differences between the mean vectors of the three populations.* To investigate this we now postulate that the three dispersion matrices are identical in the populations and then proceed to apply the test devised by Wilks (1932).

The statistic serving as appropriate criterion is

$$\Lambda = \frac{|W|}{|B+W|}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where W is the pooled matrix of sums of squares and products within all the samples, around the individual sample centroids, and B is the matrix of sums of squares and products between the samples, around the centroid of all observations.

For the present data $\Lambda = 0.3456$. The significance of this value is tested by referring

$$F = \frac{1-\sqrt{\Lambda}}{\sqrt{\Lambda}} \cdot \frac{146}{3} = 34.12$$

to the F tables with 6 and 292 degrees of freedom (Rao 1952, pp. 260, 345). This turns out to be significant at the 0.1 per cent. level. We conclude that the mean vectors of the populations are fundamentally different.

(c) *Internal Analysis*

Assuming trivariate normal distribution of W, L, and P, a common dispersion matrix, and different mean vectors in the three populations, we can visualize a three-dimensional space, with three probability masses representing the three populations (two parental species and one hybrid). Within each of these masses the surfaces of equal probability are concentric, similar, and similarly situated ellipsoids; the centroids of the three masses are different. The three systems of concentric ellipsoids are similar and similarly situated, and the dimensions of the corresponding ellipsoids of the three systems are the same.

The parameters specifying this system are reliably estimated by the appropriate statistics calculated from samples of 50 individuals each. The arrangement of the centroids will be considered in the next section.

The internal structure of the masses is described by the lengths and directions of the principal axes of the ellipsoids. These were estimated

---

* We represent the situation by a three-dimensional space in which the centroids of the samples are points having as coordinates the sample means of W, L, and P. The assumption underlying the test employed is that all displacements between these three points are due to chance alone, i.e. that the three samples are random samples from the same multivariate population. The test, when yielding a significantly small critical value, refutes this assumption.

(following Pearson 1901) by calculating the latent roots and latent vectors of the pooled correlation matrix, i.e. of the matrix formed by the last row of Table 5, with units in the diagonal. The latent roots and latent vectors of this matrix are given in Table 6.

The latent vectors represent the directions of the principal axes in the w, l, p coordinate system, obtained on dividing the original variates W, L, P by their estimated standard deviations. The elements of these vectors are the cosines of the angles between the principal axis and the appropriate coordinate axis. The square roots of the latent roots estimate the half-lengths of the principal axes of the iso-probability surface whose distance from the centroid is unity when measured along any of the three original axes.

Tests of significance devised by Bartlett (1950, 1951) showed that the above values of the latent roots can be accepted as evidence that in the population (1) the ellipsoids are eccentric, i.e. they are not spheres; and (2) the length of the second largest principal axis is not zero, i.e. the ellipsoids do not degenerate into lines.

TABLE 6

LATENT ROOTS AND VECTORS OF THE SAMPLE DISPERSION MATRIX

Roots	w	l	p
2.169	0.583	0.627	0.517
0.604	—0.545	—0.170	0.821
0.227	0.602	—0.761	0.243

No significance tests exist to show that the length of the third principal axis is not zero, i.e. that the ellipsoids do not in fact degenerate into ellipses. We accept the non-zero estimate merely to render the visual image more suggestive.

(*d*) *Arrangement of Population Centroids*

(i) *Collinearity*.—The sample centroids were found to be non-collinear. If the population centroids are collinear, then the population value of the second latent root of the matrix $W^{-1}B$ is zero (Fisher 1938). If the sample value of the first latent root of this matrix is highly significantly large and the population value of the second root is zero, then the quantity

$$n \log_e(1 + a),$$

(where n is one less than the total number of individuals and a is the sample value of the second latent root) is distributed approximately as χ^2 with three degrees of freedom (Marriott 1952).

We obtain the following three roots, 1.145, 0.024, 0.000, the first of which is highly significantly large. Hence to test whether the non-collinearity of the sample centroids is compatible with an assumption of collinear population centroids we calculate

$$149 \log_e (1 + 0.024) = 3.55,$$

which is not significant, so that the assumption of collinearity is justified.

(ii) *Symmetry.*—The sample centroid of the hybrid swarm was slightly nearer to the centroid of *E. goniocalyx* than to that of *E. elaeophora.* To test for symmetry of the population centroids, a simple generalization of Wilks's test (see Rao 1952, p. 270) is applied.

The statistic used as criterion is

$$\Lambda' = \frac{| W |}{| W + B' |}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where B' is the matrix of the sums of squares and products due to asymmetry between the sample centroids. The significance of Λ' is tested by referring

$$F = \frac{1 - \Lambda'}{\Lambda'} \cdot \frac{147}{3}$$

to the F tables with 3 and 147 degrees of freedom.

Here $\Lambda' = 0.955$, $F = 2.33$, which is not significant. Hence we find no evidence that the arrangement of the population centroids is other than symmetrical.

(*e*) *Taxonomy*

The appropriate tests of significance showed that omission of any one of the three variates decreases significantly the separation of the sample centroids (Rao 1952, p. 252). Hence classification should be based on all the three variates.

As the population centroids are assumed collinear, a single discriminant function is all that is required. This function is a linear combination of W, L, and P, with coefficients proportional to the elements of the latent column vector which belongs to the largest latent root of the matrix $W^{-1} B$. The function obtained from the present data is

$$-0.9702W + 0.16117L + 0.05775P. \quad \ldots\ldots\ldots \quad (3)$$

The coefficients of this function are adjusted so that its estimated variance is unity. On rewriting in terms of the original measurements it becomes

$$\begin{aligned} &-0.9702 \times (\text{fruit weight in mg})^{\frac{1}{3}} \\ &+ 16.117 \times \log_{10} (\text{fruit length in mm}) \\ &+ 0.5775 \times (\text{peduncle length in mm})^{\frac{1}{2}}. \quad \ldots\ldots\ldots \quad (4) \end{aligned}$$

The mean values of this discriminant function in the three samples are shown in Table 7.

When applying the discriminant function to classify an individual tree, that tree is assigned to the population whose mean is nearest to the value calculated by substituting into the discriminant function (4) the average of the measurements taken on that tree. Accepting the sample means as the best estimates of the population means, a rule of classification is obtained. This rule is shown in Table 8.

TABLE 7

MEANS OF DISCRIMINANT FUNCTION

Population	Sample Mean of Discriminant Function
E. elaeophora	8.616
Hybrid swarm	10.567
E. goniocalyx	11.870

TABLE 8

CLASSIFICATION RULE BASED ON SAMPLE MEANS ONLY

Observed Value of Discriminant Function	Classification of Individual
⩽ 9.591	*E. elaeophora*
9.592 — 11.217	Hybrid
⩾ 11.218	*E. goniocalyx*

If we assume, on genetical grounds, that the centroid of the hybrid population is midway between the centroids of the parental populations, then we get an alternative rule of classification (Table 9). The two rules lead to different decisions only over two short intervals; in both intervals the classification is rather unreliable.

TABLE 9

CLASSIFICATION RULE BASED ON SAMPLE MEANS UNDER ASSUMPTION OF SYMMETRY

Observed Value of Discriminant Function	Classification of Individual
⩽ 9.428	*E. elaeophora*
9.429 — 11.056	Hybrid
⩾ 11.057	*E. goniocalyx*

Whichever of these two rules is used, misclassifications will almost always occur because the populations overlap. The expected percentage rate of misclassification is a measure of the reliability of the method. This rate is estimated by a method due to Wilks (1943, p. 144). These estimates are given in Table 10.

It can be seen that even when a combination of three measurements is used, the hybrids overlap considerably with the parents, especially with *E. goniocalyx*. The two parental species, however, separate well. This is shown in Figure 3, which may be compared with Figure 1.

In considering the overlap of the hybrid and parental populations it should be remembered that the sample presumed as coming from the hybrid swarm included all accessible trees in the stand studied, and so would have included trees from the parental species had these been present. Although no such individuals were observed, if they nevertheless occurred in the sample, then the estimated expected percentage rates of misclassification would understate the taxonomic value of the linear discriminant function.

Table 10

ESTIMATED EXPECTED PERCENTAGE RATES OF MISCLASSIFICATION

Tree Species	Tree Misclassified as	Rate, when Using Rule in	
		Table 8	Table 9
E. goniocalyx	Hybrid	25.8	21.2
E. goniocalyx	*E. elaeophora*	1.2	0.8
Hybrid	*E. elaeophora*	16.5	21.2
Hybrid	*E. goniocalyx*	25.8	21.2
E. elaeophora	Hybrid	16.5	21.2
E. elaeophora	*E. goniocalyx*	0.5	0.8

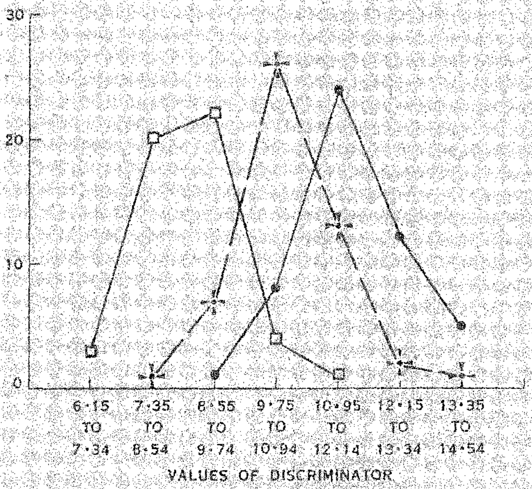


Fig. 3.—Frequency polygon for the discriminator in the three samples. Legend as in Figure 1.

III. Conclusion

It has been demonstrated that the data are consistent with the hypothesis that the tree populations are each trivariate normal with identical dispersion matrices, and with collinear and equally spaced centroids. Each centroid is the centre of a probability mass and the loci of equal probabilities are similar and similarly situated ellipsoids.

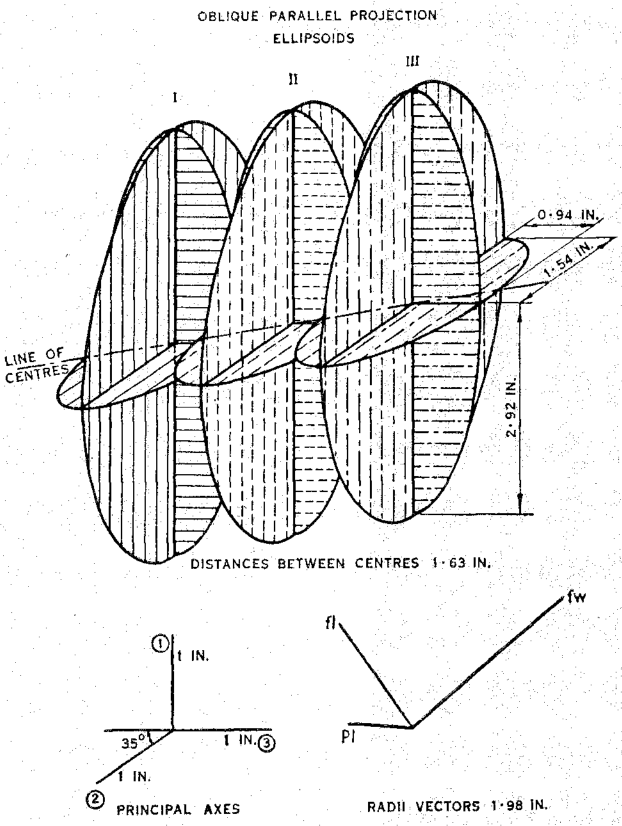


Fig. 4.—Isoprobability surfaces.

To obtain a simple illustration of the geometrical relationship between the three populations, the estimated dimensions of these ellipsoids for one selected probability, namely 1.992×10^{-4}, were calculated. A diagram of these ellipsoids is shown in Figure 4*. The axial lengths are 95 per cent. (two-sided) tolerance limits. Each ellipsoid contains 72 per cent. of the total probability mass in each instance.

The angles between the three principal axes and the line connecting the centres are 81°, 51°, and 139°. The direction of this line corresponds to the vector on which the taxonomic discriminant function is based.

Figure 4 does not add anything to the statistical analysis, all the relevant information being contained in Tables 2-10. The diagram is merely

---

* The symbols *fw*, *fl*, and *pl* denote the variates *w*, *l*, and *p*, as defined in the text. One in., according to the scale adopted, represents one unit of a variate.

intended as a pictorial representation of the overlap of the three populations and of the orientation of axes determining the internal structure of the three probability masses in relation to the arrangement of the centroids.

IV. ACKNOWLEDGMENTS

Our thanks are expressed to Professor J. S. Turner, for suggesting the problem, to Associate Professor M. H. Belz, for assistance in preparation of the paper, to Mr. D. Ashton, Mr. J. Robbins, Mr. A. Gregson, Mr. T. Arthur, Mr. K. Harrop, Mr. A. Hoyd, Mr. B. Davidson, Mr. C. D. Boomsma, Mr. R. Specht, Miss C. Eardley, and Miss K. Pike, for assistance in collecting the material, to Mr. W. M. Holmes, for preparing the diagram, and to Miss A. G. Doig, for assistance in the computations.

V. REFERENCES

BARTLETT, M. S. (1941).—The statistical significance of canonical correlations. *Biometrika* **32**: 29.

BARTLETT, M. S. (1950).—Tests of significance in factor analysis. *Brit. J. Psychol. Statist. Sect.* **3**: 77.

BARTLETT, M. S. (1951).—A further note on tests of significance in factor analysis. *Brit. J. Psychol. Statist. Sect.* **4**: 1.

BOX, G. E. P. (1949).—A general distribution theory for a class of likelihood criteria. *Biometrika* **36**: 334.

CLIFFORD, H. T. (1953).—On the distribution of the species of *Eucalyptus* in the region of the Dandenong Range, Victoria. *Proc. Roy. Soc. Vict.* **65**: 30.

CLIFFORD, H. T. (1954).—A method of analysing hybrid swarms with special reference to two *Eucalyptus* species. *Heredity* 8 (in press).

FISHER, R. A. (1936).—The use of multiple measurements in taxonomic problems. *Ann. Eugen., Lond.* 7: 179.

FISHER, R. A. (1938).—The statistical utilization of multiple measurements. *Ann. Eugen., Lond.* 8: 376.

MARRIOTT, F. H. C. (1952).—Tests of significance in canonical analysis. *Biometrika* **39**: 58.

MATHER, K. (1938).—"Biometrical Genetics." Ch. 3. (Methuen and Co. Ltd.: London.)

PEARSON, K. (1901).—The lines of closest fit to a system of points. *Phil. Mag.* **2**: 559.

RAO, C. R. (1952).—"Advanced Statistical Methods in Biometric Research." (John Wiley & Sons Inc.: New York.)

SNEDECOR, G. W. (1946).—"Statistical Methods." 4th Ed. (State Collegiate Press: Ames, Iowa.)

WILKS, S. S. (1932).—Certain generalizations in the analysis of variance. *Biometrika* **24**: 471.

WILKS, S. S. (1943).—"Mathematical Statistics." (Princeton University Press.)

APPENDIX B

Reprinted from HEREDITY, Volume 8 Part 2 pp. 259-269 August 1954

ANALYSIS OF SUSPECTED HYBRID SWARMS IN THE GENUS *EUCALYPTUS*

H. T. CLIFFORD
Botany School, University of Melbourne

Received 4.vi.53

I. INTRODUCTION

For arborescent species with long periods between successive generations and flowers borne on slender branches at great height, it is desirable to develop indirect methods for the study of cross breeding. Such a problem arises frequently in the Australian genus *Eucalyptus*, for which hybrid swarms have often been reported (Brett, 1938; Pryor, 1950). The following method of analysing such swarms differs from most of those previously described (Anderson, 1949) in that it requires a knowledge of the variability of the progeny of individual members of the hybrid swarm rather than a knowledge of the variability of the swarm itself.

2. EXPERIMENTAL MATERIAL

The two tree species, *E. elaeophora* F. Muell. and *E. goniocalyx* F. Muell. have been regarded as closely related by some (Bentham, 1866) and by others as belonging to different sections of the genus (Baker and Smith, 1920; Blakeley, 1934). They are not difficult to distinguish, but at several localities in Victoria they are linked by a series of intermediate forms. The relationship between the two species has been investigated by raising seedlings from selected parent trees and measuring their juvenile characteristics, also by study of the field distribution of the species and their suspected hybrids.

The species names are here accepted and used in the sense in which they are defined in the *Flora of Victoria* (Ewart, 1930), although a study of the type material of each would suggest that the name *E. elaeophora* should be suppressed in favour of *E. goniocalyx* and that the plant generally known as *E. goniocalyx* ought to be redescribed under another name. However, in Australia there exists no confusion as to which species is meant when the current names are used and the author (like Maiden, 1914) does not propose that they should be changed.

(a) Description of species

Eucalyptus goniocalyx is a large tree which may attain a height of up to 200 feet with a diameter of 6-8 feet at breast height. The bark is smooth, except at the base of the trunk where it may be rough and flaky for several feet. Although usually grey it is sometimes strongly mottled with yellow. Its typical habitat is a well-drained soil, usually on hillsides in areas receiving an annual rainfall of 40-50 inches. Such conditions are common on the seaward slopes of the Eastern Highlands and here *E. goniocalyx* is often a co-dominant of the forest.

Eucalyptus elaeophora, on the other hand, is a small tree seldom growing to more than 80 feet, with a diameter of about 2 feet. It has a rough flaky bark on all but the smallest branches. The usual habitat is on well-drained hillsides in places receiving an annual rainfall of about 18-30 inches. Except for a few localities in South Australia and Southern Victoria, the species is confined to the inland slopes of the Eastern Highlands, fig. 1.

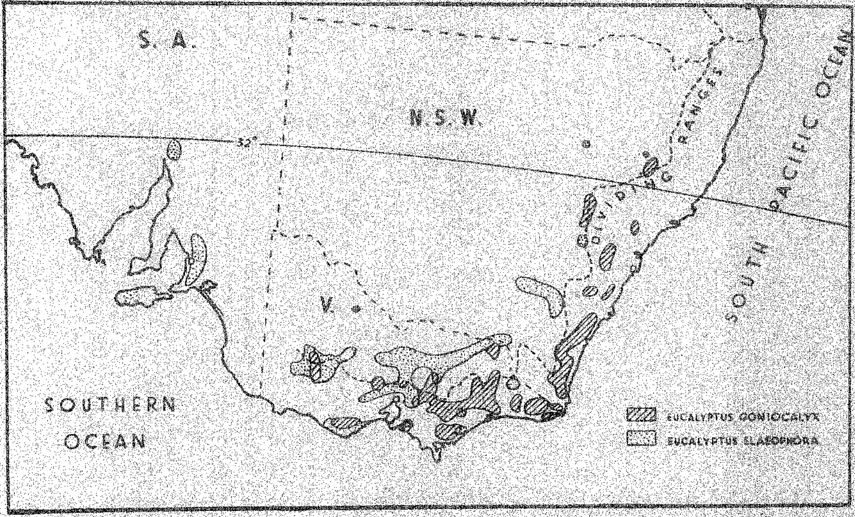


FIG. 1.—The distribution of *Eucalyptus elaeophora* and *Eucalyptus goniocalyx*. (Data in part from Carter, 1945.)

The species differ in several other respects, the most important of which are illustrated in fig. 2.

(b) *Field studies*

In the course of mapping the distribution of the Eucalyptus species in the region of the Dandenong Range, Victoria, it was observed that *E. elaeophora* and *E. goniocalyx* graded into each other. So complete was this gradation that it was impossible to define accurately the boundary between the species (Clifford, 1953). Investigation of other areas showed that such intergrading was common where the habitats of the two species adjoined (fig. 3). Intermediate trees have been observed on the Dandenong Range, in several places on the hills about the Kinglake Plateau, and further east on the Cathedral

Range. They are also reported to occur in the Grampian Ranges (L. D. Pryor in litt.).

In zones of intergradation the intermediate trees were variable *inter se*, some had a habit and bark suggesting an affinity with *E. elaeophora*, others had an appearance suggesting relationship with *E. goniocalyx*. The leaves on seedlings and on coppice shoots arising

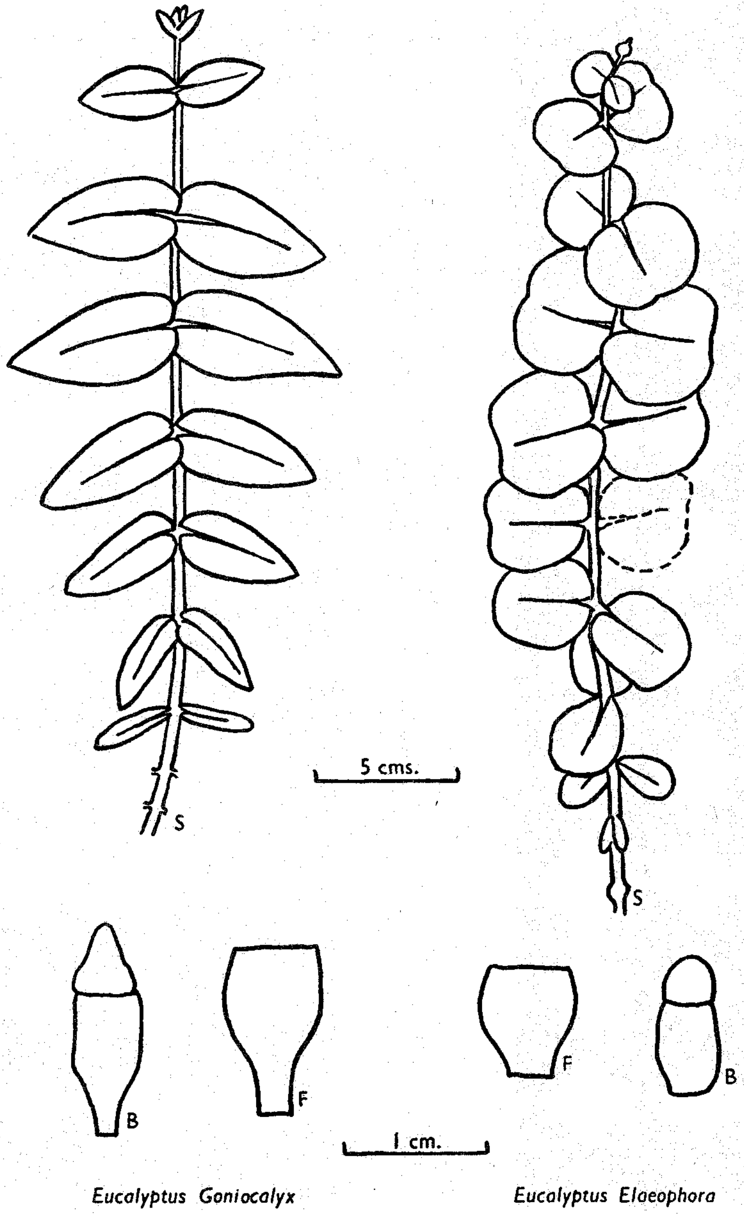


Fig. 2.—Some characteristic features of *Eucalyptus elaeophora* and *Eucalyptus goniocalyx*. S. seedling ; B. bud ; F. fruit.

from the stools of felled trees in these zones were also very variable in shape. In stands of either *E. elaeophora* or *E. goniocalyx* there was little variability of the coppice foliage between different trees, or in the shape of the seedling leaves.

No transplant experiments have been attempted, but each species has been observed growing in a variety of habitats. In each instance the species has retained its identity, even when grown in the habitat of the other, which confirms that they are not merely environmental forms of the one genotype.

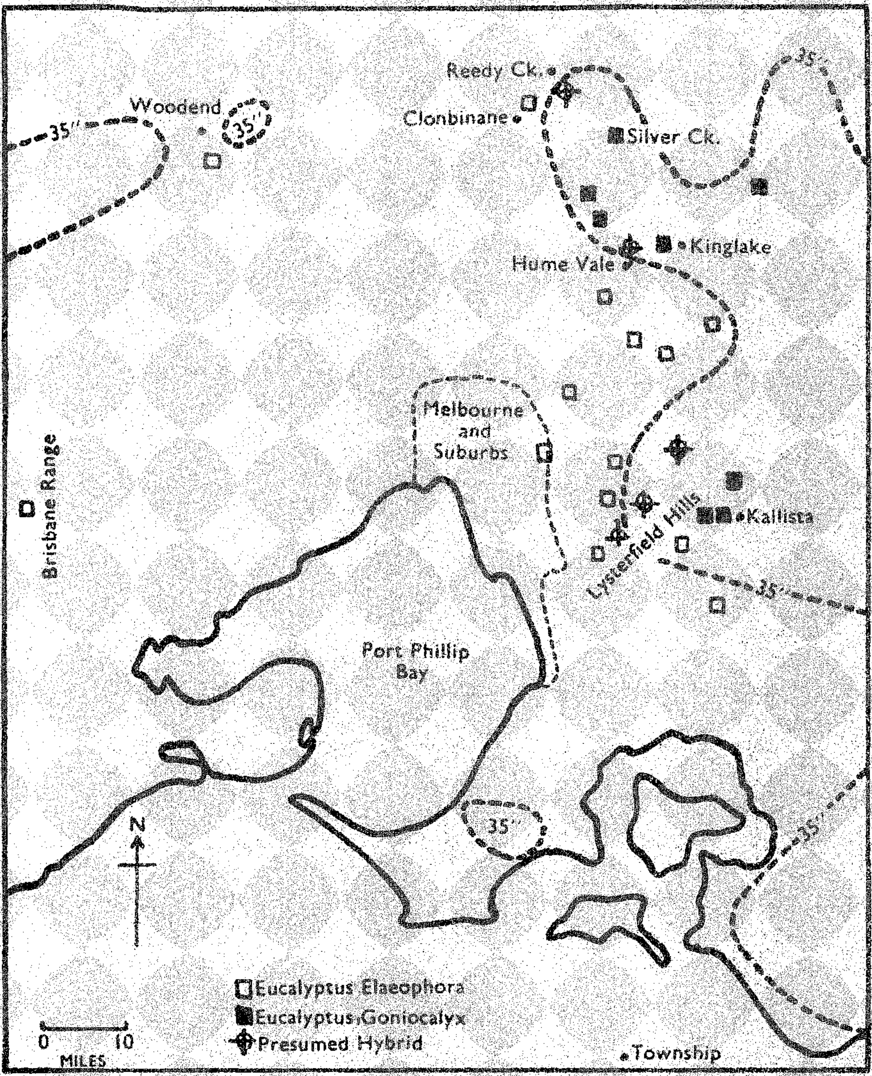


FIG. 3.—The distribution of *Eucalyptus elaeophora*, *Eucalyptus goniocalyx* and forms intermediate between them, in some localities near Melbourne.

(c) *Progeny studies*

The seedling leaves of *E. elaeophora* and *E. goniocalyx* are very different in shape, and a comparison of these and the seedling leaves of the " intermediate " trees was undertaken. Seeds were collected from several trees typical of either one or the other species and also from trees that were intermediate between them. The seeds were sown in 8 in. pots filled with a friable red-loam and placed in a heated glasshouse. Approximately 30 seeds were sown in each pot and within a fortnight most of these had germinated. When the seedlings had grown about 2 pairs of leaves the number of plants per pot was reduced to twelve. The thinning was designed to leave these twelve plants evenly spaced. No other conscious selection was practised except to remove any very stunted or diseased plants. The seedlings were finally harvested when they showed 6-8 pairs of fully expanded leaves. Altogether the progeny of fourteen trees were raised.

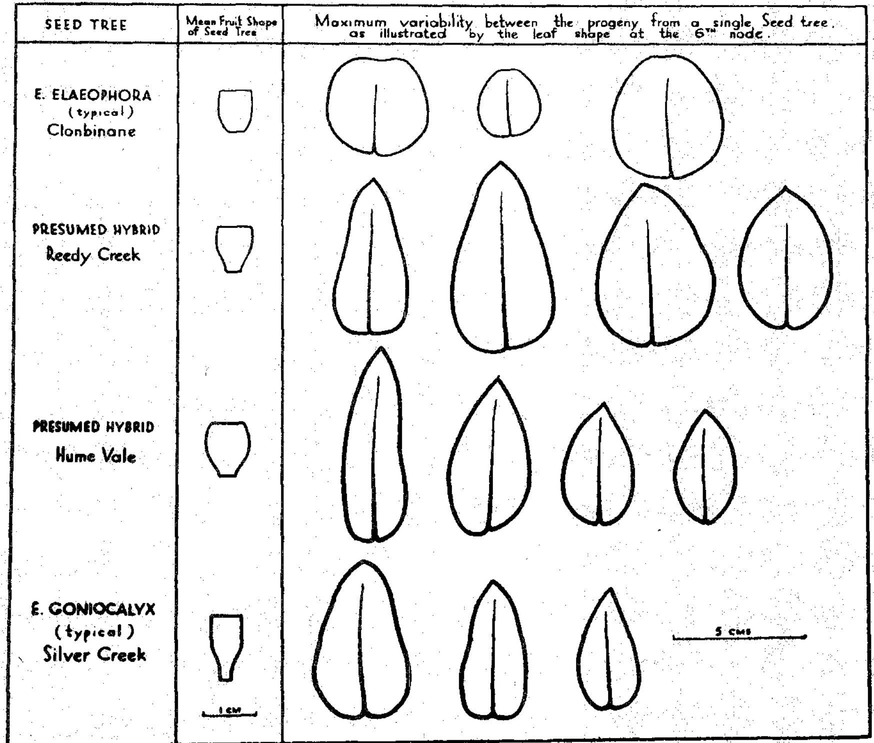


FIG. 4.—The variability between seedlings from the same parent, as illustrated by the leaf shape at the sixth node.

An examination of the seedlings grown indicated that there was less variability between those raised from parents typical of either *E. elaeophora* or *E. goniocalyx*, than between those from parents with an appearance intermediate between those species. This is illustrated in fig. 4, where leaves from the 6th node of four different seedlings from each of several parents, have been drawn. The leaves were chosen to show the maximum variability between the progeny raised from each parent.

If the seedlings from parents typical of each of the three groups studied were pooled and the variance of the length-breadth ratio of the leaves calculated, it was greater for the progeny of "intermediate" trees than for those from either *E. elaeophora* or *E. goniocalyx* at 5 of the 6 nodes measured. This is shown in table 1, from which it is evident that at the later nodes the variability of the progeny of the intermediate trees is maintained whereas the progeny of each of *E. elaeophora* and *E. goniocalyx* tend to become more uniform.

TABLE 1

The relative variability of the length-breadth ratio of seedling leaves of progeny from trees typical of E. elaeophora, E. goniocalyx *and trees intermediate between them*

Parent	Weighted mean variance of the length-breadth ratio of leaves at successive nodes above the cotyledons					
	1	2	3	4	5	6
E. elaeophora						
1. Yan Yean	0·81	0·29	0·19	0·21	0·14	0·14
2. Clonbinane . . .						
3. Brisbane Rge. . . .						
4. Wattle Glen . . .						
Intermediate trees						
1. Reedy Creek . . .	0·95	0·44	0·31	0·46	0·63	0·49
2. Hume Vale . . .						
3. Lysterfield Hills 1, 2, 3, 4 .						
E. goniocalyx						
1. Silver Creek . . .	0·83	0·44	0·27	0·31	0·28	0·26
2. Kinglake						
3. Kallista						
4. Mt. Wilson, N.S.W. . .						

The analysis of the variability in a population containing species and presumed hybrids may be carried further as follows. Consider the inheritance in pure lines and hybrids, of a character controlled by 4 genes. Assume that the parents (P_1 and P_2) are each homozygous for different alleles; that the viability and number of gametes produced by each is similar; that the F_1 hybrid between them is fully fertile; that the influence of the environment is negligible and that the overall mode of action of the genes is additive on a suitable scale. The mean measurements and their variances for the character in the parents and hybrid derivatives are shown in table 2.

When plotted graphically these values outline a triangle (fig. 5), where dimensions will depend upon the character measured. Linkage

TABLE 2

The mean measurement and its variance of a character controlled by four genes whose effects are additive

Parents	Number of genotypes	Mean numerical value of character	Variance of mean
$P_1 \times P_1$	1	x	0
$P_2 \times P_2$	1	y	0
$P_1 \times P_2$ (F_1)	1	$\frac{x+y}{2}$	0
$F_1 \times F_1$ (F_2)	81	$\frac{x+y}{2}$	v
$F_1 \times P_1$	16	$\frac{1}{4}(3x+y)$	$\frac{v}{2}$
$F_1 \times P_2$	16	$\frac{1}{4}(3y+x)$	$\frac{v}{2}$

does not alter the triangular shape nor do other parental combinations such as an F_1 crossed with an F_2 segregate, or an F_1 90 per cent. selfed and 10 per cent. outbred, all have mean-values and variances which fall within the triangle. Such a situation might arise in a population undergoing active introgression.

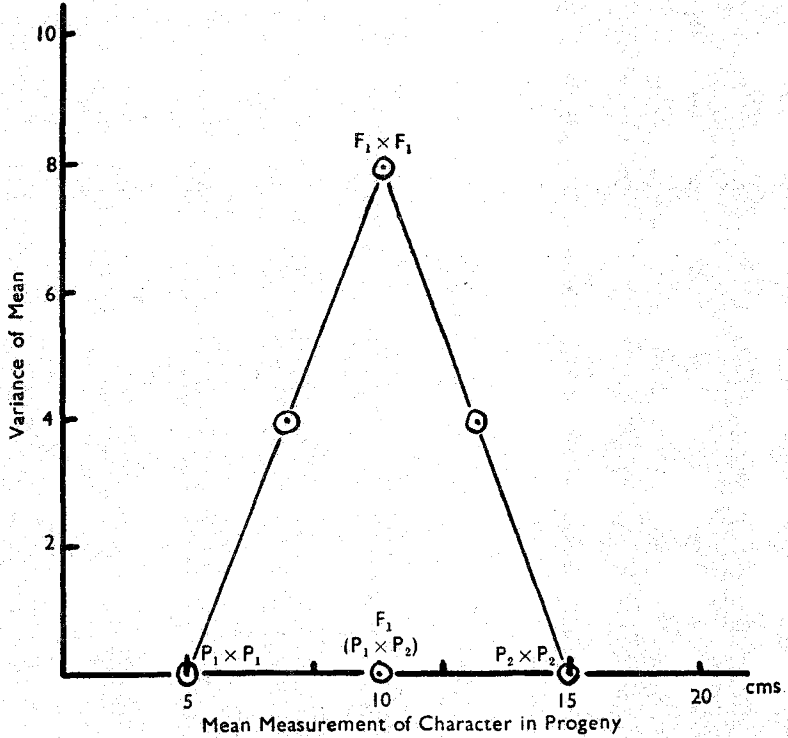


FIG. 5.—The theoretical distribution of the means and variances for a character measured on the families whose parents are given.

This theoretical hypothesis can be tested with the progeny of selfings and hybrids of species, varieties and so on, if they yield fertile hybrids, for it can be assumed for most of these that they differ principally in possessing different allelic genes. Suitable data is provided by East (1950) for cob length in maize. His numerical values are shown in table 3 and are graphed in fig. 6, from which it is seen, that for the progeny whose parents are indicated in the table, the mean and its variance for cob length mark out a triangular area.

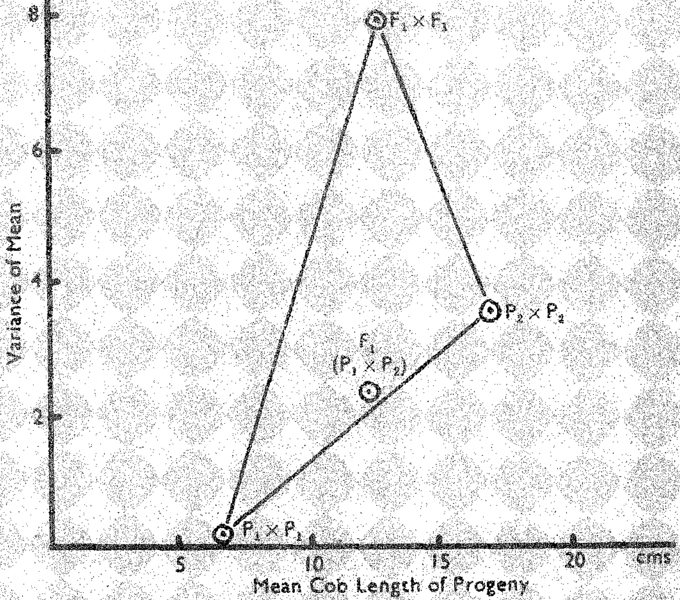


FIG. 6.—The distribution of the means and variances for cob length in maize measured on the families whose parents are given.

The application of this method of presenting data may be illustrated with the results for *E. elaeophora* and *E. goniocalyx*. When the mean length-breadth ratios of the leaves from seedlings raised from each tree studied are plotted against their variances, the co-ordinates were included within a triangle. This is shown in fig. 7 which is based upon the data of table 4.

TABLE 3

The mean and its variance for cob length in the parents and two hybrid generations of maize

Parents	Mean cob length of progeny in cms.	Variance of mean
$P_1 \times P_1$	6·6	0·66
$P_2 \times P_2$	16·8	3·49
$P_1 \times P_2$ (F_1) . . .	12·1	2·28
$F_1 \times F_1$ (F_2) . . .	12·6	7·89

TABLE 4

The mean and variance for the length-breadth ratio of the leaves for all the seedlings raised from individual parent trees

Parent tree	Locality	Mean length-breadth ratio ± the variance at successive nodes above the cotyledons					
		1	2	3	4	5	6
E. elaeophora, typical	Yan Yean	3·21±0·54	2·13±0·12	1·75±0·07	1·45±0·20	1·23±0·14	1·06±0·13
	Clonbinane	3·46±0·37	2·49±0·16	2·04±0·28	1·36±0·23	1·21±0·15	1·09±0·14
	Brisbane Rge.	5·45±2·09	3·37±0·43	2·77±0·24	2·08±0·19	1·73±0·12	1·72±0·20
	Wattle Glen	4·90±0·52	3·53±0·55	2·79±0·27	2·14±0·25	1·75±0·16	1·45±0·09
E. goniocalyx, typical	Silver Creek	4·47±0·33	3·73±0·32	3·41±0·43	2·75±0·48	2·30±0·28	2·16±0·35
	Kinglake	4·48±0·42	3·87±0·47	3·19±0·34	2·83±0·21	2·70±0·11	2·63±0·03
	Kallista	4·48±1·87	4·21±0·49	3·49±0·14	2·83±0·44	2·69±0·41	2·37±0·34
	Mt. Wilson, N.S.W.	3·86±0·92	3·36±0·39	3·29±0·19	3·08±0·20	3·00±0·30	2·73±0·20
Intermediate between *E. elaeophora* and *E. goniocalyx* (presumed hybrids)	Reedy Creek	4·06±1·42	3·08±0·32	2·54±0·53	2·23±0·46	2·08±0·94	1·83±0·25
	Hume Vale	4·73±1·52	4·03±0·68	3·23±0·49	2·70±0·59	2·28±0·45	2·19±0·48
	Lysterfield Hills 1	4·06±1·75	3·13±0·28	2·75±0·21	2·43±0·40	2·10±0·43	1·92±0·76
	2	3·79±0·24	3·02±0·24	2·53±0·12	2·28±0·33	1·95±0·31	1·84±0·25
	3	3·30±0·18	3·08±0·39	2·58±0·22	2·13±0·44	1·90±0·40	1·70±0·67
	4	4·62±1·12	3·59±0·65	2·55±0·10	2·21±0·45	2·00±1·27	1·90±0·67

The co-ordinates within this triangle are distributed in such a way that the progeny of trees acceptable as *E. elaeophora* or *E. goniocalyx* tend to occur at the basal angles and those from the " intermediate " trees tend to occupy the centre and apex of the triangle. Such a distribution of means and variances would be expected if the " intermediate " trees were hybrid derivatives of *E. elaeophora* and *E. goniocalyx*.

When the mean-variance diagrams are compared at successive nodes it is observed that the parental trees are not similarly disposed in each; but an analysis of the causes of this variability would be difficult, and is better postponed until more data are available, and direct proof obtained that hybridisation is occurring in accordance with the premises underlying the theory.

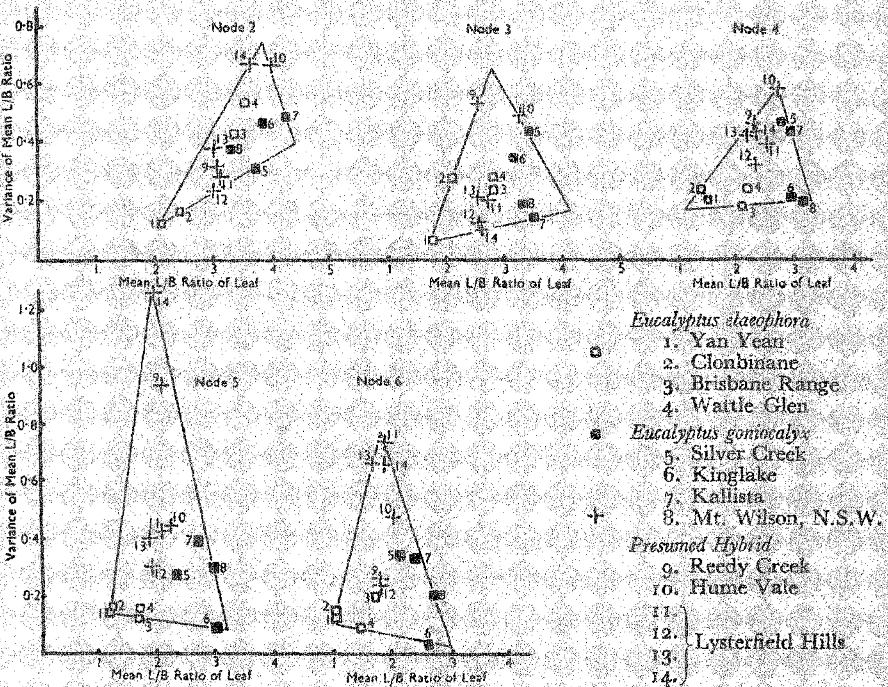


FIG. 7.—The distribution of the means and variances for the length-breadth ratios of seedling leaves in families from several *Eucalyptus* trees whose taxonomy is indicated. The data for the first node above the cotyledons are omitted as they do not conform to the general pattern.

3. DISCUSSION

Both the general appearance of the " intermediate " trees and their occurrence along the boundary where the habitats of *E. elaeophora* and *E. goniocalyx* meet, suggest that they might be hybrid derivatives of those species. The flowering seasons of the two species are concurrent from about February to June, so opportunity for hybridisation would exist. Furthermore, the progeny of the trees intermediate between *E. elaeophora* and *E. goniocalyx* show segregation of characters towards these species, if leaf shape is considered (fig. 4). From the variety of leaf shapes obtained at comparable nodes of seedlings from each " intermediate " parent, it would appear that many genes control this character.

That the two species concerned can interbreed has been established with reasonable certainty from the progeny studies. No simple ratios for different leaf shape have been obtained and nothing is known concerning the extent of inbreeding under natural conditions. It is suggested, however, that the application of the " triangle " method illustrated above supplies confirmation for the hybrid origin of the " intermediate " trees and might be used to analyse other hybrid populations.

4. SUMMARY

1. Several stands of trees with characters intermediate between *E. elaeophora* and *E. goniocalyx* have been studied.

2. Segregation of characters amongst the seedlings from individual " intermediate " trees suggested that they were of hybrid origin.

3. Further support for this interpretation was afforded by field studies, which showed that " intermediate " trees occurred only in localities where *E. elaeophora* and *E. goniocalyx* grew nearby.

4. The means and variances of the length-breadth ratios of the seedling leaves for the progeny of selected trees have been used to illustrate a possible method of determining the parentage of hybrid swarms.

Acknowledgments.—The writer has pleasure in thanking Professor J. S. Turner of the Botany School, Melbourne University for his help and encouragement during the course of this work, and Miss N. Mirams of the University Department of Visual Aids for assistance in preparing the diagrams.

5. REFERENCES

ANDERSON, E. A. 1949. *Introgressive Hybridisation.* John Wiley & Sons.

BAKER, R. T., AND SMITH, H. G. 1920. *A Research on the Eucalpyts especially in regard to their Essential Oils.* 2nd Ed. Govt. Print., Sydney.

BENTHAM, G. 1866. *Flora Australiensis, 3,* 229. Lovell Reeve and Co.

BRETT, R. G. 1938. A survey of the Eucalyptus species in Tasmania. Papers and *P.R.S. Tas.,* 1937, 75.

CARTER, C. E. 1945. *The Distribution of the more important Timber Trees of the Genus* Eucalyptus. Commonwealth Forestry Bureau. Atlas No. 1.

CLIFFORD, H. T. 1953. On the distribution of the species of Eucalyptus in the region of the Dandenong Range, Victoria. *P.R.S. Vic., 65,* 30.

EAST, E. M. 1950. As quoted in *Principles of Genetics* by E. W. Sinnott *et al.,* 1950. McGraw Hill Book Coy.

EWART, A. J. 1930. *Flora of Victoria.* Melbourne University Press.

MAIDEN, J. H. 1914. *A Critical Review of the Genus* Eucalyptus, *2,* 267. Govt. Print., Sydney.

PRYOR, L. D. 1950. A hybrid Eucalyptus. *Proc. Linn. Soc., N.S.W., 75,* 96.

PRINTED IN GREAT BRITAIN BY OLIVER AND BOYD LTD., EDINBURGH