A biometric survey of *Limonium vulgare* Miller and *L. humile* Miller in the British Isles

H. J. DAWSON and M. J. INGROUILLE

Biology Department, Birkbeck College, Malet Street, London, WC1E 7HX

ABSTRACT

Limonium vulgare Miller and *L. humile* Miller (Plumbaginaceae) are closely related species that grow on saltmarshes around the coasts of northern Europe including the British Isles. They are often found together in the same marsh. Morphometric analyses were performed on plants collected from sites around the British Isles. This demonstrated the close relationship of these two species and provided strong evidence for hybridization and introgression in sites where both species were present.

KEYWORDS: hybridization, introgression, self-incompatibility, population, speciation.

INTRODUCTION

Limonium vulgare Miller and the closely related *L. humile* Miller, of the family Plumbaginaceae, are found in saltmarshes around the coast of Ireland and Britain northwards to southern Scotland. They have been recognised as distinct species from the time of Ray (1724) as *Limonium majus vulgatius* and *Limonium Anglicum minus, caulibus ramosioribus, floribus in spicis rarius sitis. L. vulgare* has short spikes with densely arranged spikelets and *L. humile* is more laxly branched with long spikes and spikelets distant from each other (Salmon 1905a & b). *L vulgare* does not occur on the coast of Ireland, where it is replaced by *L. humile*. Both occur on the coasts of north-western continental Europe. They grow mixed together in some marshes but *L. humile* is more frequent in the lower marsh than *L. vulgare* (Boorman 1966, 1967).

The difficulties of the identification of the two species have been discussed by Dawson (1988). The confusion in identification between *L. humile* and *L. vulgare* has led to misleading reports and records from some areas and may have obscured the true distribution of both species across the British Isles. This confusion has arisen because of the high degree of variability in *L. vulgare* which has, in the past, led to the description of a wide variety of forms and variants. The variability is not environmentally determined as was thought previously (Clapham 1987). Boorman (1966) used comparative cultivation and transplant experiments to demonstrate that some of the variation is genotypic in origin. Genotypic variation is emphasised in marshes because of the extent of clonal reproduction by rhizomatous spread in *L. vulgare*. Large homogeneous patches with distinct morphologies are found growing next to each other.

The species differ for the most part in chromosome number, *L. vulgare* is a tetraploid $2n = 36$ and *L. humile* a hexaploid $2n = 54$ (Dawson 1990a). There is aneuploid variation in *L. humile* and some plants have $2n = 36$, like *L. vulgare*.

The species possess different breeding systems; *L. vulgare* is an obligate outbreeder and *L. humile* is a facultative inbreeder. Outbreeding in *L. vulgare* is enforced by a self incompatibility system accompanied by dimorphism of pollen and stigmas, with either 'A' pollen (coarsely reticulate) and Cob stigma or 'B' pollen (finely reticulate) and Papillate stigma. The A/Cob morph is heterozygous (AC.ac) and the B/Papillate morph homozygous (ac.ac). *L. vulgare* is also slightly heterostylous, although this character is very variable. *L. humile* is a self-compatible homostylous monomorphic species with an 'A' Papillate morph with the stigma compatible to B pollen as well as A pollen ($Ac^1.Ac^1$). The monomorphic condition is found rarely elsewhere in the genus, and is also found in the related genus *Armeria* (Miller) Willd. and is always derived from the normal dimorphic system (Vekemans *et al.* 1990).

The two species, *L. vulgare* and *L. humile*, provide excellent material for the examination of speciation and the origins and outcomes of different breeding systems.

SAMPLING AND METHODS

Plants were sampled from 52 sites across Britain and Ireland (Fig. 1) some of which had populations of both species present. Details of sample sites are recorded in Dawson (1990b). Material was sampled from sites to represent the whole range of variation present. Some populations were either very small and/or uniform and could be effectively surveyed with a small sample. Different numbers

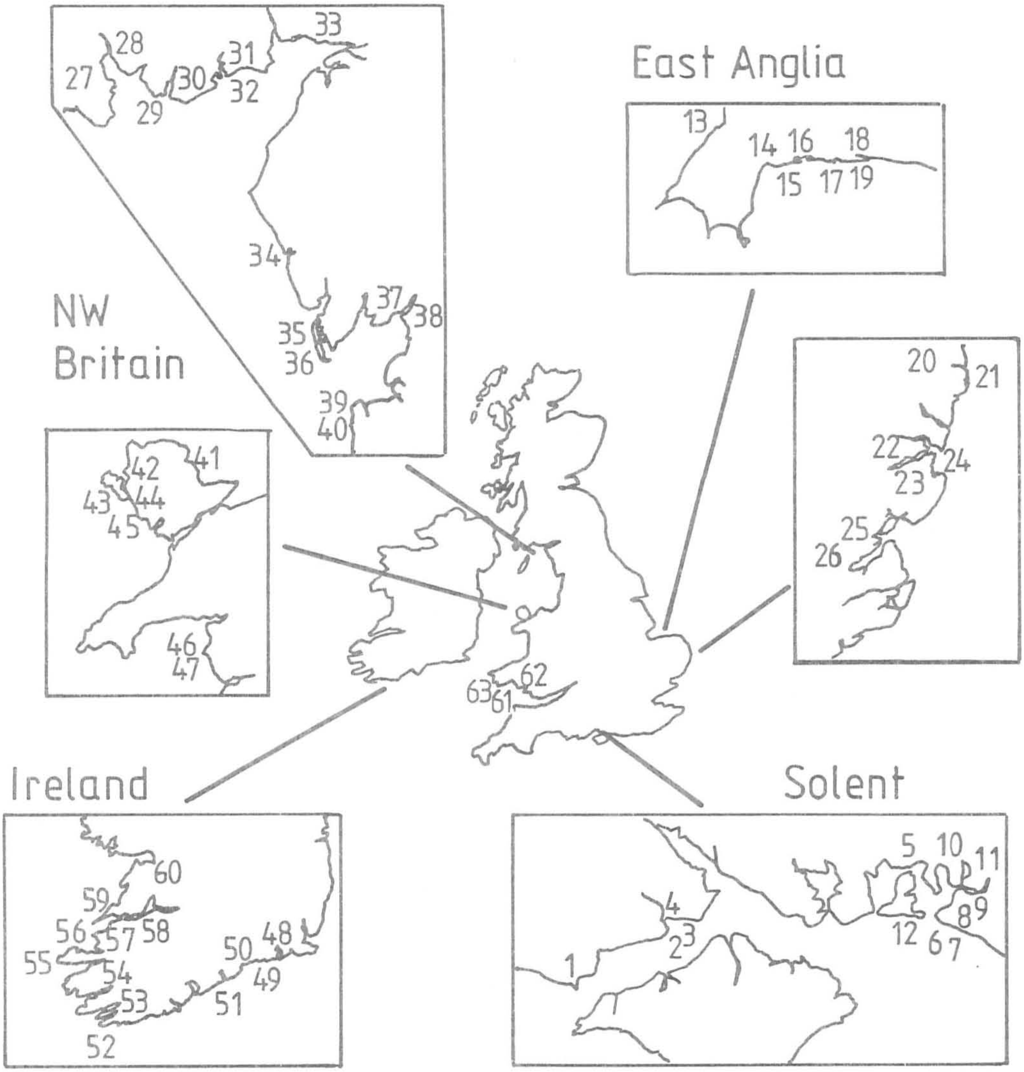

FIGURE 1. Geographical distribution of sampled sites of *Limonium* spp. (Population codes for British Isles as for O.T.U.s in Fig. 6).

of plants were collected at different sites. For the main survey of variation in the British Isles 580 plants were sampled and scored.

For the purpose of analysis, where a large number of populations were sampled, a number of geographical regions were circumscribed; Ireland, North West Britain (Scotland, Cumbria and Lancashire, North Wales), South Wales, the Solent Region, and East Anglia (Essex, Suffolk, Norfolk and Lincolnshire).

Plants from each site were first pressed and dried. Flowering spikes were removed from each plant on collection, labelled correspondingly and preserved separately in 70% ethanol.

The difference in pollen and stigma morph combination has been used to allocate plants to species in this work: A/Papillate = *L. humile*, A/Cob and B/Papillate = *L. vulgare*. This is however complicated by the mutated morphology of the Papillate stigma in *L. humile* plants, which is variable and somewhat intermediate between the Cob and Papillate morphology of the stigmas in *L. vulgare* (Dawson 1990b). There is also some variation in pollen morphology. Variation in stigma and pollen will be reported elsewhere. Pollen and stigma characters and those associated with heterostyly were not used in the multivariate analyses to investigate similarity.

An initial study was made on plants from two sites, chosen because they provided large, pure populations of each of the species: *L. vulgare* from Oxwich, Gower (v.c. 41, SS/514. 877) and *L. humile* from Dale, Dyfed (v.c. 45, SM/812.070). After this study an initial set of 33 scored characters was reduced to exclude those characters which proved difficult to measure accurately.

Following the methods described in Tabachnick & Fidell (1989) a number of characters were eliminated because of very high correlations with another character. Very high correlations may arise because of the logical correlation of characters. For example the length of secondary branches and the length of primary branches were very highly correlated ($r>0.90$) so that length of primary branches only has been included as a measure of branchiness. The length of secondary branches then becomes a superfluous character. In this situation the inclusion of superfluous characters can weight particular aspects of the morphology.

A set of characters which were highly correlated are branch length, spike length, number of spikelets and the distance between the lowest two spikelets. This is a general relationship perhaps relating to the potential to lengthen internodes. However in previous work it has been clear that *Limonium* species vary independently in these characters (Ingrouille 1984; Ingrouille & Stace 1985). For example, some species have an uneven distribution of spikelets on the spike with a relatively large distance between the first two spikelets. Others with a similar length of spike have an even distribution of spikelets on a spike.

Other high correlations between characters in the data set were between scape height and other vegetative measurements. It was clear that there was an overall size effect. Nevertheless scape height was clearly an important distinct character from overall size, one aspect of the 'gestalt' of the plant. A striking feature of variation on marshes is clones of markedly different 'gestalt', growing adjacent to each other (Dawson 1990b) and others with a similar 'gestalt' but differing in overall size. Scape height is only one aspect of the 'gestalt' but should nevertheless be included.

The inclusion of composite variables such as ratios between characters as well as the characters used to construct them may lead to inflated correlations (Tabachnick & Fidell 1989). In this work this has been avoided so that outer-bract length and outer bract width were included but not a ratio of outer-bract length to width. However this general rule has been relaxed in the analysis of 'leaf shape' which is a ratio of leaf length to leaf width. Leaf width, although significantly correlated to leaf shape, has also been included as a measure of maximum leaf size. In an examination of clones it was clear that leaf shape varied in two distinct ways: by the maximum size of leaf achieved by any clone and also by the relative narrowness of the leaf. Leaf length has not been included separately in the analysis.

Significant correlations between the characters remaining in the data set were the rule but it was neither possible nor desirable to try to abstract a set of characters which were not significantly correlated to each other. Significant correlations occur in a complex multidimensional way. In this large data set with just two species, significant correlations between characters were expected because particular sets of character measurements were associated with particular taxa or variants. It would not be desirable, in an analysis designed to measure the extent of relationship between taxa, a priori to limit the range of characters used on the basis of the presumed distinctiveness of those taxa.

It is not the level of statistical significance of correlations which is important but the actual level of the correlation. A data set with many very high correlations (say $r>0{\cdot}8$) lacks information content.

For the major survey 13 metric characters were used in the multivariate analysis. (Table 1, Fig. 2). There were no missing data. Correlations between these characters are reported in Table 2. The data were screened for the presence of outliers and non-normality and following the recommendations of Tabachnick & Fidell (1989) those characters which had high levels of skewness or kurtosis were transformed to their natural logarithmic value (Table 1). A few outliers were recoded as maximum or minimum values within the normal range. Characters were transformed to Z-scores.

Several different univariate and multivariate analyses were performed. Hierarchical analysis of variance of variables for plants within populations within regions was carried out to compare the patterns of variability in species. Multivariate analyses included discriminant function analysis,

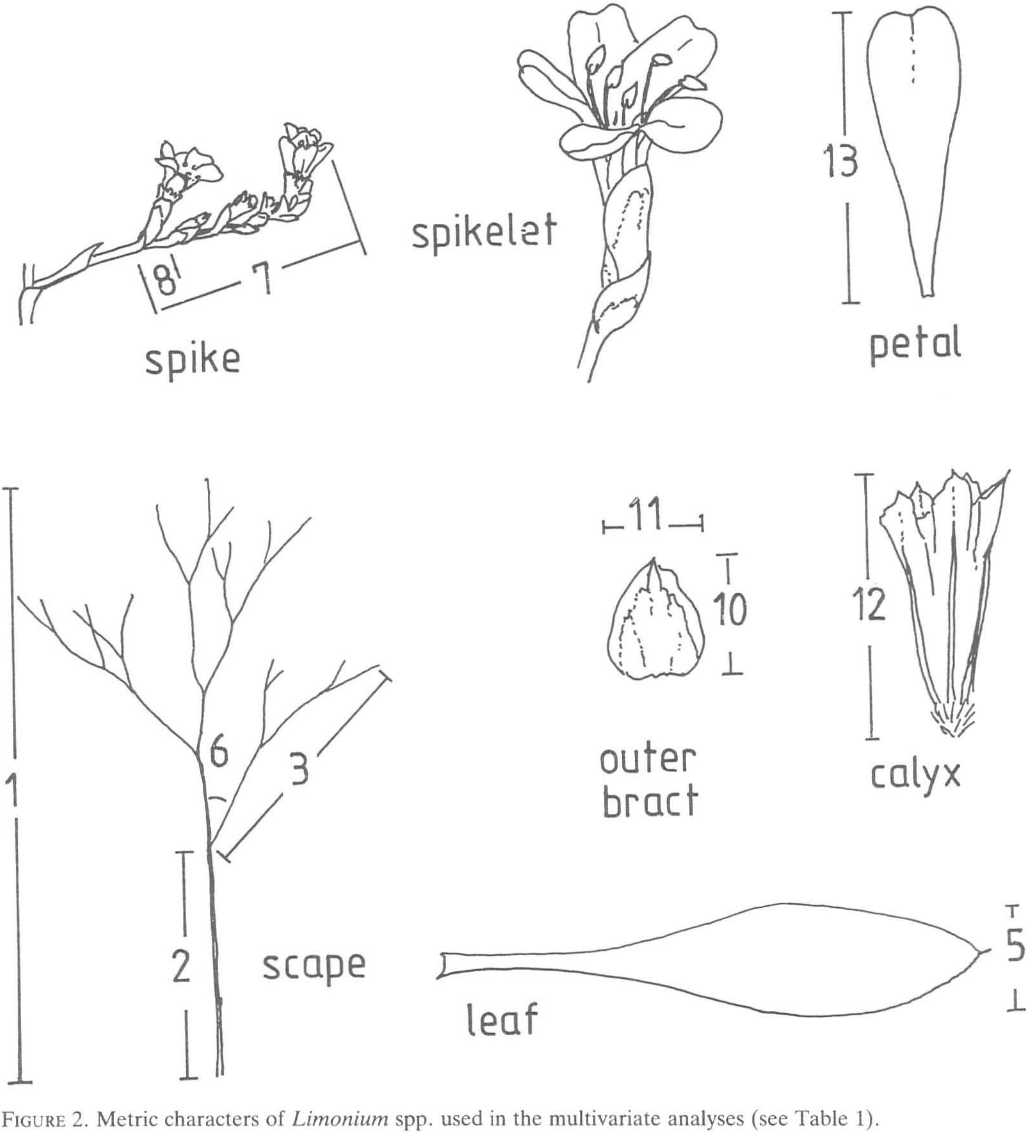

FIGURE 2. Metric characters of *Limonium* spp. used in the multivariate analyses (see Table 1).

TABLE 1. METRIC CHARACTERS OF *LIMONIUM* SPP. USED IN MULTIVARIATE ANALYSES

Character	Transformed (natural log)	No. of outliers* recoded of 580 cases
1. Scape height	yes	0
2. Height to first branching node	yes	6
3. Primary branch length	yes	1
4. Leaf shape	yes	0
5. Leaf width	yes	2
6. Branch angle	no	0
7. Spike length	yes	1
8. Spikelet distance	yes	2
9. Number of spikelets per spike	yes	1
10. Outer bract length	yes	2
11. Outer bract width	yes	1
12. Calyx length	no	0
13. Corolla length	no	2

* An outlier has a Z score >3.00.

TABLE 2. CORRELATION MATRIX BETWEEN CHARACTERS OF *LIMONIUM* SPP.
Probability in parentheses (n = 580)

	Characters											
	1	2	3	4	5	6	7	8	9	10	11	12
2	−0·00											
	(0·99)											
3	0·64	−0·10										
	(0·00)	(0·02)										
4	0·07	−0·15	0·12									
	(0·09)	(0·00)	(0·01)									
5	0·50	0·02	0·19	−0·09								
	(0·00)	(0·55)	(0·00)	(0·03)								
6	0·26	−0·02	0·17	0·08	−0·47							
	(0·00)	(0·69)	(0·00)	(0·05)	(0·00)							
7	0·21	−0·30	0·49	0·21	−0·11	0·16						
	(0·00)	(0·00)	(0·00)	(0·00)	(0·01)	(0·00)						
8	0·19	−0·12	0·08	−0·03	0·25	0·03	0·48					
	(0·00)	(0·00)	(0·07)	(0·53)	(0·00)	(0·49)	(0·00)					
9	0·19	−0·24	0·57	0·28	−0·25	0·18	0·80	0·02				
	(0·00)	(0·00)	(0·00)	(0·00)	(0·00)	(0·00)	(0·00)	(0·58)				
10	0·18	−0·04	0·29	0·06	0·08	0·01	0·41	0·22	0·36			
	(0·00)	(0·29)	(0·00)	(0·17)	(0·06)	(0·87)	(0·00)	(0·00)	(0·00)			
11	0·02	−0·11	0·24	0·20	−0·12	0·02	0·55	0·15	0·54	0·63		
	(0·64)	(0·01)	(0·00)	(0·00)	(0·01)	(0·56)	(0·00)	(0·00)	(0·00)	(0·00)		
12	0·10	−0·12	0·33	0·17	−0·04	−0·01	0·37	−0·12	0·47	0·45	0·43	
	(0·02)	(0·01)	(0·00)	(0·00)	(0·31)	(0·81)	(0·00)	(0·00)	(0·00)	(0·00)	(0·00)	
13	0·06	−0·03	0·01	−0·02	0·27	−0·17	−0·02	0·08	−0·10	0·20	0·07	0·28
	(0·15)	(0·44)	(0·88)	(0·63)	(0·00)	(0·00)	(0·00)	(0·55)	(0·05)	(0·02)	(0·08)	(0·00)

1=Scape height; 2=Height to first branching node; 3=Primary branch length; 4=Leaf shape; 5=Leaf width; 6=Branch angle; 7=Spike length; 8=Spikelet distance; 9=Number of spikelets per spike; 10=Outer bract length; 11=Outer bract width; 12=Calyx length; 13=Corolla length.

principal component analysis (P.C.A.) and cluster analysis on individuals and/or population means of British and Irish material. Cladistic methods were deemed unsuitable for the analysis of continuously varying characters.

Each multivariate method simplified the complex data set in different ways. Discriminant analysis was used to assess the level of intermediacy (hybridization/introgression) between species. P.C.A. and cluster analysis were used to detect patterns without any a priori allocation of plants to species. P.C.A. was carried out with VARIMAX rotation of the axes to maximise the interpretation of components in terms of the original characters. Cluster analysis was carried out on population means as O.T.U.s using both transformed character scores and also factor scores of factors with an eigenvalue greater than one. Several different measures of distance and clustering methods were tried including Ward's method of minimum variance clustering after the calculation of Squared Euclidean Distance.

Analyses were carried out using SPSSX (Norusis 1985) and NTSYS-pc (Rohlf 1990) statistical packages where appropriate.

RESULTS

Hierarchical analysis of variance of individual characters shows a contrasting pattern of variation in each species. An example is reported for the character corolla length in Table 3 and summarised in Table 4 for all 13 characters. *L. humile* is slightly more likely to have populations within regions more distinct than expected by chance from individual plant variation within populations, and *L. vulgare* is slightly more likely to have regions more distinct than expected by chance from variation between populations within regions. A comparison of amounts of variability which can be allocated to each source (Table 4) shows that within populations *L. vulgare* plants are significantly more variable than *L. humile* plants in seven characters. No characters are more variable for *L. humile* within populations.

No single character or pair of characters is effective for separating species. Character distributions of three important transformed characters are illustrated in Fig. 3.

Discriminant analysis using a combination of all characters is successful in identifying the two taxa (Table 5, Fig. 4). An analysis of variance of discriminant scores was highly significant. Characters highly weighted in the discriminant function include spike length and distance between the first two spikelets, previously used to identify species and also the outer bract width and calyx length. None of these characters, either individually or in combination effects a perfect separation of species (Fig. 3). Indeed even using all characters there is an overlap in discriminant scores, with 14 *L. humile* plants and 16 *L. vulgare* plants allocated incorrectly. Of the 16 mis-allocated *L. vulgare* eleven have the A/Cob pollen stigma combination. A statistically significant proportion (34) of the 50 *L. vulgare*

TABLE 3. HIERARCHICAL ANALYSIS OF VARIATION FOR COROLLA LENGTH IN *LIMONIUM* SPP.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-ratio
L. humile				
Between regions	7	966	138·00	1·53
Between populations within regions	34	3075	90·44	4·34*
Between plants within populations	268	5584	20·84	
Total	309	9625		
L. vulgare				
Between regions	8	2683	447·17	7·38*
Between populations within regions	27	1636	60·59	1·43
Between plants within populations	236	10005	42·39	
Total	271	14324		

* F-ratios significant at $p \leq 0.05$.

TABLE 4. VARIABILITY OF *LIMONIUM VULGARE* AND *L. HUMILE* COMPARED; FREQUENCY OF SIGNIFICANT F-RATIOS (AT P≤0·05 LEVEL) IN 13 CHARACTERS

	No. of significant F-ratios (13 maximum)	
	L. humile	*L. vulgare*
F-ratio within species		
Between regions vs between populations within regions	6	8
Between populations within regions vs plants within populations	9	7
	L. humile/ *L. vulgare*	*L. vulgare*/ *L. humile*
F-ratio between species		
Regions	0	1
Populations within regions	3	1
Plants within populations	0	7

plants with the least discriminating scores (i.e. the 50 most similar to *L. humile*) are A/Cob (Table 6). Mis-allocated plants and plants with small discriminant scores are found almost entirely in mixed populations and concentrated in particular regions (Fig. 5).

Species distinctiveness differs markedly between regions (Table 7). Within species there are significant differences between regions for discriminant score (Table 8). The most distinct *L. humile* is found in the extreme north and west, in Ireland and Scotland. The most distinct *L. vulgare* is found in South Wales and East Anglia. In part this is related to the relative abundance of plants of each species in each region. It is most difficult to identify species in mixed populations in the Solent region, where in four mixed populations there are plants which are mis-allocated and in three of these marshes mean discriminant scores for different species are not or only just significantly different: probabilities of a significant difference are East Head p≤0·089, Itchenor p≤0·050, Chidham p≤0·134. Elsewhere discriminant scores are significantly different even in mixed populations. The Solent Region has 66% of all mis-allocated plants in the discriminant analysis.

Within regions some populations are significantly different for discriminant score. Commonly this is related to whether the populations come from mixed sites or not. For example Cumbria/ Lancashire Ravenglass *L. humile* is distinct from the other populations of *L. humile* which are from mixed sites. Similarly the populations of *L. vulgare* found growing with *L. humile* in the Solent Region and Norfolk are statistically distinct from pure *L. vulgare* ones in the same regions. Elsewhere, occasional individual populations can be significantly distinct. Exceptionally *L. humile* plants from the far west of Ireland, where only pure *L. humile* populations are found have low discriminant scores. One *L. humile* plant from Poulnasherry Bay in County Clare was mis-allocated by discriminant analysis.

Cluster analysis of Squared Euclidean Distance by Ward's method proved most effective at clustering species separately. A small number of populations were clustered with populations of the other species, but often close to those from the same region. There were only minor differences between clustering using characters or factor scores. Clustering using character scores was slightly more successful than clustering factor scores; only five populations are mis-clustered compared to six using factor scores (Fig. 6). Only clustering using character scores is described in more detail here.

Mis-clustered *L. vulgare* populations are all from mixed sites. Itchenor and Needs Ore Point *L. vulgare* populations from the Solent are clustered in the major *L. humile* cluster in a small sub-cluster with the populations of *L. humile* also from the Solent. The Holme Island *L. vulgare* population clusters with the *L. humile* populations from its region of Lancashire and Cumbria. Two pure *L. humile* populations are mis-clustered: Treaddur Bay in North Wales and Rine Point in the far west of Ireland.

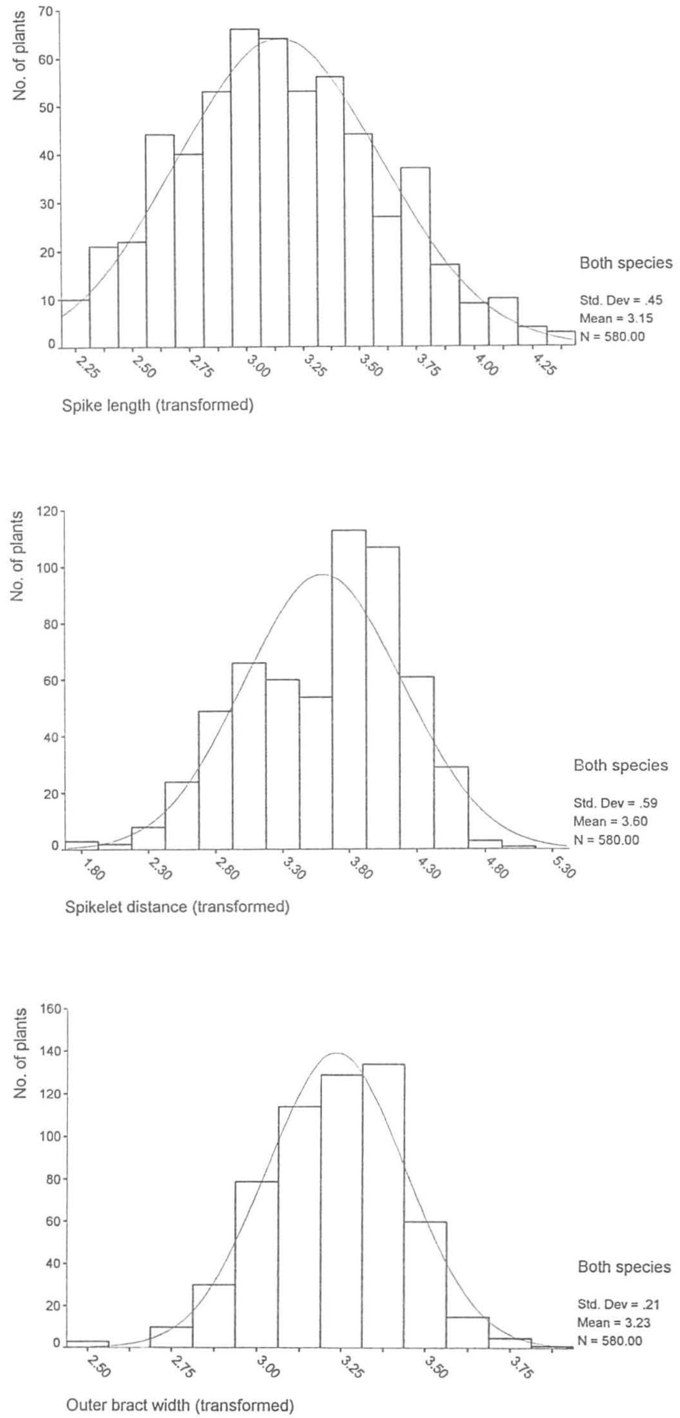

FIGURE 3. Histograms of transformed character measures of three characters: Spike length, Spikelet distance and Outer bract width of *Limonium* spp.

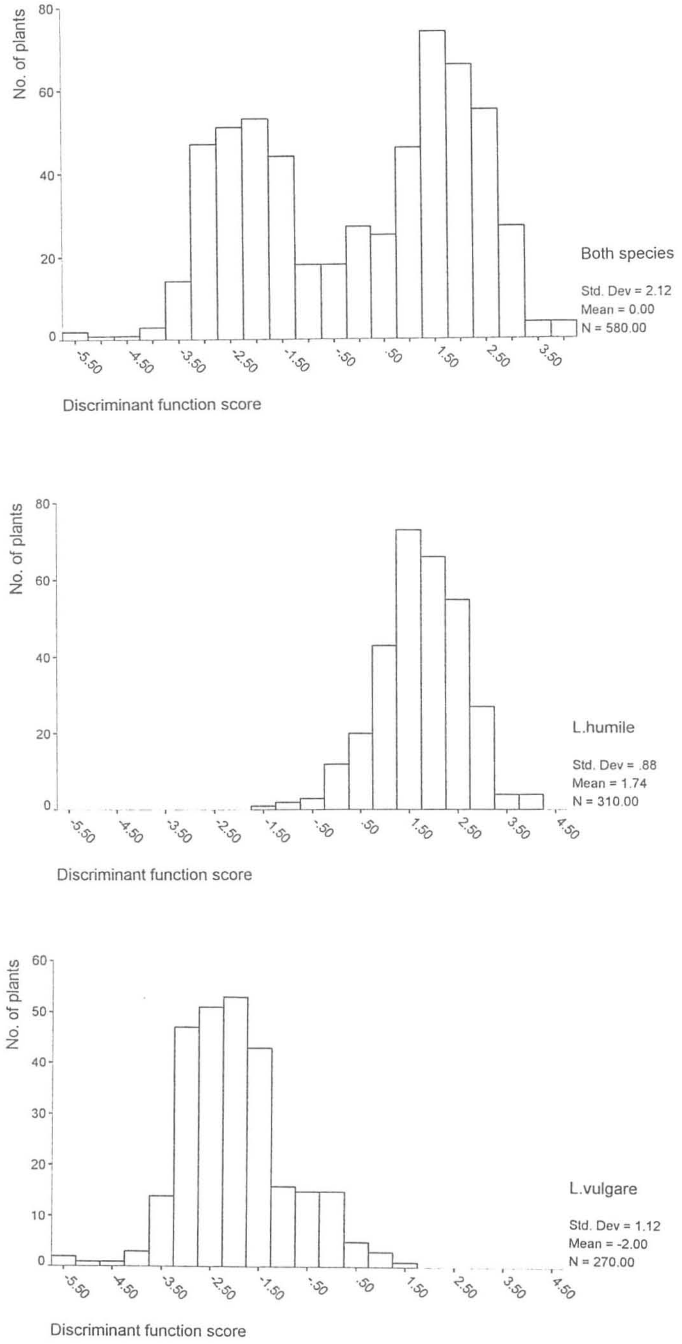

FIGURE 4. Histogram of discriminant scores for: all plants of both species, *Limonium humile* plants only and *L. vulgare* plants only.

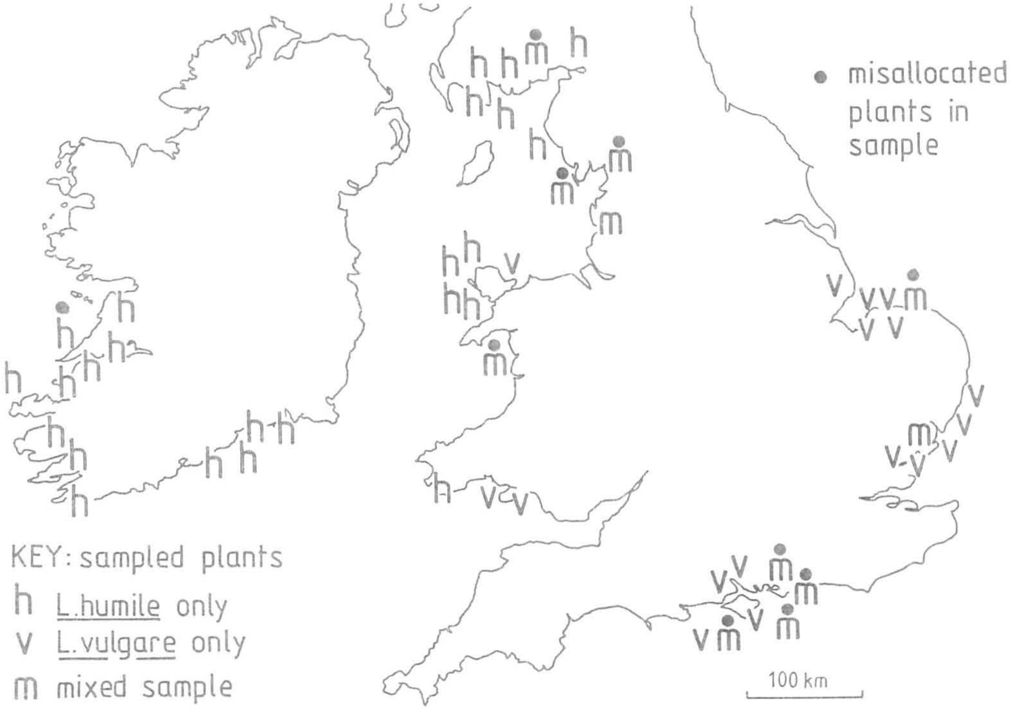

FIGURE 5. Summary of geographical origin of plants of *Limonium* spp. mis-allocated by the discriminant analysis.

TABLE 5. CHARACTERS OF *LIMONIUM* SPP. ORDERED BY CORRELATION WITHIN THE DISCRIMINANT ANALYSIS FUNCTION

Character	Pooled within groups correlations between characters and canonical discriminant functions
8. Spikelet distance	0·63
7. Spike length	0·34
11. Outer bract width	0·27
12. Calyx length	0·26
5. Leaf width	−0·24
3. Primary branch length	0·22
9. Number of spikelets per spike	−0·17
6. Branch angle	0·17
13. Corolla length	−0·14
10. Outer bract length	0·11
4. Leaf shape	0·09
2. Height to first branching node	−0·09
1. Scape height	−0·02

TABLE 6. ANALYSIS OF THE DISTRIBUTION OF STIGMA/POLLEN MORPHS IN THE MOST INTERMEDIATE *L. VULGARE* PLANTS

Stigma/pollen morph	Low Discriminant Scores	High Discriminant Scores	Totals
A/Cob	34	99	133
B/Papillate	16	121	137
Totals	50	220	270

Chi square = 8·62; $p \leq 0·01$.

TABLE 7. DISCRIMINANT SCORES FOR *L. HUMILE* AND *L. VULGARE* IN DIFFERENT REGIONS

Region	Mean score *L. humile*	Mean score *L. vulgare*	Difference between means	Standard error of difference
1. Norfolk/Lincolnshire	1·49 (n=7)	−2·39 (n=61)	3·88	0·366 (df=66)
2. Suffolk/Essex	1·51 (n=7)	−2·22 (n=60)	3·73	0·356 (df=65)
3. Solent	0·89 (n=21)	−1·44 (n=62)	2·33	0·311 (df=81)
4. South Wales	1·53 (n=26)	−2·60 (n=38)	4·13	0·226 (df=62)
5. North Wales	1·68 (n=55)	−1·50 (n=23)	3·18	0·218 (df=76)
6. Cumbria/Lancashire	1·24 (n=24)	−1·74 (n=17)	2·98	0·307 (df=39)
7. Scotland	2·24 (n=40)	−0·90 (n=9)	3·14	0·317 (df=47)
8. Ireland	1·91 (n=130)			
Total	1·74 (n=310)	−2·00 (n=270)		

n = number of plants; df = degrees of freedom.
All differences significant at $p \leq 0·01$.

TABLE 8. DIFFERENCES BETWEEN REGIONS FOR DISCRIMINANT SCORES OF *LIMONIUM HUMILE* AND *L. VULGARE*, REGIONS ORDERED BY SIZE OF DIFFERENCE

	Region							
L. humile	3	6	1	2	4	5	8	7
3. Solent								
6. Cumbria/Lancashire								
1. Norfolk/Lincolnshire								
2. Essex/Suffolk								
4. South Wales	★							
5. North Wales	★							
8. Ireland	★	★			★			
7. Scotland	★	★			★	★	★	

	Region						
L. vulgare	4	1	2	6	5	3	7
4. South Wales							
1. Norfolk/Lincolnshire							
2. Essex/Suffolk							
6. Cumbria/Lancashire	★	★					
5. North Wales	★	★	★				
3. Solent	★	★	★				
7. Scotland	★	★	★				

Duncan test, ★ = $p \leq 0·05$.

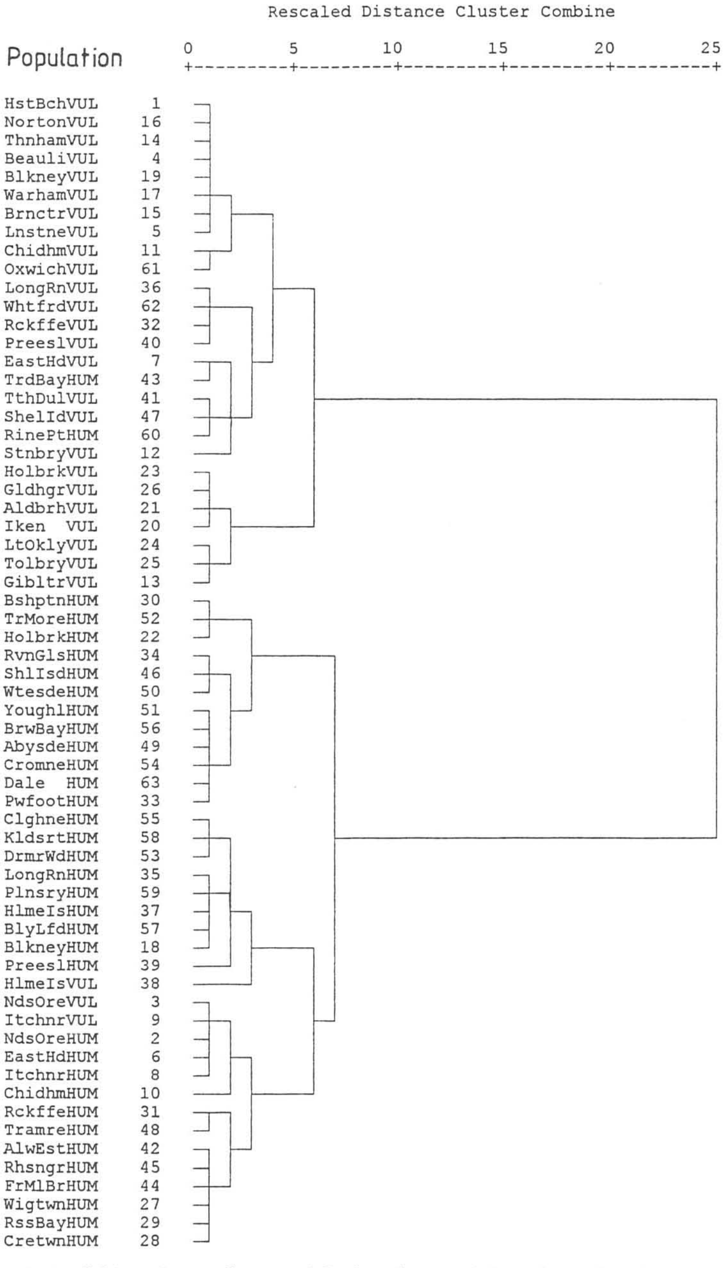

FIGURE 6. Phenogram of *Limonium vulgare* and *L. humile* populations from the British Isles. O.T.U. code numbers as in Fig. 1. (HUM = *L. humile*, VUL = *L. vulgare*.)

There is some clustering together of populations from the same region. For example, all six populations of *L. vulgare* from Essex and Suffolk cluster together with a population from Lincolnshire. All five *L. vulgare* populations from North Norfolk and four (of eight) from the Solent cluster together along with one from South Wales. Four (of six) *L. humile* populations from Scotland cluster with three others from North Wales and one from Ireland.

P.C.A. produces axes of variation which partially separate plants of different species. The first component was strongly correlated with the same characters discovered by discriminant analysis to be important discriminators. However multi-dimensional plots of factor scores do not effectively separate the two species but serve only to emphasise the overlap in species morphology. The second principal component was strongly correlated to overall size.

DISCUSSION

It is clear that *L. vulgare* and *L. humile* are morphologically very close. The ability to transform characters in a very simple way to produce near normal distributions of characters from the combined data set of both species is remarkable evidence for the continuity of variation between the two species. Of the best species discriminators only Spikelet Distance shows any trace of bimodality after transformation (Fig. 3b). No simple combination of easily measured characters can be used to identify species precisely. The best discriminating character is Spike Length but it is a character of debatable value; in eastern England and the Solent, Spike Length is plainly longer in *L. humile* than in *L. vulgare* but taxometric analyses of *L. humile* from several pure populations in Ireland show that here it is not necessarily significantly different from *L. vulgare* (Dawson 1990b).

The shape of the outer bract character given by Clapham (1987) is, in practice, unworkable. Although outer bract width is generally greater in *L. humile* there is very considerable overlap. Another character which has been used in keys is the degree of branching above or below the middle of the stem. This is one of the weakest discriminants.

Patterns of variation may be complicated by hybridization and introgression. Putative hybrid plants, intermediate plants as determined by discriminant analysis, are concentrated where both parental species are present in a marsh. This is strong evidence for hybridization, and it could have arisen because of the presence of intermediate habitats in these marshes. Nevertheless a concentration of intermediacy is the Solent region where a high degree of aneuploidy has also been detected. This is in plants with the *L. humile* stigma/pollen morph combination. *L. humile* is normally $2n=54$. Aneuploid plants are generally intermediate in gross morphology (Dawson 1990b). Intermediate *L. vulgare* plants have $2n=36$.

Intermediate/hybrid plants do have reduced pollen stainability though there is a broad range of values and some hybrids may be partly fertile. Those with the *L. humile* pollen/stigma morph have mean stainability of 68% (standard deviation = 18·9, n = 10) and those with the *L. vulgare* morphs have stainability of 84% (standard deviation = 14·4, n=15). This compares to stainabilities of 95% and 96% for the pure species *L. humile* and *L. vulgare* with little variation (Dawson 1990b).

Artificial hybrids are vigorous and at least partly fertile. The results of a small number of crossing experiments (Dawson 1990b) have shown that hybridization between the species is unidirectional and is only successful when *L. vulgare* is used as the male parent and *L. humile* as the female. This unidirectional inter-specific incompatibility is normal in other groups in crosses between a self-incompatible and self-compatible species; pollen from the parent with the intact self-incompatibility mechanism germinates successfully on the stigma of the self-compatible species but not the other way around.

However there is little evidence in nature that introgression is unilateral. Approximately equal numbers of intermediate plants are found in each species. Of the 40 plants mis-clustered in a cluster analysis of plants (Dawson 1990b) 17 were *L. humile* and 23 *L. vulgare*. Of the 30 plants misclassified by the discriminant analysis 14 were *L. humile* and 16 *L. vulgare* using their pollen/stigma combination.

There is a significant difference in the pollen/stigma morph of intermediate *L. vulgare* (Table 4). They more commonly have the A/Cob morph than the B/Papillate morph. This is expected because hybrids of a cross between an A/Cob *L. vulgare* plant (genotype AC/ac) and a *L. humile* plant (genotype Ac^1/Ac^1) produce A/Cob plants (genotype AC/Ac^1) and A/Papillate plants (genotype Ac^1/ac) in equal proportions.

It is unlikely that A/Cob intermediate plants can self-pollinate because they have a functioning Cob stigma gene. Nevertheless they may be able to act as males in backcrosses to either parental species: to *L. humile* because it has a mutated stigma gene and to *L. vulgare* because of the presence of B/Papillate plants. The consequences of having an unmutated stigma allele in intermediate A/

Papillate plants is not known. The A pollen could act in the same way as A pollen from the A/Cob intermediates. If selfing is possible it would give rise to a range of genotypes identical for pollen/stigma morph to pure B/Papillate *L. vulgare* or A/Papillate *L. humile* as well as hybrids, with genotypes in proportion 1 ac/ac(B/Cob):2 Ac^1/ac(hybrid A/Papillate):1 Ac^1/Ac^1 (A/Papillate *L. humile* type.

Another possibility is shown by *Armeria maritima* (Miller) Willd., which has an identical incompatibility system including some similar A/Papillate monomorphic populations. It also has some dimorphic populations, found on polluted soils, in which both A/Cob and B/Papillate morphs are partly self-compatible (Vekemans *et al.* 1990). In these populations it is the papillate morph which is the better selfer but the cob morph may also self effectively.

In *L. vulgare*/*L. humile* there is some evidence that introgression occurs in different directions in different regions. In North Wales and Scotland *L. vulgare* is the more intermediate in mixed populations. In East Anglia neither species has a high level of intermediacy in mixed populations. In Cumbria and Lancashire it is *L. humile* which is more intermediate in mixed populations. In the Solent Region both species have a high level of intermediacy in mixed populations.

The selective pressures that cause the breakdown of self-incompatibility systems are difficult to identify (Jain 1976) but are frequently associated with the colonization of marginal habitats (Stebbins 1950; Moore & Lewis 1965; Barrett 1988). In these circumstances the more assured production of seed shown by *L. humile* is an advantage. A generally lower position down the marsh may be for *Limonium* a more marginal position. *L. humile* is found in more open communities, often lower down the marsh than *L. vulgare*, where competition between species is lower. Its distribution is positively correlated with patches of bare mud (Boorman 1967, 1968, 1971).

Regional patterns of variation within each species are complicated by the different abundance of each species. The presence of both species within a marsh indicates a greater range of habitats, including intermediate habitats where plants with an intermediate morphology can grow successfully. There may have been selection within the gene pool of either species, enlarged or not by hybridization, for characteristics which allow them to grow within the ecological range of the other species. Different relative abundance also alters the potential for hybridization and introgression in different regions. *L. vulgare* is absent from Ireland and in Scotland and North Wales *L. humile* is the much commoner species. In East Anglia it is *L. vulgare* which is much more abundant.

Clear patterns of geographical variation in salt-marsh vegetation have been noted in Britain both on a broad geographical scale and more locally (Adam 1978). Part of this is clearly related to climate. For example, Scottish marshes are distinct because they are beyond the climatic limits of many important southern salt-marsh species. They are also more frequently subject to fresh-water run-off from the dry-land. Another important factor is the varying importance of grazing in marshes. Those around the Irish Sea are often very grassy as a result of grazing. They are also sometimes narrow or have a small altitudinal range over a large part of them. All these factors may influence the patterns of variation within and between regions. However apart from the continuum of variation between *L. vulgare* and *L. humile* there is no broad geographical pattern of variation, say from south-east to north-west. Differences in variation are more haphazard, but here too may reflect local variation between marshes. This kind of variation has been noted in *Salicornia* even in some of the same marshes sampled here (Ingrouille & Pearson 1987; Ingrouille *et al.* 1990).

It has been frequently suggested that inbreeding species and obligate outbreeders have contrasting patterns of variation (Loveless & Hamrick 1984). Inbreeders are homogeneous within populations but have distinct populations. Outbreeders are more variable within populations with less distinct populations. This pattern has been found in the *Limonium* species pair described here. Neighbouring individual plants of *L. vulgare* are more likely to be distinct than neighbouring plants of *L. humile* (Table 3). This observable difference is exaggerated because *L. vulgare* grows as large homogeneous clonal patches producing tens or hundreds of genetically identical flowering scapes. *L. humile* plants are smaller and more discrete with fewer scapes. This contrast is part of the differing strategies exhibited by the species. It will be described in detail elsewhere.

There are clearly two distinct species if one resorts to stigma and pollen morph to identify them. Since these are characters of considerable biological importance, relating to reproductive isolation, it is taxonomically worth maintaining two distinct species. However this kind of difference in *Armeria maritima* is not normally considered significant enough to merit specific recognition. Northern populations of *A. maritima* are monomorphic like *L. humile* with the A/Papillate morph.

There is considerable gross morphological variation within *A. maritima* which might be used to distinguish species in characters such as hairiness, calyx size and leaf shape for example (Lefebvre 1971; Philipp 1974). The monomorphic variant of *Armeria* is sometimes given specific rank as *A. sibirica* but is only one of several subspecies of *A. maritima* recorded in *Flora Europaea* (Gorentflot & Roux 1972; Pignatti 1972).

The correct identification of *L. vulgare* and *L. humile* can be fraught with difficulties for the non-expert even if pollen/stigma morph is used. This was graphically illustrated to M.J.I. when a class of final year B.Sc. students asked to identify the species from stigma and pollen morph routinely made an incorrect identification even with the aid of microscopes. Their difficulty was not a result of poor observation, but because stigma and pollen morphology is more variable than has been recognised previously. The stigma of *L. humile* is somewhat intermediate between the clearly contrasting Cob and Papillate morphs of *L. vulgare* (Dawson 1990b) and the 'A' pollen is variable.

Nevertheless, despite extensive hybridization where they grow together, *L. humile* and *L. vulgare* are distinct species, with different chromosome numbers and, perhaps related to this, some restriction on their ability to produce fully fertile hybrids.

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