AN ANALYSIS OF POPULATIONS OF IRISH *EUPHRASIA* L.

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ABSTRACT

Data from population samples of Irish *Euphrasia*, selected as representative of homogenous morphological types, were projected on horizontal axes in a natural ordering for 34 characters. The 32 wild populations so treated were found to occur in groups, with varying degrees of proximity, along the gradients of several characters.

The characters were investigated for their diagnostic value in delineation of groups of populations. The resulting groups were analysed for their degree of separation and were found to relate to the following species: *E. micrantha, E. scottica, E. tetraquetra, E. nemorosa, E. brevipila, E. rostkoviana, E. confusa, E. salisburgensis, E. pseudokerneri.*

All characters except one, glandular hairs, were continuously variable; the majority of characters tested were stable in cultivation. For most species, habitat appears to have been an important factor in speciation and is often an aid in identification.

Variation within this section of the genus is essentially continuous and intermediate populations are of frequent occurrence; nevertheless, narrowly separated but 'good' species have emerged from the study.

INTRODUCTION

Euphrasia, as suggested by Webb (1963), 'is a difficult genus, in which the delimitation of species and their distribution in Ireland are still subject to much uncertainty'. The purpose of this study was to investigate the nature of the variation within the genus as represented in Ireland, and to delineate the taxa present.

Pugsley's (1930) revision of the British *Euphrasiae*, based on Wettstein's (1896) monograph, is the most recent taxonomic treatment of the section of the genus occurring in the British Isles. The species of the British Isles all belong to Section *Semicalcaratae* Bentham of the type Subgenus *Euphrasia*. The section consists of four subsections, two of which include species of the British Isles, Subsection *Angustifoliae* Joergensen which includes one species in the British Isles, and Subsection *Ciliatae* Joergensen. Subsection *Ciliatae* consists of eight series; four of these include species of the British Isles: Series *Nemorosae* Pugsl., Series *Latifoliae* Pugsl., Series *Brevipilae* Pugsl., Series *Hirtellae* Pugsl. (Pugsley 1930).

Pugsley admitted 24 species and several varieties to the British *Euphrasiae*. One additional species was included in the British Flora (Warburg 1962) which follows the treatment of Pugsley. A total of fifteen species are recorded for Ireland; Webb (1963) treated thirteen species.

Among the twenty species Yeo (1954) considers indigenous to the British Isles, only two chromosome numbers occur, the diploid, $n = 11$, which occurs in five species, and the tetraploid, $n = 22$, which is common to all other species. Barriers to hybridization are essentially lacking and there are numerous records of hybrids as well as reports of successful cross-fertilizations in cultivation (Yeo 1959, 1966). Apomixis is considered not to be involved in reproduction of *Euphrasia* (Yeo 1966).

One of the difficulties in the determination of species of *Euphrasia* is due to the lack of certainty of the diagnostic value of the characters. An initial step, therefore, in this study, was a biometric analysis of characters in the Irish *Euphrasia*. This resulted in the structuring of groups of morphological types which finally were related to previously described species.

METHODS

Samples, ranging from 37 to 70 plants with an average of 58 plants, were taken from each of 32 wild populations (Table 1), and 21 characters of each plant were measured. An effort was made to sample as widely and completely as possible the total range of

TABLE 1. Population numbers, localities and sample size.

Population	*Locality*	*Sample size (n)*
A1	Stepaside, Co. Dublin	60
A2	Ballyknockan, Co. Wicklow	43
A3	Ballyknockan, Co. Wicklow	68
A4	Caherconnell, Co. Clare	55
A5	Carron, Co. Clare	66
A6	Forth Mountain, Co. Wexford	60
B1	Kilcoole, Co. Wicklow	70
B2	Gort, Co. Clare	70
B3	Ballinstraw, Co. Wexford	60
C1	Mannin Bay, Co. Mayo	53
C2	Achill, Co. Mayo	60
C3	Ballyvaughan, Co. Clare	60
C4	Lady's Island, Co. Wexford	60
C5	Lady's Island, Co. Wexford	60
C6	Lady's Island, Co. Wexford	60
D1	Caherconnell, Co. Clare	37
D2	Caherconnell, Co. Clare	60
D3	Caherconnell, Co. Clare	47
E1	Poulsallagh, Co. Clare	51
E2	Lackagh, Co. Galway	58
E3	Baur, Co. Clare	46
F1	Aghavannagh, Co. Wicklow	40
F2	Malahide, Co. Dublin	60
G1	Glencree, Co. Dublin	60
G2	Lough Bunny, Co. Clare	60
G3	Oughterard, Co. Galway	50
H1	Taum, Co. Galway	70
H2	Ballinrobe, Co. Clare	70
H3	Corranroo, Co. Clare	60
J1	Portmarnock, Co. Dublin	60
J2	Lahinch, Co. Clare	70
J3	Roadford, Co. Clare	70

variation within the genus as represented in Ireland, and in so doing to include all the taxa reported for the country. This resulted in collecting in all the habitats recorded for the Irish *Euphrasia*, with the exception of mountain tops. Only those populations were sampled that were considered to be homogeneous, that is, composed of one morphological type. Populations which appeared to consist of more than one type or species, and those in which intermediate forms occurred, were not sampled. Two populations of one morphological type were sampled; all other morphological types were represented by samples of at least three populations.

Each population studied was paced systematically and collections of five plants were made at approximately every five metres. In collecting, an effort was made to reduce the variation caused by grazing, host establishment, and age of plant. Grazed plants if inadvertently collected were subsequently eliminated after inspection of dried material. Plants obviously weak and poorly developed, possibly due to inadequate or late host

establishment (Yeo 1959) were not collected. At the same time an effort was made to avoid gathering only the large most vigorous plants. Gatherings were made of plants at the same stage of maturity in that they were in full flower and had set seed in the lower capsules.

In measuring each plant as large a sampling of morphological structures was made as was practical with the proposed statistical methods. Twenty-one characters of each plant were measured and, in addition, combinations of these direct measurements, including ratios, differences between the measurements of pairs of characters, and correlations of pairs of characters, were investigated. In the selection of characters an effort was made to include those used by previous workers, to quantify characters previously used subjectively and finally to include characters not formerly used but which as a result of field studies were suspected of having some diagnostic value (Table 2).

TABLE 2. Characters selected for analysis (* = original measurement).

*1. Number of glandular hairs per 1·5 mm
*2. Length of longest glandular hair
*3. Plant height
*4. Plant width
5. Plant height/width
*6. Length of stem to first branch
*7. Length of stem to first flower
8. Length of stem to first branch/plant height
9. Length of stem to first flower/plant height
*10. Number of primary branches
*11. Angle of branching
12. Number of primary branches/plant width
13. Angle of branching/plant width
*14. Number of flowers and capsules
15. Number of flowers and capsules/plant height
*16. Leaf width
*17. Leaf length
18. Leaf length/width
*19. Leaf teeth
20. Leaf width/number of teeth
*21. Lower corolla lip width
*22. Lower corolla lip plus tube length
*23. Upper corolla lip width
*24. Upper corolla lip plus tube length
25. Flower size (lower corolla lip width × lower corolla lip plus tube length)
26. Lower corolla lip plus tube length/width
27. Lower/upper corolla lip plus tube length
*28. Flower colour
*29. Capsule width
*30. Capsule length
*31. Capsule apex indumentum
*32. Calyx length
33. Leaf length minus calyx length
34. Calyx length minus capsule length

All measurements of the plants were made after they were pressed and dried. Means and standard deviations were calculated and means for each character then were plotted on histograms, with the populations arranged along the horizontal axis in ascending order of the means (Bobear 1964). These diagrams were then examined for discontinuities in the range of variation and for repeated occurrence in proximity of the same populations. As a result of this examination certain groups of populations were found to cluster consistently for several characters.

The groups* of populations were further analysed and tested for their overall affinities. Populations which proved at the conclusion of the study to have clustered for several characters were considered to be of similar morphological type and are here assigned a letter, indicating the group to which they belong, and a number representing the population number within the group (Table 1).

Seed from the plants of wild populations used in the study was planted in November of the year in which collections were made. All pots were placed in cold frames at the time of planting and were moved out of doors in April. Adventive species were retained as host plants of *Euphrasia*. The work on the semi-parasitic habit of *Euphrasia* by Yeo (1959, 1961) and Wilkins (1958, 1963) indicates non-specificity of host preference and therefore steps were not taken to provide particular hosts.

The cultivated plants were harvested and pressed in July and August, care being taken that they were collected in a stage of development similar to that of the wild populations; that is, they were in full flower with some mature capsules. Thirteen cultivated populations were examined in detail and an average of 21 plants for a population were measured for the same characters and in the same manner as were the wild populations.

Results—Characters

As a result of analysis of the variation of characters, four categories of characters were established. Category I consists of characters exhibiting discontinuities in variation for groups of populations similar for several other characters. In category II are those characters continuous in variation but resulting in clustering, along the gradient, of several groups of morphologically similar populations. Category III consists of characters continuous in variation and resulting in clustering of one or very few populations of similar morphological type. The distinction between Categories II and III is necessarily somewhat arbitrary. Category IV consists of characters most of which show little if any clustering of populations which occur in proximity in the variation for other characters. Characters of this category are considered of little or no diagnostic value.

A degree of genetic stability of characters in all categories was indicated through cultivation results, with three exceptions, plant width, capsule length, number of primary branches. Correlation coefficients were calculated between wild and cultivated populations for fourteen characters as given in Table 3.

Table 3. Correlation coefficients (r) and corresponding probabilities (P) for means of characters in wild and cultivated populations.

Character	*r*	*P*
Number of glandular hairs	+0·9537	<0·001
Capsule width	+0·8545	<0·001
Plant height	+0·7034	<0·01
Plant width	+0·1159	>0·10
Lower corolla lip width	+0·8426	<0·001
Lower corolla lip plus tube length	+0·8832	<0·001
Upper corolla lip width	+0·6928	<0·01
Upper corolla lip plus tube length	+0·8870	<0·001
Leaf width	+0·8426	<0·001
Leaf length	+0·7652	<0·001
Calyx length	+0·6928	<0·01
Capsule length	+0·3672	>0·10
Number of primary branches	+0·1792	>0·10
Angle of branching	+0·7242	<0·01

*To simplify the presentation, the 'Groups' which emerged at the conclusion of the analysis have been referred to freely in the following sections on character variation. See p. 85.

(*a*) Characters of Category I

The only characters of Category I, those having discontinuities in variation, are related to features of glandular hairs: their presence or absence, if present their length and number.

Number of glandular hairs

The presence or absence of glandular hairs has been considered a useful but variable character in distinguishing species of *Euphrasia* (Pugsley 1930).

In this study data for the character were obtained through sampling of the relative abundance of glandular hairs by counting the glandular hairs present along a 1·5 mm segment of the mid-vein on the dorsal surface of the leaf measured.

Discontinuities occurred in the variation of populations measured for number of glandular hairs, as seen in Fig. 1, first in the presence or absence of the hairs. A second discontinuity occurs between the mean of population means of 13·04 hairs/1·5 mm representing Group A, and the mean of population means of 5·93 hairs/1·5 mm representing Group B. These two groups are separated from all others and from each other. The character proved of little value for those groups of low mean for number of glandular hairs since variation within this portion of the range for the character is continuous (Fig. 1a).

Based on the number of glandular hairs, four morphological types are distinguishable.

Numerous glandular hairs ($\bar{x} = 13 \cdot 04$)	—Group A
Less numerous glandular hairs ($\bar{x} = 5 \cdot 93$)	—Group B
Scarce glandular hairs ($\bar{x} = 1 \cdot 12$)	—Groups C and D
No glandular hairs	—Groups J, G, F, E, H

Length of glandular hairs

A second measurement of glandular hairs was made, the length of the longest glandular hairs among those counted. Two distinct mean lengths were found. Mean length of $\bar{x} = 0 \cdot 60$ mm distinguished Group A, and $\bar{x} = 0 \cdot 20$ mm was found in all other populations bearing glandular hairs.

There was a correlation in length and number of glandular hairs in that populations at the highest end of the range for number of glandular hairs were also at the highest end of the range for the length of glandular hairs. Group A was thus separated from other types by a discontinuity of variation for both number and length of glandular hairs (Fig. 1a).

(*b*) Characters of Category II

Eleven characters constitute the second category in which the variation is continuous but several groups of populations originally believed to be similar occur in proximity within a relatively limited range of the gradient. The characters of this category were found to have diagnostic value for several morphological types.

Capsule width

Capsule width, not formerly used as such but apparently included in descriptions of capsule shape, was measured at the widest section of a matured capsule which had not yet dehisced. The measurements were small and therefore the differences were slight, but capsule width proved a successful character in distinguishing six of the nine morphological types, Groups E, A, C, H, B, D respectively (Fig. 1b).

Plant height

Plant height has been consistently used by previous workers and frequently precise measurements have been given (Wettstein 1896, Townsend 1897, Bucknall 1917, Pugsley 1930, Warburg 1962, Yeo 1962).

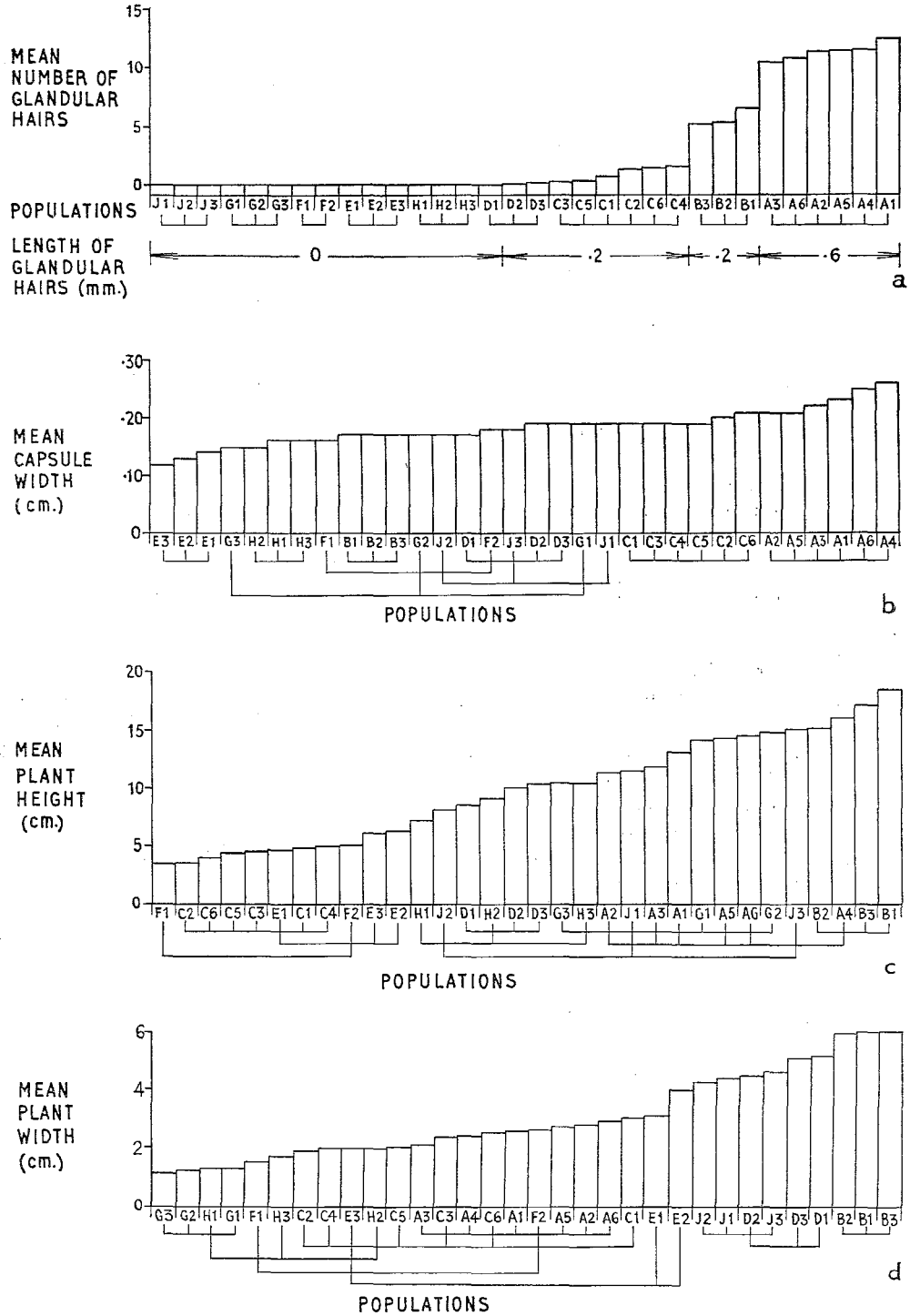

Fig. 1. Histograms of characters of Category I (a) and Category II (b, c, d).

The measurement of plant height was here made from the base of the stem, indicated by withered cotyledons or their scars, to the tip of the floral spike.

The members of six groups of populations, Groups C, E, F, B, A, D, similar in other morphological characters were similar also in plant height (Fig. 1c). In addition tall meadow forms remained tall and low-growing pasture plants remained low in cultivation.

Plant width

Plant width has not been directly used by other workers, although reference has been made by Pugsley (1930) to 'slender' plants and his and Wettstein's (1896) 'early summer habit' and 'late summer habit' usually included the width of the plant.

Plant width was measured at the widest point of the plant, which was in most cases between the extremes of the most spreading branches.

Plant width varies continuously but the populations of three Groups, B, D, J, consistently occurred at the high end of the range and two Groups, G and H, occurred so at the low end, while a sixth group of populations, Group A, clustered in the centre of the gradient (Fig. 1d). Plant width was one of three characters that did not remain stable in cultivation; nevertheless it proved a consistent character in geographically isolated wild populations of similar morphological type.

Plant height/width ratio

Plant height/width ratio was not found to have been used in the past as a quantitative character but was generally referred to in description of habit.

For this ratio, six Groups, G, H, A, C, D, B, of morphologically similar populations were distinguishable for their clustering within the gradient. Group G was discretely distinct (Fig. 2a).

Lower corolla-lip width

Floral characters being considered of importance have been used in various ways. Wettstein (1896) divided the Section *Semicalcaratae* into three Series based on the elongation of the corolla tubes, a characteristic later not considered of value (Chabert 1902, Pugsley 1930). Bucknall (1917) considered corolla-length variable and unreliable as a diagnostic character, while Pugsley accepted the importance of overall corolla size. Yeo (1962, 1966) and Wilkins (1958) both used floral characters, especially those of the lower corolla lip.

All measurements in this study were made on a flower chosen from among the largest on each plant and believed to have recently dehisced anthers.

The width of the lower corolla-lip was measured at the widest part of the lip. Plotting of the means of populations for this character resulted in seven groups of populations occurring in proximity. Groups D, A, and B, respectively, were at the high end of the range, and Groups G, E, F, H, at the lower, with Group J in the centre of the range. Group C proved most variable for this character (Fig. 2b).

Lower corolla-lip plus tube length

The measurement of the lower corolla-lip plus tube length was taken from the base of the tube to the tip of the middle lobe of the lower corolla lip.

The distinguishing of groups in the gradient for length of lower corolla-lip plus tube is similar to that for the lower corolla-lip width in that the same six groups are found to occur in proximity but there is slightly less variation in the latter character (Fig. 2c).

Lower corolla-lip plus tube length/width ratio

This ratio, not previously used, proved useful in the sorting of the large-flowered forms with small differences in length and width at one extreme of the range, Groups D, A, B, and the small-flowered forms with large differences in the two measurements at the other end of the range, Groups G, E, F. Six groups then were proximal for this ratio. One, Group C, was conspicuously varied for length-width ratio of the lower corolla (Fig. 2d).

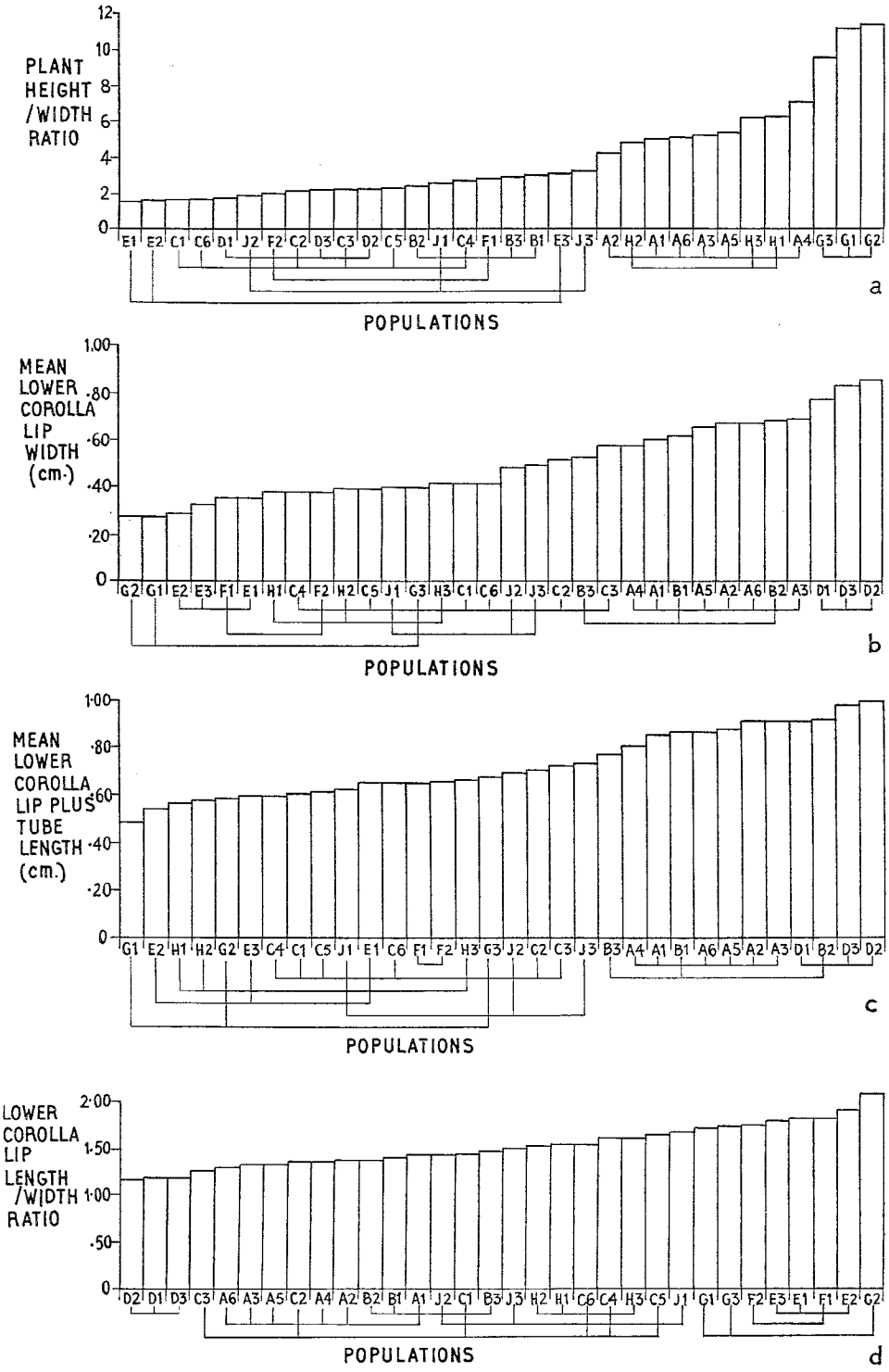

Fig. 2. Histograms of characters of Category II.

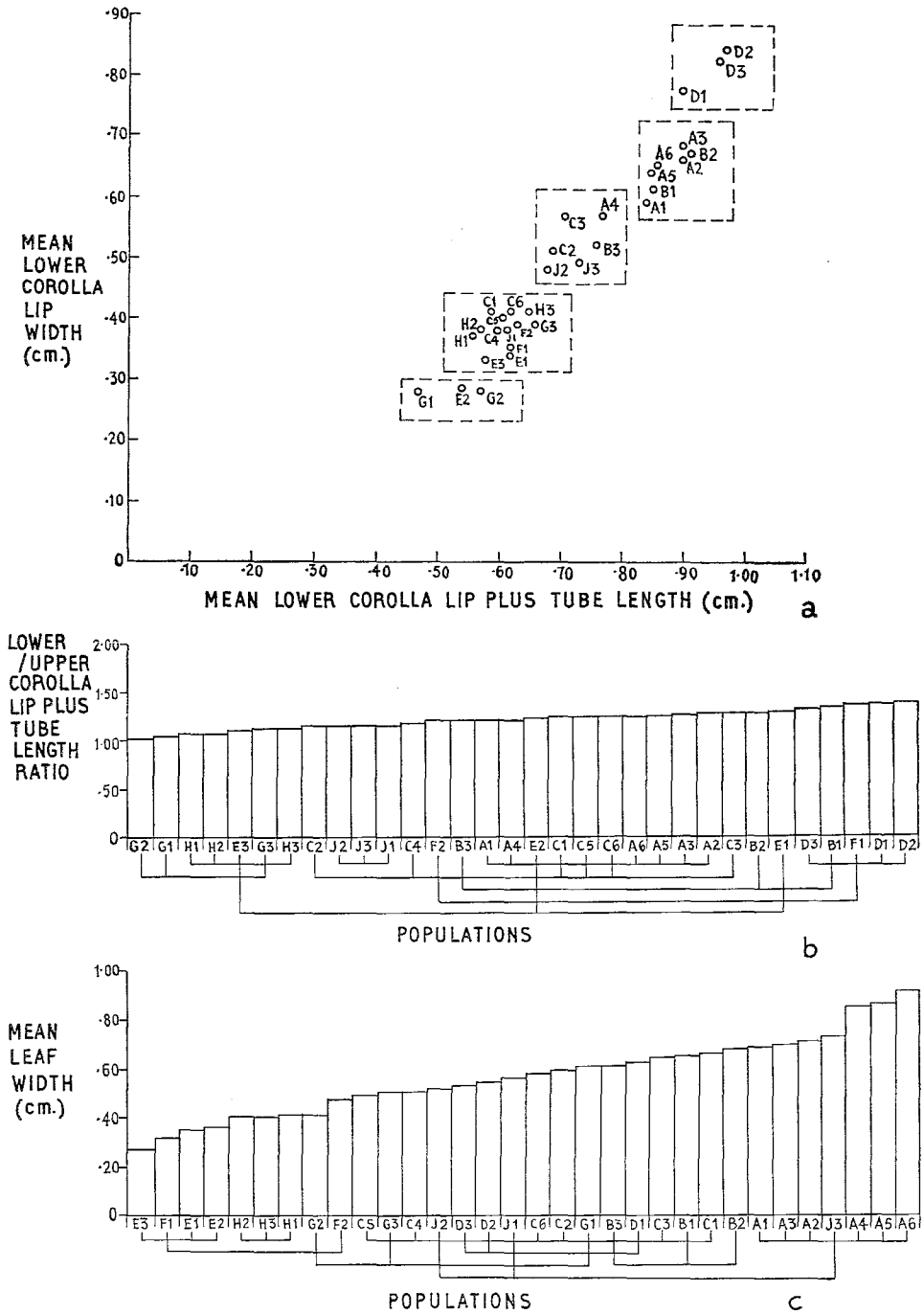

Fig. 3. Scatter diagram and histograms of characters of Category II.

Lower corolla-lip width × lower corolla-lip plus tube length (Flower Size)

Lower corolla-lip width and lower corolla-lip plus tube length were plotted against each other on rectangular axes, and were used as an estimate of flower size (Fig. 3a).

The continuous nature of the variation of the characters is apparent as well as the positive correlation between the two characters, but such an arrangement of data is useful

in revealing three categories of groups of populations, which may be distinguished by flower size, small, medium and large. There is also a small degree of discontinuity in mean measurements for the three groups:

Lower corolla-lip	*Small*	*Medium*	*Large*
Mean length	0·47–0·66 cm	0·68–0·91 cm	0·90–0·97 cm
Mean width	0·28–0·41 cm	0·47–0·68 cm	0·77–0·82 cm

Lower/upper corolla-lip plus tube length ratio

The relative lengths of the lower and upper corolla-lip plus tube were consistently used by Pugsley (1930) in his descriptions of the British *Euphrasiae*, and it appears in both the British and Irish Floras (Warburg 1962, Webb 1963), but without quantification.

The difference in length of the two corolla-lips is useful for two groups, G and H, of similar populations with small differences for the ratio; and for one group, Group D, with a large difference for the ratio. Two groups of populations, J and A, near the centre of the gradient are also proximal but this character is not as useful in distinguishing these groups as it is for those at the extremes of the range (Fig. 3b).

Leaf width

Leaf shape has been used by previous workers as a diagnostic character with measurements often given for leaf length (Bucknall 1917, Pugsley 1930, Warburg 1962). Leaf width has been used in relation to the length with an approximate ratio.

The leaf selected for measurement was the one subtending the capsule measured; it was measured at its widest point including the teeth.

Leaf width is a useful character resulting in the distinguishing of six groups of morphological types, Groups E, H, F at the lower end of the gradient, and Groups A and B at the upper end, with Group D populations proximal near the centre of the gradient (Fig. 3c).

Leaf length

Leaf length was measured along the mid-vein from the leaf base or point of attachment to the stem, to the tip of the leaf.

Leaf length is similar to leaf width in its value as a diagnostic character in that the same six groups are proximal for leaf length, Groups H, E, F, A, B, D (Fig. 4a).

(*c*) Characters of Category III

The third category of character variation consists of seven characters for which the clustering of morphological types within the continuous gradients of variation is infrequent. The characters are of less diagnostic value than those in the preceding two categories, since fewer morphological types are distinguishable by use of the following characters.

Capsule length

Capsule shape, with some measurements given for capsule length (Pugsley 1930) has been previously used as a diagnostic character.

Capsule length was measured from the base to the tip of the capsule, not to the central notch. The capsule measured was the one also measured for capsule width.

When arranged in order of population means for capsule length, Groups E and H were proximal at the lower end of the range, Groups B and F at the upper centre. The character proved variable for all other morphological groups (Fig. 4b).

Upper corolla-lip width and upper corolla-lip plus tube length

The width of the upper corolla-lip was measured at the widest part of the lip. The length was measured from the base of the tube to the tip of the lobes of the upper lip.

For the gradient of both characters populations of similar morphological type occur within proximity for five groups, but these characters of the upper lip proved less efficient in distinguishing groups than did comparable measurements of the lower lip (Figs. 4c, 4d).

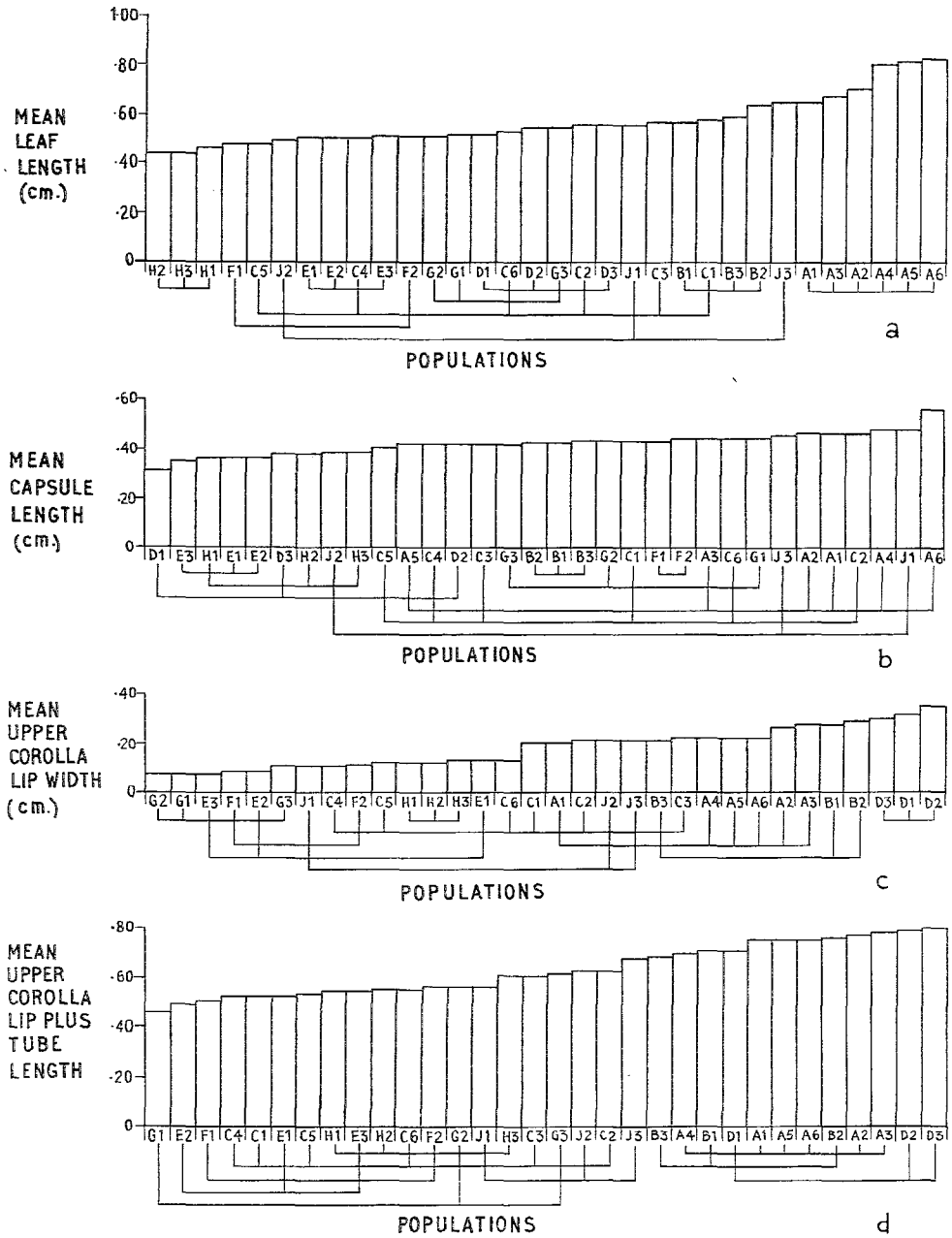

Fig. 4. Histograms of characters of Category II (a) and Category III (b, c, d).

The saccate shape of the upper lip (which did not press well), and variations in the tendency of the margins to curl in drying, rendered the upper lip of the corolla less amenable to measurement than the lower lip.

Leaf length/width ratio

Leaf length/width ratio has been considered of value (Pugsley 1930) as a distinguishing character, although numerical values have not been given.

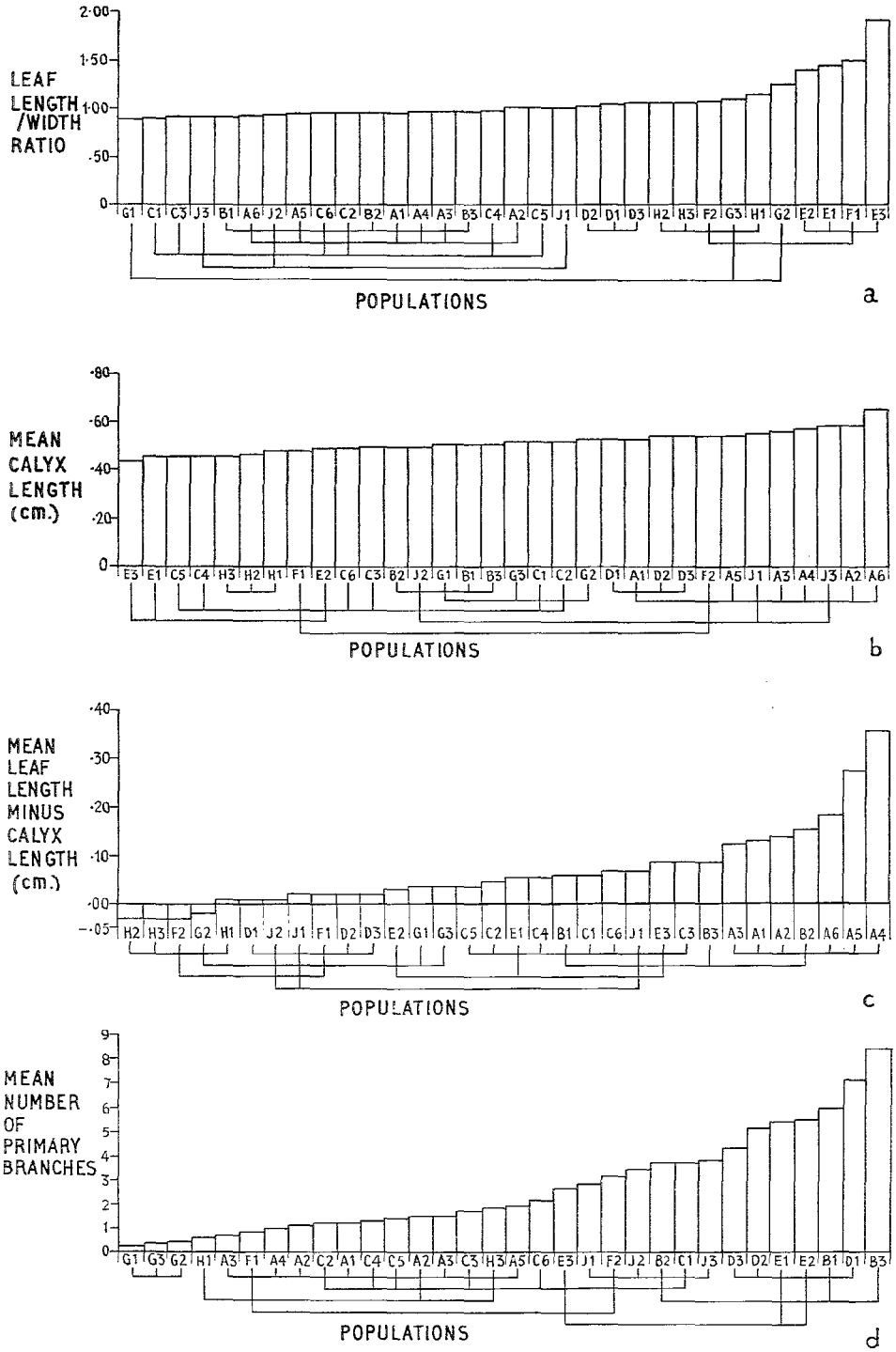

Fig. 5. Histograms of characters of Category III.

The leaf length/width ratio was here found useful as a distinguishing character for four morphological types, Groups E, H, D, F. The character was variable in the remaining five groups of populations (Fig. 5a).

Calyx length

Calyx length appears in Pugsley's (1930) work as being shorter, longer, or as long as either the capsule or subtending leaf.

It was measured, for this study, from the base of the calyx to the tip of the calyx teeth.

Five groups demonstrate varying degrees of clustering for this character. Two groups, B and G, were overlapping in the centre of the range and the variation of groups at either end of the gradient, Groups E, H, A, was greater than in most preceding characters (Fig. 5b).

Leaf length minus calyx length

The difference in length of leaf and calyx has been expressed as calyx 'exceeding greatly' or 'equalling or exceeding' (Pugsley 1930).

In this study the character was found to be continuous in variation with three groups of populations proximal within the gradient, Groups H, A, D. The character therefore is of value for three morphological types (Fig. 5c).

Number of primary branches

The degree of branching had been found useful by Pugsley (1930) and has been expressed as 'few branches' or 'bushy habit'.

The mean number of primary branches was found to be consistently less than one for Group G, and relatively high for Groups D and B. The character has diagnostic value for these three groups (Fig. 5d).

The number of primary branches is a character which appeared as a result of cultivation trials to lack stability (Table 3); but, as with plant width, it was consistent in wild material.

Plant width and the number of primary branches were found to be strongly correlated as demonstrated by plotting of mean values on abscissa and ordinate, and also by calculation of the correlation coefficient, $r = +0{\cdot}8306$ ($P<0{\cdot}001$).

(*d*) Characters of Category IV

The characters of category IV have the least diagnostic value since groups of populations occurring in proximity for other characters show considerable variation for the following characters. In some other cases two characters were found to be too closely correlated for both to be of value as individual diagnostic features.

Angle of branching

Angle of branching, implied in the terms 'erect' or 'ascending' or 'suberect', has been used by Pugsley (1930) and those who followed his treatment of the genus.

An estimate of the average angle of branching was obtained by laying a protractor on the plant and reading the angle common to the majority of branches.

Within the continuous variation of means of populations for angle of branching two groups, G and A, cluster at the lower end of the range and two, Groups B and D, at the upper centre. The total range of variation of angle of branching is small and the character is difficult to apply except for Group G at the lowest end of the gradient (Fig. 6a). A plotting of values for angle of branching and width of plant revealed a lack of correlation between the two characters.

Calyx length minus capsule length

Pugsley (1930) consistently referred to the relative lengths of the calyx and capsule as a descriptive feature of each species.

Plotting of the means for this difference resulted in the proximity of one group of populations at the upper end of the continuous gradient, Group D, and two groups, C

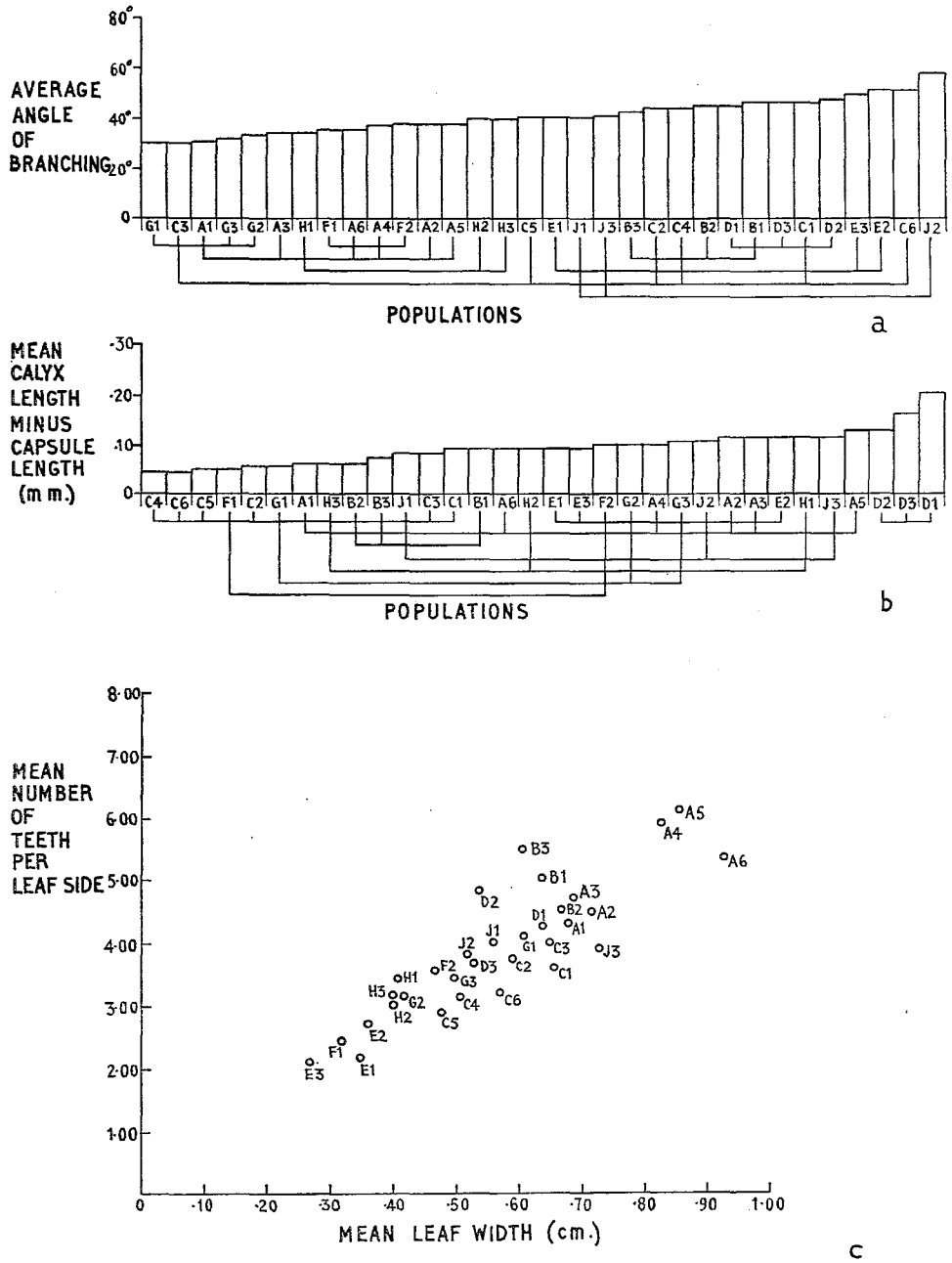

Fig. 6. Histograms and scatter diagram of characters of Category IV.

and B, near the lower end. All other groups showed considerable variation for this characteristic. The results are not unexpected since variation within groups was high for both capsule and calyx lengths (Fig. 6b).

Leaf teeth

The number of leaf teeth on a side of a leaf appeared in each of Pugsley's (1930) species descriptions.

The number of teeth on a side were plotted (abscissa) against the width (ordinate) of the leaf. The two characters were found to correlate with a value of $r = +0{\cdot}8659$ ($P<0{\cdot}001$). The few populations which appeared to deviate from the general trend of correlation of the majority, belonged to several different morphological groups (Fig. 6c).

Length of stem to first branch

The height of branching was frequently used and referred to as being near the base or the midpoint of the stem (Pugsley 1930). Warburg (1962) refers to the number of the node at which branching commences.

In this study the length of the stem to the first branch was measured from the base of the stem to the first branch. This was considered to be a more reliable quantification of height of branching than a counting of nodes which may be difficult to determine with precision.

Populations of Group G were conspicuously clustered at the upper end of the range and Groups F and C were proximal at the lower end.

Plotting of length of stem to first branch (ordinate) against plant height (abscissa) indicates that the characters are positively correlated. The populations of Group G are the only ones of a single group that consistently demonstrate lower branching for their height. This character then is of particular value in characterizing Group G, while all others correlate with plant height (Fig. 7a).

Length of stem to first flower

Length of stem to first flower is and has been a frequently used character. Pugsley (1930) used the number of the pairs of leaves at which flowering begins. The node at which flowering begins is commonly used by Warburg (1962) to indicate the length of stem to the first flower.

This character was determined here by measuring the length of stem from its base to the first flower or capsule of each plant.

Variation is essentially continuous for length of stem to first flower with populations of Group B clustering at the upper end of the gradient, Groups D and E at upper and lower ends of centre respectively, and Groups C and F at the lower end (Fig. 7b).

While these five groups did occur in proximity, plotting of the mean values of plant height (abscissa) against length of stem to first flower (ordinate) revealed a correlation of the two characters (Fig. 7b).

The correlation coefficient between the two characters is $r = +0{\cdot}8887$ ($P < 0{\cdot}001$) The height of flowering appears to have minimal diagnostic value.

Flower colour

Pugsley (1930) recognized variability of flower colour, but thought it to be useful in providing 'some indication of species'. It was used by him in his key as well as in descriptions and has appeared in subsequent floras based on his treatment of the genus.

In the present study, in order to render colour of flower more amenable to biometric treatment, a colour scale of three values was prepared. Flowers of each plant were compared with the colours of the chart and were scored using a combination of the two elements of the following key:

I – white	U – upper lip only
II – light purple	L – lower lip only
III – dark purple	UL – both lips

Flower colour has the same pattern of continuous variation as is typical of all but one other character examined in *Euphrasia*. Flowers within individual populations may range from white to dark purple. Three populations were composed entirely of plants with white flowers, and one entirely of plants with dark purple flowers. The majority of populations, however, were varied from all white through varying degrees of purple and white on single flowers to all dark purple flowers. Even in the flowers of an individual plant, there is often considerable variability in colour. In general, populations found to be related in respect to other characters were not distinguished by flower colour.

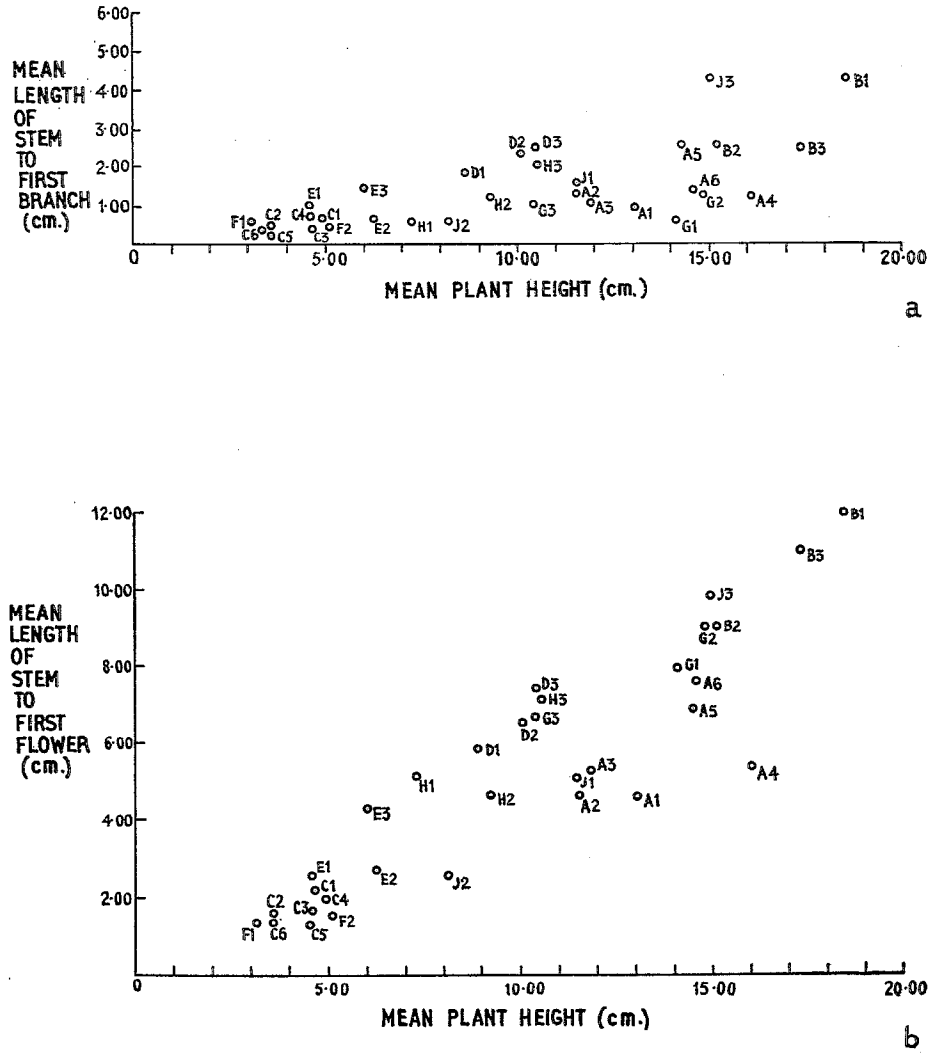

Fig. 7. Scatter diagrams of characters of Category IV.

The flower colour patterns of wild populations were unchanged by cultivation.

Flower colour appears to have little diagnostic value, with the exception of one morphological type which is predominantly white flowered. Also, the one all dark purple population studied occurred within the group for which this flower colour has frequently been reported.

Number of flowers and capsules

The number of flowers and capsules were counted to discover, in particular, whether this characteristic in relation to plant height would be a distinguishing character of a low-growing form of *Euphrasia* from an exposed habitat. While populations of this morphological type did occur in proximity at the upper end of the gradient, the difference was not sufficiently distinct from other low-growing forms for this relationship to be of diagnostic value.

Capsule-apex indumentum

The capsule apices of all plants were examined and found to be ciliate with the exception of a single morphological type, Group E, in which they were glabrous.

(*e*) Habitat

While ecological studies were not a part of this work, some recordings were made of soil pH, height of sward, and associated species. In analysing this data it was discovered that the populations of seven of the groups were consistent in habitat to the following degree. Classification of vegetation follows that of Tansley (1939).

Group G — wet habitat (fen and flush)
Group H — heath
Group E — limestone pasture (average height of sward 7 cm)
Group C — dune grassland (grazed)
Group A — meadow (average height of sward 46 cm)
Group D — meadow
Group B — meadow

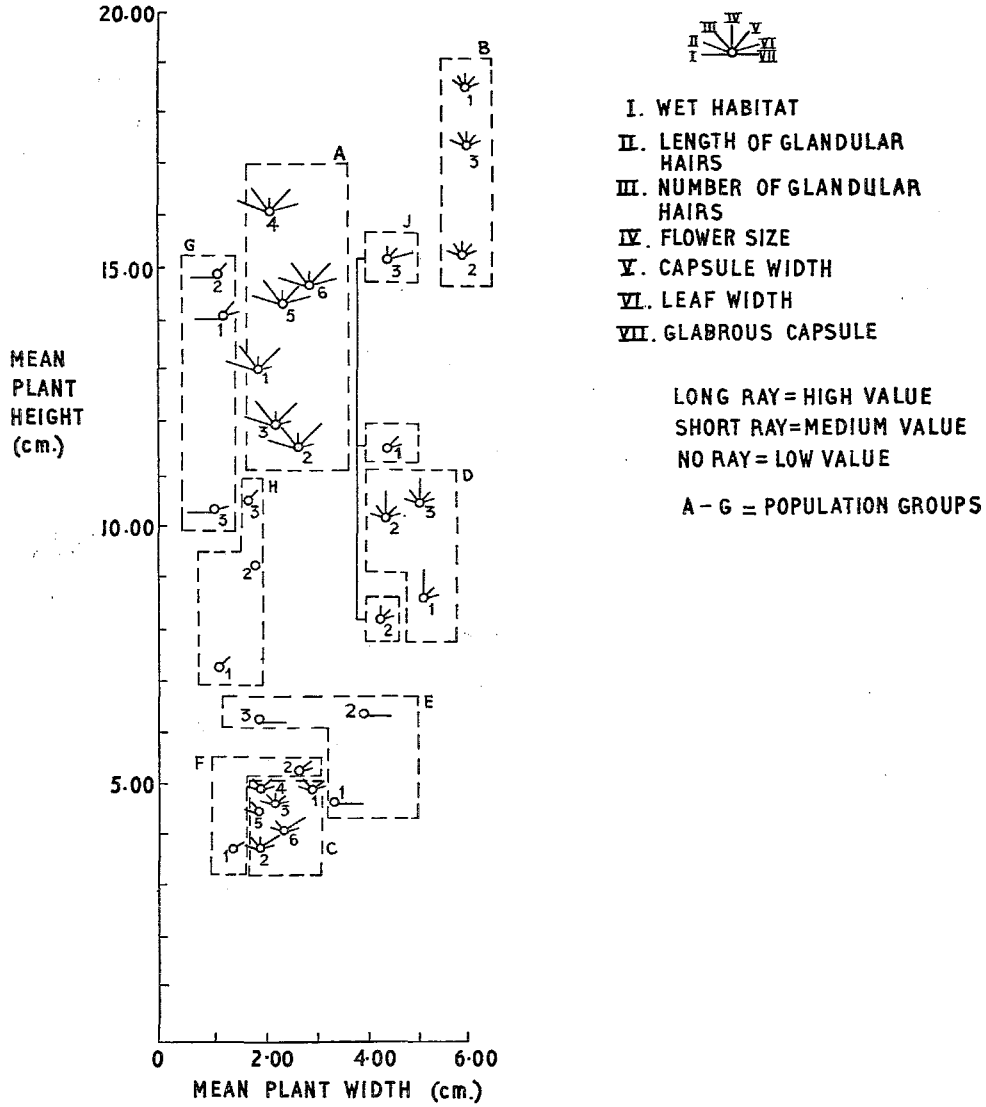

Fig. 8. Populations diagrammed for 9 characters.

RESULTS—GROUPS

As a result of the preceding analysis of characters, nine of the characters which appeared likely to be most informative as to the composition of possible population groups were selected for investigation on a co-variation diagram (Fig. 8) as described by Anderson (1957).

On the basis of the results as revealed by the diagram and the additional evidence of character variation presented above but not included on the diagram, nine morphological types are distinguishable. These groups of populations, Groups A to J, were identifiable at this stage of the study, although they have been used, in this paper, in the preceding section on character variation and in Table I, for simplicity of presentation.

In order to determine further the relative importance of differences between groups, *t*-tests were performed between the mean values of adjacent groups for six of the most discriminating characters. The points of interest which emerged were the predominantly continuous variation in the mean values of groups for the characters considered, and secondly the inconsistency in occurrence of significant differences between the same pairs of groups for different characters. The results of the *t*-tests are illustrated in a diagram of the type developed by Roberts (1961).

In the diagram (Fig. 9), groups are arranged vertically for each of the six characters in order of magnitude of the mean values, the high values being uppermost. Discontinuities in the variation of each character are indicated by interruptions in the columns. The variation of characters for groups enclosed within resulting blocks is continuous. The lines connecting blocks indicate the position of each group within the gradient for each character.

The reticulate pattern of variation of the groups for the six characters is evident. However there is indication that the characters do not vary independently; some co-variation is evident through the number of parallel lines linking groups between characters.

Group B is the most sharply differentiated group; Groups A, E, H, G, D are also differentiated from each other and remaining groups. Groups C, F, J are less well differentiated but can be defined in terms of particular ranges of values for several continuous variables.

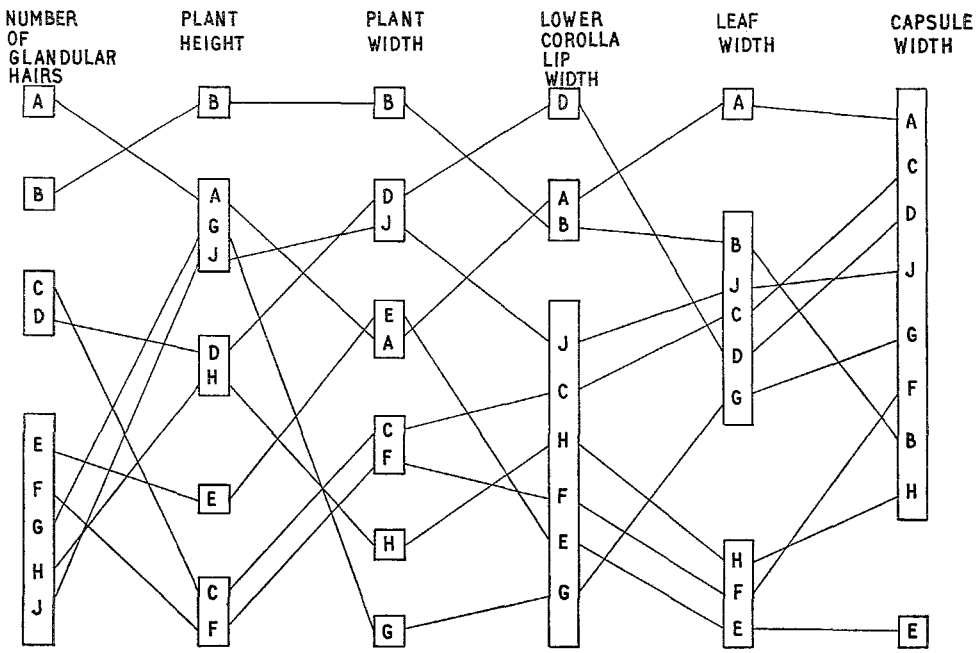

Fig. 9. A natural ordering from high to low value of Groups of populations (A–J) for six characters.

Results—Taxonomic

Fifteen species of *Euphrasia* have been recorded for Ireland (Hart 1887, 1898, Stewart & Corry 1888, 1938, Colgan 1904, Praeger 1909, 1934, Scully 1916, Pugsley 1930, Brunker 1950, Warburg 1962, Webb 1963).

Examination of the whole of the data considered above indicates the following relationships between the previously described species and the nine morphological groups recognized in this investigation.

E. micrantha Reichb.	Group H
E. scottica Wettst.	Group G
E. tetraquetra (Bréb.) Arrondeau (*E. occidentalis* Wettst.)	Group C
E. nemorosa (Pers.) Wallr.	Group J
E. brevipila Burnat & Gremli	Group B
E. rostkoviana Hayne	Group A
E. confusa Pugsley	Group F
E. salisburgensis Funck	Group E
E. pseudokerneri Pugsley	Group D
E. frigida Pugsley	
E. curta (Fr.) Wettst.	
E borealis Wettst.	
E. anglica Pugsley	
E. hirtella Jord. ex Reuter	
E. montana Jord.	

The semi-alpine habitat of *E. frigida* was not visited. Localities reported for *E. curta* were investigated but *E. brevipila* was always found. No collections fitting the descriptions of *E. borealis* were discovered and all herbarium material seen could either be placed in *E. nemorosa* (one specimen), or, except for the lack of glandular hairs, could be identified as *E. brevipila.*

One population studied was found to exhibit in some degree the lower node of first flowering, wider leaves, smaller corolla and wider capsules described (Pugsley 1929) for *E. anglica*, but it was not sufficiently distinct from the others of Group A to justify its separation as a discrete morphological type.

E. hirtella and *E. montana* have been distinguished (Pugsley 1930) from *E. rostkoviana* by an unbranched habit which was found to be characteristic of all populations of Group A. These two species were also distinguished by leaf and flower size, characteristics of considerable variation in Group A. No single populations of Group A could be separated from the others on the basis of Pugsley's (1930) descriptions for these two species. It appears possible that the variation of populations of Group A included that of *E. hirtella* and *E. montana.*

The following key and descriptions were prepared for the nine species as delineated by this study. Each description includes only those characters which were most efficient diagnostically for particular species, while the asterisk (*) indicates the characters of greatest diagnostic value.

Key to Species

1. Glandular hairs present, at least on some plants 2
   Glandular hairs lacking 5
2. Glandular hairs maximum length 0·60 mm; mean number of glandular hairs per 1·5 mm *c.* 13 ..... *E. rostkoviana*
   Glandular hairs maximum length 0·20 mm 3
3. Glandular hairs present on all plants; mean number of glandular hairs per 1·5 mm *c.* 6 ..... *E. brevipila*
   Glandular hairs not always present on all plants; mean number of glandular hairs per 1·5 mm < 2 4

4. Mean plant height *c.* 5 cm; mean lower corolla-lip width <5 mm; mean number of glandular hairs per 1·5 mm 1·98 ..... *E. occidentalis*
   Mean plant height *c.* 10 cm; mean lower corolla-lip width *c.* 8 mm; mean number of glandular hairs per 1·5 mm 0·16 ..... *E. pseudokerneri*
5. Capsule apex glabrous ..... *E. salisburgensis*
   Capsule apex ciliate 6
6. Mean plant height *c.* 4·5 cm ..... *E. confusa*
   Mean plant height >7 cm 7
7. Mean plant width *c.* 4 cm; mean lower corolla-lip width >4 mm ..... *E. nemorosa*
   Mean plant width <2 cm; mean lower corolla-lip width *c.* 3·5 mm 8
8. Mean lower corolla-lip width *c.* 3 mm; habitat fen or flush ..... *E. scottica*
   Mean corolla lip width *c.* 4 mm; habitat heath ..... *E. micrantha*

Euphrasia rostkoviana Hayne (Group A)

Character	*Mean*	*Range*
* Length of longest glandular hairs	0·60 mm	
* Number of glandular hairs per 1·5 mm	13·04	7–24
Plant height	13·61 cm	5·50–31·50 cm
Plant width	2·59 cm	0·60–15·00 cm
Plant height/width ratio	5·28	
Number of primary branches	1·27	0–10
* Capsule width	0·23 cm	0·15– 0·32 cm
Lower corolla-lip width	0·63 cm	0·40– 1·00 cm
Lower corolla-lip plus tube length	0·85 cm	0·52– 1·25 cm
* Leaf width	0·78 cm	0·40– 1·00 cm
Leaf length	0·74 cm	0·40– 1·40 cm
Leaf length/width ratio	0·95 cm	
Leaf length minus calyx length	0·19 cm	
Habitat—meadow		

Euphrasia brevipila Burnat & Gremli (Group B)

Character	*Mean*	*Range*
* Length of longest glandular hairs	0·20 mm	
* Number of glandular hairs per 1·5 mm	5·93	1–14
* Plant height	16·98 cm	7·60–35·00 cm
Plant width	5·93 cm	0·60–21·00 cm
* Number of primary branches	5·91	0–17
* Capsule width	0·17 cm	0·11– 0·26 cm
Lower corolla-lip width	0·60 cm	0·40– 1·00 cm
Lower corolla-lip plus tube length	0·84 cm	0·52– 1·40 cm
Leaf width	0·64 cm	0·30– 1·20 cm
Leaf length	0·60 cm	0·40– 0·90 cm
Leaf length/width ratio	0·94	
Habitat—meadow		

Euphrasia tetraquetra (Bréb.) Arrondeau (Group C)

Character	*Mean*	*Range*
* Length of longest glandular hairs	0·20 mm	
* Number of glandular hairs per 1·5 mm	1·98	0–10
* Plant height	4·70 cm	2·00–10·60 cm

Plant width	2·20 cm	0·80– 6·00 cm
Plant height/width ratio	1·64	
Number of flowers and capsules/plant height	4·78	
* Capsule width	0·19 cm	0·13– 0·30 cm
Lower corolla-lip width	0·44 cm	0·26– 0·72 cm
Lower corolla-lip plus tube length	0·63 cm	0·42– 0·90 cm
Leaf width	0·57 cm	0·24– 1·00 cm
Leaf length	0·54 cm	0·35– 0·83 cm
Leaf length/width ratio	0·95	
Habitat—sand dunes		

Euphrasia pseudokerneri Pugsl. (Group D)

Character	*Mean*	*Range*
Glandular hairs usually lacking, but if present:		
Length of longest glandular hairs	0·20 mm	
Number of glandular hairs per 1·5 mm	0·25	0–2
Plant height	9·74 cm	5·00–14·80 cm
Plant width	4·84 cm	0·80–17·00 cm
* Plant height/width ratio	1·70	
Number of primary branches	5·57	0–25
Capsule width	0·18 cm	0·13– 0·30 cm
* Lower corolla-lip width	0·81 cm	0·50– 1·00 cm
* Lower corolla-lip plus tube length	0·94 cm	0·62– 1·20 cm
* Lower lip plus tube length/width ratio	1·16	
* Lower/upper lip plus tube length ratio	1·21	
Leaf width	0·56 cm	0·36– 0·90 cm
Leaf length	0·54 cm	0·40– 0·82 cm
Leaf length-width ratio	1·03	
Calyx length minus capsule length	0·17 cm	
Habitat—meadow		

Euphrasia salisburgensis Funck (Group E)

Character	*Mean*	*Range*
* Capsule apex glabrous		
Glandular hairs lacking		
Plant height	5·73 cm	2·90–10·10 cm
Plant width	2·93 cm	0·40– 9·00 cm
Plant height-width ratio	2·14	
* Capsule width	0·13 cm	0·10– 0·22 cm
Capsule length	0·35 cm	0·26– 0·50 cm
Lower corolla-lip width	0·32 cm	0·20– 0·50 cm
Lower corolla-lip plus tube length	0·58 cm	0·40– 0·80 cm
* Lower corolla-lip plus tube length/width ratio	1·79	
Flower colour—high percentage of white flowers		
* Leaf width	0·33 cm	0·10– 0·70 cm
Leaf length	0·50 cm	0·30– 0–70 cm
* Leaf length-width ratio	1·57	
Habitat—limestone pasture		

Euphrasia confusa Pugsl. (Group F)

Character	*Mean*	*Range*
Glandular hairs lacking		
* Plant height	4·40 cm	2·00– 9·00 cm
Plant width	1·97 cm	0·50– 6·00 cm

	Plant height/width ratio	2·37	
	Capsule width	0·17 cm	0·12– 0·22 cm
*	Capsule length	0·44 cm	0·30– 0·60 cm
	Lower corolla-lip width	0·36 cm	0·24– 0·60 cm
	Lower corolla-lip plus tube length	0·62 cm	0·50– 0·90 cm
*	Lower corolla-lip plus tube length/width ratio	1·73	
	Leaf width	0·39 cm	0·20– 0·64 cm
	Leaf length	0·44 cm	0·35– 0·70 cm
	Leaf length minus calyx length	0·02 cm	
	Habitat variable—sand dunes, acidic pasture		

Euphrasia nemorosa (Pers.) Wallr. (Group J)

Character	*Mean*	*Range*
Glandular hairs lacking		
Plant height	11·59 cm	5·00–24·00 cm
Plant width	4·25 cm	0·30–14·30 cm
Capsule width	0·18 cm	0·12– 0·26 cm
Lower corolla-lip width	0·44 cm	0·30– 0·70 cm
Lower corolla-lip plus tube length	0·67 cm	0·45– 0·90 cm
Leaf width	0·60 cm	0·30– 1·00 cm
Leaf length	0·56 cm	0·35– 0·90 cm
Leaf length/width ratio	0·94	
Habitat variable—sand dune, basic meadow		

Euphrasia scottica Wettst. (Group G)

Character	*Mean*	*Range*
Glandular hairs lacking		
Plant height	13·19 cm	6·00–24·70 cm
Plant width	1·16 cm	0·60– 4·60 cm
* Plant height/width ratio	10·67	
* Number of primary branches	0·29	0–4
Length of stem to first branch	5·56 cm	2·40–10·00 cm
Number of flowers and capsules	11·42	2–43
Capsule width	0·17 cm	0·13– 0·30 cm
Lower corolla-lip width	0·31 cm	0·20– 0·58 cm
Lower corolla-lip plus tube length	0·56 cm	0·38– 0·80 cm
* Lower corolla-lip plus tube length/width ratio	1·80	
Lower/upper corolla-lip plus tube length ratio	1·04	
Flower colour—may have high percentage of all white		
Leaf width	0·51 cm	0·20– 1·20 cm
Leaf length	0·53 cm	0·30– 0·80 cm
Habitat—wet, fen or flush		

Euphrasia micrantha Reichb. (Group H)

Character	*Mean*	*Range*
Glandular hairs lacking		
Plant height	8·99 cm	2·80–15·70 cm
Plant width	1·60 cm	0·40– 7·00 cm
Plant height/width ratio	5·70	
Number of flowers and capsules	12·72	3–52
Capsule width	0·16 cm	0·12– 0·20 cm
Capsule length	0·36 cm	0·22– 0·49 cm
Lower corolla-lip width	0·39 cm	0·20– 0·60 cm
Lower corolla-lip plus tube length	0·59 cm	0·42– 0·83 cm

	Lower/upper corolla-lip plus tube length ratio	1·05	
	Flower colour may be dark purple		
	Leaf width	0·40 cm	0·20– 0·62 cm
	Leaf length	0·44 cm	0·30– 0·70 cm
*	Leaf length minus calyx length	(—) 0·02 cm	(—) 0·03– 0·00 cm
	Habitat—heath		

Conclusions

The characters of *Euphrasia* were found, with the exception of glandular hairs, to be continuously variable and also inconsistently variable within each species. That is, a character such as lower corolla lip width was variable within a large range for some species and within a small range for others. Therefore characters were found to have diagnostic value for varying numbers of species, some being useful for several, others for only one or two species. No single character was consistently useful for all morphological groups.

Vegetative characters were efficient in distinguishing species. Plant height was consistent for particular morphological types in both wild and cultivated populations. Plant width, while unstable in cultivation, proved a reliable character in wild populations, a fact which appears consistent with Davis & Heywood's (1963) observation that 'adaptive characters will remain stable as long as the environmental conditions to which they are adapted remain constant'. This is particularly applicable to *Euphrasia*, most species of which appear to be specific for habitat.

It is interesting that, while the indications are that *Euphrasia* demonstrates ecological specificity, this at the same time usually fails to function as a barrier to hybridization, since habitats themselves are continuously variable, permitting spatial proximity of differing morphological types. Interbreeding is apparently of frequent occurrence, isolating mechanisms being virtually lacking (Yeo 1966), and yet it seems characteristic of the genus that, despite apparent lack of barriers to gene flow, segregation does occur.

It was found that data for many individuals from homogeneous populations disclosed the existence of morphological types distinct in their particular combination of correlating characters. However it is clear that species of *Euphrasia* are less well separated than would be desirable for efficient taxonomic handling of the group. It must also be accepted, as has been long recognized (Anderson 1949, Clausen 1951, Heslop-Harrison 1960, Davis & Heywood 1963), that the use of relatively large population samples rather than a few individuals is necessary for a realistic approach to identification.

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