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The pattern of morphological variation in the *Salicornia europaea* L. aggregate (Chenopodiaceae)

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ABSTRACT

A numerical taxonomic analysis of diploid *Salicornia* L. (Chenopodiaceae) plants from four salt marshes in W. Sussex (v.c. 13) and Essex (v.cc. 18 & 19) was carried out by minimum variance clustering (Ward's Method) and principal components analysis. The pattern of morphological variation both between and within marshes provides no evidence for the separate recognition of *Salicornia europaea* L. and *S. ramosissima* J. Woods.

INTRODUCTION

In the British flora, the distinction between perennial and annual species, and between diploid (2n=18) and tetraploid (2n=36) species of *Salicornia* L. (Chenopodiaceae) is well established. The woodiness of *Salicornia perennis* Miller (=*Arthrocnemum perenne* (Miller) Moss) clearly identifies it. Diploid and tetraploid annual species may be distinguished by a number of characters. The number of stamens, the shape of the spike-segment, the length of the terminal spike, the angle of the upper branches to the main stem and the size of the lateral flowers in relation to the central flowers are all useful characters in the field (Ball & Tutin 1959; Ball 1964; Ball & Brown 1970). It is within each ploidy level where difficulties arise, especially for the non-expert.

A major difficulty in *Salicornia* taxonomy emerged in the course of an ecophysiological study on salt marshes in Norfolk, Essex, W. Sussex and Pembrokeshire. In a few locations it proved difficult to distinguish taxa, especially the diploids, even when typical representatives of each species were present within a single marsh.

The first edition of *Flora of the British Isles* (Clapham *et al.* 1952) records three diploid species in the *S. stricta* Dumort aggregate (=*S. europaea* L.), and three diploid species in the *S. prostrata* Pallas aggregate, as well as *S. disarticulata* Moss (=*S. pusilla* J. Woods). The second edition (Clapham *et al.* 1962) reduces these species to four: *S. europaea*, *S. ramosissima* J. Woods (including the *S. prostrata* agg.), *S. pusilla* and *S. obscura* P. W. Ball & Tutin. This trend in the reduction of numbers of species recognized was outlined by Ball (1964) in *Flora Europaea*, where *S. obscura* is recorded as a probable variant of *S. europaea*. Of the three British diploid species presently recognized, *S. pusilla* is distinctive in having a single floret in each cyme rather than the normal three florets of *S. europaea* and *S. ramosissima*. This study is confined to the last two species as these are often the most difficult to distinguish in the field.

Of the distinguishing features between *S. europaea* and *S. ramosissima*, emphasis is placed on the width of the lower fertile segments of the terminal spike, the width of the scarious border and the colour of the segments and cymes (Ball & Tutin 1959; Clapham *et al.* 1962; Ball 1964). However, there is a range of overlap and variation in these characters that make their use difficult. Furthermore, any segment colouring serves to highlight the scarious border and perhaps over-emphasize its importance. It was found that identification of the two species at any one location was generally reliable if the habitat and colour differences were distinct. For example, at Itchenor in W. Sussex (v.c. 13), *S. europaea* is found on the lower marsh associated with bare ground and the tetraploid *S. dolichostachya* Moss, and here its colour is generally dark green with some red round the cyme. *S. ramosissima* is found on the middle and upper parts of the marsh, largely associated with *Puccinellia maritima* (Hudson) Parl., with the segments and cymes coloured purple. The two populations are separated by a broad, almost pure zone of *Spartina anglica* C. E.

Hubbard. Less well-defined marshes, or marshes heavily dissected with creeks, often make such identification more difficult because the habitats are less clearly defined. However, phenological and genotypic studies have shown that differences between the two species at these levels do exist and have emphasized the habitat differences (Jeffries *et al.* 1981; Jeffries & Gottlieb 1982). Jeffries & Gottlieb (1982), using electrophoresis, found that six out of 30 isozymes tested in diploid species at several locations were consistent with two distinct homozygous genotypes, representing *S. europaea* and *S. ramosissima*.

The aim of this study was to carry out a numerical taxonomic study on the morphology of several populations of both species and to determine whether inter-location and intra-location differences could be detected. Any such investigation suffers from the difficulty of maintaining in cultivation, on a regular basis, *Salicornia* plants. Although germination of seeds is easy, maintaining representative plants in terms of succulence and growth form has proved impossible so far. As a result this work has been carried out entirely on collections of wild material. The lack of any testing of genotype – environment interaction for any of the morphological characters measured is a severe drawback.

Of necessity, a classification of *Salicornia* must relate to the pattern of variation in nature since one of the most important requirements of a general purpose classification is that species can be identified by the field botanist. Although classifications can be erected for many purposes, a classification in which it is impossible to identify named taxa except by specialist techniques has a very restricted value.

MATERIALS AND METHODS

SAMPLED POPULATIONS

Both *S. europaea* and *S. ramosissima* are recorded as being common and locally abundant or dominant in the parts of Essex (Jermyn 1974) and W. Sussex (Hall 1980) that have been sampled.

Four salt marshes were sampled (Table 1), three in Essex (v.cc. 18 and 19) and one in W. Sussex (v.c. 13). Marshes were chosen that include typical representatives of *S. europaea* and *S. ramosissima*. At each locality the marsh was sampled at two sites at different levels. Plants were selected from the lower marsh sites from the most seaward populations of the mudflats at St Peter's, Canvey and Itchenor. At Tollesbury, the lower marsh is foreshortened by erosion and at this location plants were collected from the bare mud next to the eroded cliffs. Middle or upper marsh sites were those areas with the most landward group of plants of a reasonable size.

At each site on the marsh a relatively small area was sampled, keeping to the same altitude as much as possible. Underdeveloped or damaged plants and those outside the *S. europaea* group were ignored. For the purpose of this study the *S. europaea* group was defined as in *Flora Europaea* (Ball 1964), consisting of all 3-flowered diploid plants.

Plants were collected over a two week period in late September and kept in polythene bags at 4°C until being scored. It proved possible to keep plants fresh for 2–3 weeks in this way without any signs of shrinkage or distortion. Scoring took place within this period.

TABLE 1. SITE DETAILS OF SAMPLED POPULATIONS

Sampled site		Number of plants	Grid reference
N. Essex, v.c. 19			
Tollesbury	lower marsh	50	52/97.10
	middle marsh	50	
S. Essex, v.c. 18			
St Peter's	lower marsh	50	62/03.08
	middle marsh	50	
Canvey	lower marsh	24	51/82.83
	middle marsh	50	
W. Sussex, v.c. 13			
Itchenor	lower marsh	50	41/78.01
	upper marsh	49	

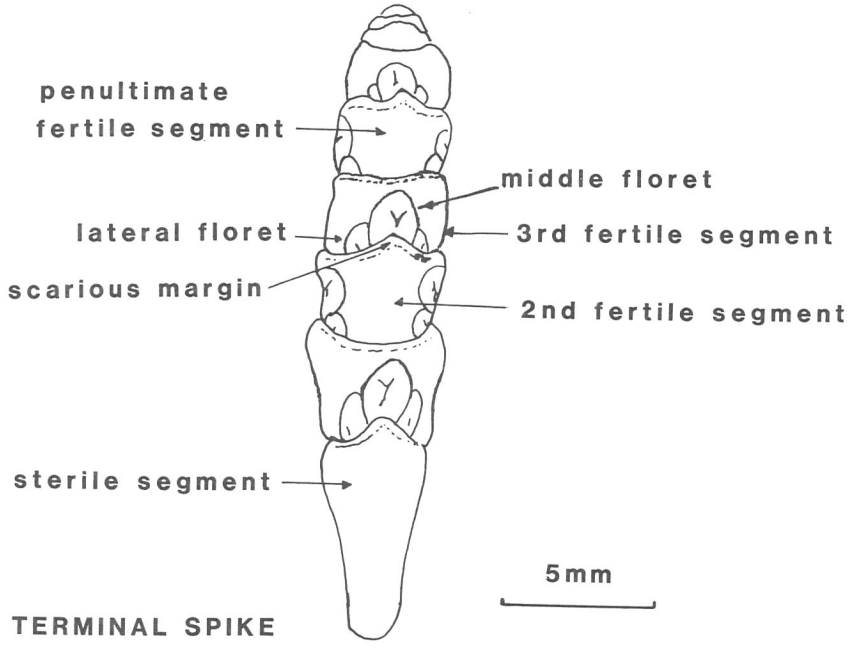


FIGURE 1. Spike characters.

SCORING OF CHARACTERS

Plants were scored for 52 characters consisting of 47 metric characters covering all parts of the plant (including the branching pattern, spike and floret size) and five multistate characters assessing the coloration of the sterile and fertile segments and of the florets of the terminal spike (Fig. 1). An additional 28 characters were derived as ratios of some of the initial 52 characters in order to assess the shape of the whole plant and parts of the terminal spike (Table 2).

In choosing characters, no attempt was made to identify 'useful' characters, nor to examine correlations between characters other than logically correlated characters, e.g. height of the spike and height of each of the segments making up the spike, which were rejected.

DATA PREPARATION AND NUMERICAL ANALYSIS

Initial data preparation was carried out on a Vax – 11/750 computer. Data sets consisting of matrices of individuals as Operational Taxonomic Units (OTUs) against characters were compiled. Characters were encoded as scored without adjustment, with ratio characters calculated and added to the sets.

Subsequent analysis was carried out on the following data sets:

a) all sites and populations consisting of 373 individuals and all 80 characters;

b) eight sets, one for each sampled site of all the individuals from a marsh and all 80 characters;

c) a reduced data set consisting of all 373 individuals and 36 characters – these being the ones relating only to the terminal spike (characters 30–52 and 64–76).

The analysis was carried out on the reduced data set as well as the whole data set in order to identify the effects of environmentally induced variability. One of the most obvious characteristics of *Salicornia* plants is the variation in height and branching pattern of plants on different parts of a marsh. This variability may be environmentally induced and leads to difficulty in the strict comparison of branch characteristics between a plant with and without branches. Terminal spike characters are strictly comparable (being always present) and may be the least environmentally labile characteristics. Subsequent data preparation was carried out by using procedures of the CLUSTAN package (Wishart 1978) release 2 version 1C on an Amdahl 470 computer. All further analysis was carried out using this package of clustering and ordination procedures.

TABLE 2. CHARACTERS SCORED FOR TAXOMETRIC ANALYSIS

GENERAL MORPHOLOGY

1. Height of plant from rooting point to apex
2. Height from rooting point to 1st branching point
3. Number of internodes
4. Length of 1st internode
5. Length of 2nd internode
6. Length of penultimate internode
7. Length of ultimate internode
8. Length of longest 1st (basal) primary branch
9. Number of fertile segments in 1st primary branch
10. Number of sterile segments in 1st primary branch
11. Length of longest 2nd primary branch
12. Number of fertile segments in 2nd primary branch
13. Number of sterile segments in 2nd primary branch
14. Length of the longest penultimate branch
15. Number of fertile segments in penultimate branch
16. Number of sterile segments in penultimate branch
17. Length of ultimate branch
18. Number of fertile segments in ultimate branch
19. Number of sterile segments in ultimate branch
20. Distance from apex to apex of ultimate branch
21. Distance from apex to apex of 1st primary branch
22. Number of secondary branches in 1st primary branch
23. Number of secondary branches in 2nd primary branch
24. Branch node with the most secondary branches
25. Maximum number of secondaries on a primary branch
26. Length of longest secondary branch
27. Number of fertile segments on the longest secondary
28. Number of sterile segments on the longest secondary
29. Length of the longest tertiary branch

TERMINAL SPIKE CHARACTERS

30. Length
31. Number of fertile segments
32. Number of sterile segments
33. Maximum width of 3rd fertile segment
34. Width of middle floret
35. Width of 3 florets
36. Width across apex of 2nd fertile segment
37. Minimum width of 2nd fertile segment
38. Maximum width of 2nd fertile segment
39. Distance between florets on 2nd fertile segment
40. Distance from tip of 3rd fertile segment to apex of middle floret
41. Height of middle floret of 3rd fertile segment
42. Height of side floret of 3rd fertile segment
43. Height of triangular apex of 2nd fertile segment
44. Width of the scarious margin of 2nd fertile segment
45. Length of last sterile segment
46. Maximum diameter of penultimate fertile segment
47. Minimum diameter of penultimate fertile segment
48. Colour of sterile segment*
49. Colour of florets*
50. Colour of fertile segments*
51. Distribution of coloration**
52. Sterile segments***

*Green or yellow=0, diffuse pink=1, red=2. **Basal or even=0, apical=1. ***Not yellow=0, yellow=1.

TABLE 2 cont'd. CHARACTERS SCORED FOR TAXOMETRIC ANALYSIS

RATIO CHARACTERS
53. 1/11
54. (1−2−8)/3
55. 54/(4+5)
56. 54/(6+7)
57. 23/8
58. 24/(8+1−2)
59. 11/14
60. 11/20
61. 17/20
62. 29/11
63. 29/32
64. 33/(40+41)
65. 34/41
66. 35/42
67. 37/43
68. 37/38
69. 35/39
70. 45/(40+41)
71. 38/46
72. 37/47
73. 46/47
74. 36/43
75. 33/37
76. 33/38
77. 45/7
78. 38/1
79. 8/20
80. 9/20

Before carrying out any statistical procedures, the data were standardized by converting to standard scores to give each measured character equal weighting. A similarity matrix with Squared Euclidean Distance as the measure of similarity between individuals (OTUs) was calculated. Subsequent ordination and clustering methods attempted to produce a low dimensional, but undistorted, simplification of this matrix.

Initially a principal components analysis was carried out and scatter diagrams of the first few components constructed.

Cluster analysis was performed using Ward's Method as the criterion for the fusion of clusters. Ward's Method attempts to find a set of clusters with the minimum total within cluster variance. Dendrograms were constructed to illustrate the cluster patterns produced.

The inclusion of so many characters, some of which are highly correlated, poses a number of problems. The inclusion of correlated characters may be justified theoretically on the basis that they each represent the phenotypic expression of a more basic but unmeasured pleiotropic gene or genes. The inclusion of different measures imposes a kind of objective weighting; the more times the expression of the pleiotropic gene is measured the more accurately it is assessed.

It is because of the problem of correlated characters that principal components analysis is so useful. Here the basis of the method is to seek to simplify the data matrix by finding character correlations. The results of a cluster analysis and a principal components analysis can validate each other.

The inclusion of many characters increases the possibility that numerical noise will conceal any patterns which exist but there is no way of choosing the best characters before the analysis is carried out except in an arbitrary, subjective manner. The alternative, as reported here, is to carry out the analysis, detect the best characters a posteriori, and then use these alone to see what pattern of variation exists. The best characters can be chosen a posteriori on the basis of having low intra-cluster variability and high inter-cluster variability, i.e. they are good cluster diagnostics.

TABLE 3. PERCENTAGE VARIABILITY ACCOUNTED FOR BY THE COMPONENTS OF THE PRINCIPAL COMPONENTS ANALYSIS

Component	Full data set (80 characters) % variability	cumulative %	Spike data set (36 characters) % variability	cumulative %
1	18.26	18.26	23.77	23.77
2	13.53	31.79	12.25	36.01
3	6.05	37.84	9.05	45.06
4	5.86	43.70	6.46	51.52
↓	↓	↓	↓	↓
15			1.87	90.76
29	0.76	90.43		

RESULTS

ORDINATION

Principal components analysis, both of the whole data set and the reduced (spike) data set, failed to identify any useful (i.e. discriminatory) components. The data were not summarized adequately by any of the first few components although the first four components accounted for about half of the total variability (Table 3). For example, the principal component scatter plots shown in Figs. 2 & 3 did not reveal any distinct groups or clusters. Rather there was a broad spread of individuals with no obviously distinct modes.

CLUSTER ANALYSIS

Clustering by Ward's method produced well defined clusters in both the whole and reduced data sets (Figs. 4 & 5) but it is necessary to validate the clustering procedure. In part this can be achieved by a comparison of different methods of analysis and by comparing the results of the one

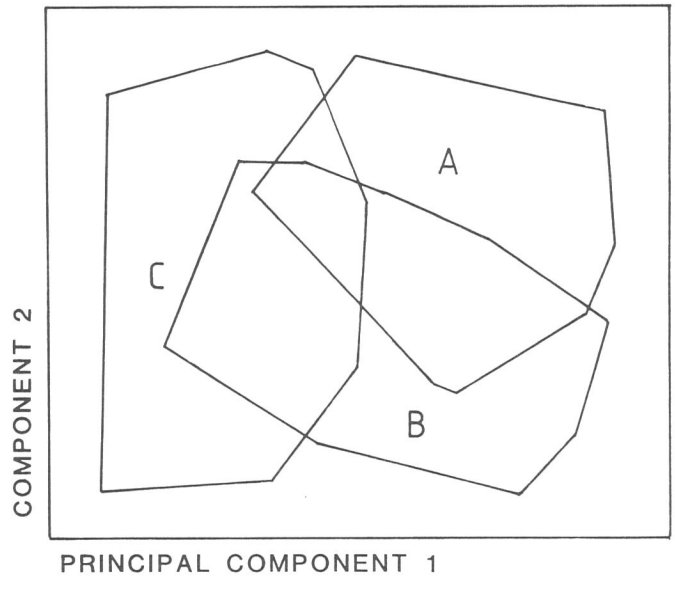


FIGURE 2. Principal component scatter diagram the first 2 components from an analysis of the whole data set. Clusters labelled as in Fig. 4 and in Table 4.

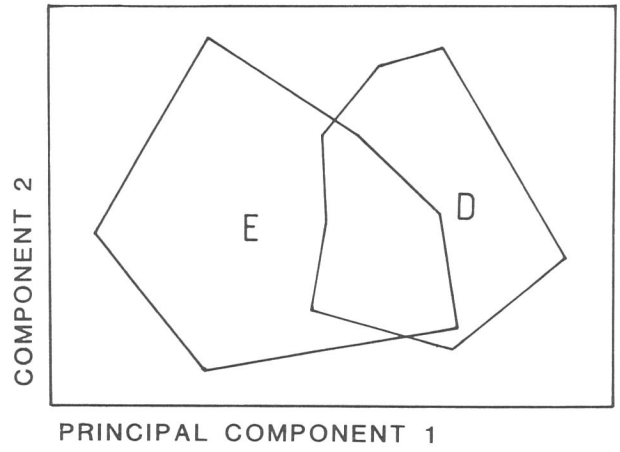


FIGURE 3. Principal component scatter diagram of the first 2 components from an analysis of the spike data set. Clusters labelled as in Fig. 5 and Table 5.

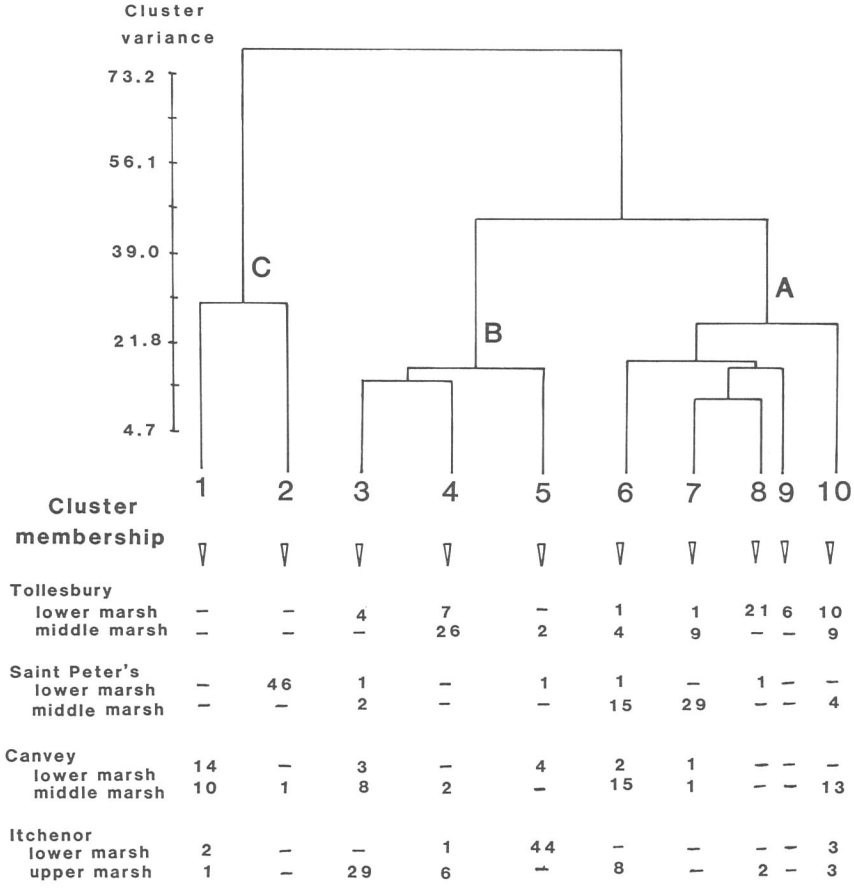
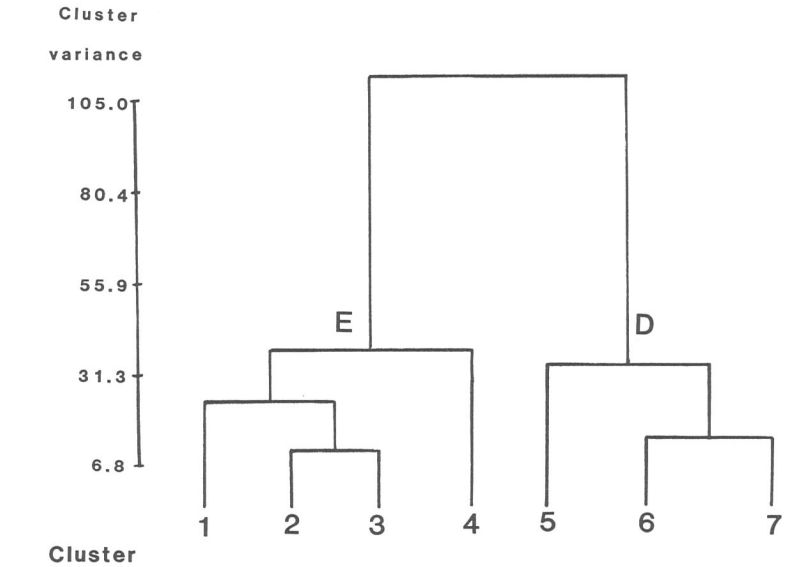


FIGURE 4. Phenogram of cluster analysis of whole data set. Clusters A, B and C defined in Table 4. Source of cluster members at the 10 cluster phenon level indicated.



Cluster membership	1	2	3	4	5	6	7
Tollesbury							
lower marsh	13	6	8	10	2	3	8
middle marsh	7	2	4	1	-	29	7
Saint Peter's							
lower marsh	2	1	-	47	-		
middle marsh	28	2	14	1	-	3	2
Canvey							
lower marsh	9	1	2	-	1	10	1
middle marsh	4	21	4	1	10	3	7
Itchenor							
lower marsh	-	-	-	1	-	5	44
upper marsh	-	12	2	2	29	1	3

FIGURE 5. Phenogram of cluster analysis of spike data set. Cluster D and E defined in Table 5. Source of cluster members at the 7 cluster phenon level indicated.

clustering method (Ward's Method) on the different data sets. Since the clusters isolate different portions of the principal components scatter plots (Figs. 2 & 3), there is a correspondence between different methods of analysis. The phenograms based on the complete data set and the reduced (spike) data set showed a good correspondence especially at the lower levels of clustering, and therefore the clustering procedure is fairly stable.

More important than this kind of internal validation is biological validation to determine whether the clusters produced have any biological significance. The following points suggest that they do.

In the analysis of the whole data set of all the individuals, small clusters regularly identify single sampling sites. 65% of OTUs are most closely grouped to a cluster which is made up only of individuals from the same site. Most of these pure clusters are small ranging, with from two to eight individuals, but four clusters, two from the lower marsh at St Peter's and one each from Itchenor lower marsh and Tollesbury middle marsh, have 13–16 individuals. There are, in addition, a number of other clusters as large or larger which would be from a single site if not for a few mismatched individuals (Fig. 4).

The best diagnostic characters which distinguish the large clusters of both phenograms (Figs. 4 & 5) are listed in Tables 4 & 5. Included is character no. 44, width of the scarious margin of fertile

segments, which has been described as being important (Ball & Tutin 1959). Here it has proved to be a very poor diagnostic. The other characters, however, do act as good diagnostics and relate very well to those used in traditional classification.

The large clusters identify variants very similar to the accepted species concept (Ball 1964) especially clusters A & C of the cluster analysis of the full data set (Fig. 4).

TABLE 4. CHARACTER DISTRIBUTION OF THE THREE CLUSTER STAGE OF THE WHOLE DATA ANALYSIS

Character	*S. europaea* sensu Ball (1964)	Cluster Means A (n=157)	B (n=142)	C (n=74)	*S. ramosissima* sensu Ball (1964)
30. Spike length	10–50 mm	34.5 mm SD=8.3	22.8 mm SD=6.2	23.4 mm SD=8.7	5–30(40) mm
37. Minimum width of 2nd fertile segment	3–5 mm	3.6 mm SD=0.3	3.0 mm SD=0.4	3.1 mm SD=0.5	2.5–4 mm
44. Width of scarious margin	0.1 mm	0.18 mm SD=0.11	0.20 mm SD=0.12	0.24 mm SD=0.09	0.1–0.2 mm
64. Fertile segment width/length		1.6 SD=0.3	1.5 SD=0.3	1.9 SD=0.4	
49. Colour of florets	Diffuse Red	1.5 SD=0.6	2.0 SD=0.8	2.3 SD=0.8	Dark Red
2. Height to 1st branch		41.8 mm SD=26.1	42.9 mm SD=30.1	11.3 mm SD=6.2	
55. Internode unevenness		0.6 SD=0.2	0.6 SD=0.4	1.1 SD=0.5	
8. Length of basal branch		52.0 mm SD=28.2	55.0 mm SD=41.4	98.9 mm SD=39.8	
25. Max. no. of secondary branches	Simple – much branched	0.4 SD=0.5	0.5 SD=0.6	1.9 SD=0.5	Typically much branched
29. Length of longest tertiary branch		0.8 mm SD=3.0	0.1 mm SD=0.6	7.5 mm SD=7.4	
1. Height of plant	(100–)150–300(–350) mm	195 mm SD=43	179 mm SD=50	161 mm SD=40	30–400 mm
Source of cluster members					
Tollesbury	Lower marsh	39	11	0	
	Middle marsh	20	30	0	
St Peter's	Lower marsh	3	2	45	
	Middle marsh	47	3	0	
Canvey	Lower marsh	4	6	14	
	Middle marsh	30	10	10	
Itchenor	Lower marsh	3	43	4	
	Upper marsh	11	37	1	

SD=standard deviation.

TABLE 5. CHARACTER DISTRIBUTION AT THE TWO CLUSTER STAGE OF THE SPIKE DATA ANALYSIS

Character	*S. europaea* sensu Ball (1964)	Cluster Means D (n=168)	E (n=205)	*S. ramosissima* sensu Ball (1964)
30. Spike length	10–50 mm	34.5 mm SD=8.0	21.6 mm SD=6.0	5–30(–40) mm
37. Minimum width of 2nd fertile segment	3–5 mm	3.6 mm SD=0.3	2.9 mm SD=0.4	2.5–4 mm
44. Width of scarious margin	0.1 mm	0.18 mm SD=0.10	0.22 mm SD=0.12	0.1–0.2 mm

SD=standard deviation.

DISCUSSION

The failure of the principal components analysis to discriminate any groups is remarkable because the first four components encompass a large part of the total variation. This failure may reflect the absence of identifiable variants or that the taxonomic structure is too complex, with very many variants, to be easily simplified.

A comparison of the contribution of spike characters and vegetative characters to the clustering is interesting. For example, at the three-cluster stage of the whole data set (Fig. 4 & Table 4), the smallest and most distinct cluster (C) exhibits many of the characteristics of *S. ramosissima*, such as being well branched and having a short spike. Cluster A exhibits the character of *S. europaea*, having a long spike and being only moderately branched. Cluster B has the spike characteristics of cluster C and the branching characteristics of cluster A.

In this cluster analysis, the vegetative characters are of equal importance to spike characters. This is not surprising, despite the wide range of phenotypic plasticity reported by Ball & Tutin (1959) in vegetative characters, because many are correlated. As a result much emphasis has been placed on spike characters. Here the 20 best diagnostic characters of the 80 recorded (derived from the cluster analysis) include eleven vegetative measurements. All of the ten best diagnostics of cluster C are vegetative.

Cluster B may, however, represent an ecophene of variant C – a poorly branched variant growing in suboptimal conditions. Tutin (in Clapham *et al.* 1962) notes that "*S. ramosissima*, though typically much branched, bushy and erect" is "often quite unbranched when growing in crowded pure stands or in competition with other plants. Crowding reduces the degree of branching and this is often accompanied by an increase in the length of the terminal spike."

This last point may explain the failure of the principal components analysis to separate the two taxa when it is carried out on the spike data alone.

There is little correlation between clustering and the level on the marsh of the sampling site. At St Peter's there is a separation of individuals of lower from middle marsh sites (Table 4). At Tollesbury this is only on the basis of spike characters. Notably here, it is the well-branched plants which grow in the lower marsh. Itchenor and Canvey show little clustering of sites within each marsh. At Itchenor this is due to the existence of a large proportion of intermediate individuals (Cluster B). At Canvey the clusters are more equally represented.

The lack of stratification of variants as shown by clusters A, B and C is not due to the inclusion of environmentally induced variation in vegetative characters. An examination of the clusters from the analysis of the spike data shows that 45% of cluster D and 48% of cluster E plants come from lower marsh sites.

The cluster analysis of both spike and full data sets does show a remarkable ability to identify local variants. At the seven-cluster stage of the spike data analysis (Fig. 5), between 44% and 75% of individuals of each cluster come from a single site. The only sites not identified are those of Canvey lower marsh, where only 24 individuals were sampled, and Tollesbury lower marsh, where the lower marsh is foreshortened by erosion. In particular, St Peter's lower marsh and Itchenor lower marsh have very distinct local variants. Such local variants are probably the result of the regular self-pollination reported by Dalby (1962). This would encourage the evolution of distinct inbred lines. The hierarchy of clusters at each site may relate to a pattern of familial relationships.

Means and standard deviations of the best cluster diagnostics for each site are shown in Table 6. As can be seen, there is little that can be said in a general way about the variation in particular characteristics between sites. Each site is defined in a different way. The degree of variation present between individuals within each site is assessed by the clustering method which finds 'spherical' minimum variance clusters. The clustering level of the final clustering procedure provides an estimate of the error sum of squares. Tollesbury and Canvey sites which are probably the least well stratified marshes have the greatest range of variant individuals and the least well distinguished variant groups.

TABLE 6. MEANS AND STANDARD DEVIATIONS IN PARENTHESES OF DIAGNOSTIC CHARACTERS FROM SITES WITHIN MARSHES. MEASUREMENTS IN MM

Character	Tollesbury		St Peter's		Canvey		Itchenor	
	Lower marsh	Middle marsh	Lower marsh	Middle marsh	Lower marsh	Middle marsh	Lower marsh	Upper marsh
30. Spike length	23.7 (5.6)	34.7 (8.4)	18.7 (5.9)	24.2 (7.8)	30.3 (8.9)	23.0 (8.0)	37.8 (6.6)	27.8 (7.1)
37. Minimum width of 2nd fertile segment	3.0 (0.3)	3.5 (0.4)	2.7 (0.5)	3.1 (0.4)	3.4 (0.4)	3.2 (0.5)	3.7 (0.3)	3.3 (0.4)
44. Width of scarious margin	0.16 (0.12)	0.11 (0.13)	0.28 (0.18)	0.29 (0.10)	0.16 (0.07)	0.20 (0.08)	0.14 (0.06)	0.25 (0.10)
64. Fertile segment width/length	1.4 (0.2)	1.4 (0.3)	1.9 (0.4)	1.5 (0.3)	1.8 (0.4)	1.7 (0.4)	1.5 (0.2)	1.7 (0.3)
2. Height to 1st branch	30.2 (19.1)	51.5 (27.0)	9.7 (5.3)	77.3 (27.4)	15.7 (6.2)	22.5 (16.7)	31.6 (17.0)	40.7 (21.5)
55. Internode unevenness	7.3 (3.1)	5.2 (3.3)	12.7 (5.1)	2.9 (1.5)	7.9 (1.9)	7.6 (3.2)	5.7 (2.4)	5.6 (2.2)
8. Length of basal branch	60.4 (36.2)	41.4 (36.2)	76.8 (30.9)	38.6 (27.7)	91.8 (46.4)	80.7 (52.5)	63.6 (36.0)	59.9 (34.5)
25. Max. no. of secondary branches	4.1 (5.4)	2.3 (4.6)	16.7 (6.0)	2.5 (3.5)	14.3 (8.5)	10.4 (9.0)	4.5 (5.8)	7.4 (6.0)
29. Length of longest tertiary branch	0.08 (0.57)	0.32 (2.26)	6.80 (7.0)	0.0 —	6.00 (7.4)	2.20 (5.6)	0.16 (1.13)	0.90 (3.14)
49. Colour of florets	2.40 (0.53)	1.42 (0.70)	2.70 (0.50)	1.94 (0.84)	1.91 (0.78)	1.74 (0.69)	1.00 —	1.76 (0.66)
Overall variability	7.45	7.29	6.75	6.62	3.51	9.90	6.73	6.62
Error Sum of Squares from cluster analysis	11.90		17.93		11.13		11.55	
Sample size	50	50	50	50	24	50	50	49

CONCLUSION

The evidence presented here suggests that by far the most important kind of variation in the *Salicornia europaea* agg. is small scale. Where sites are compared, local variants can be identified, but the variation between sites is not consistent, and thus it is not possible to find a lower marsh and a middle/upper marsh variant in every marsh. In addition, taking the plants as a whole, there is little evidence for the recognition of distinct taxa. Cluster analysis may define clusters which are very similar to the recognized taxa but this is the result of a rather arbitrary division of a more or less continuous spectrum of variation. Such clusters fulfill only one of the four criteria Stace (1980) and Davis & Heywood (1973) cite for the recognition of plant species, i.e. the first of the following:

1. The individuals should bear a close resemblance to one another such that they are always recognisable as members of that group.
2. There are gaps between the spectra of variation exhibited by related taxa.
3. Each taxon occupies a definable geographical area or ecological niche.
4. Sexual taxa should have individuals capable of interbreeding with little or no loss of fertility and there should be some reduction in the level or success (measured in terms of hybrid fertility) on crossing with other taxa.

That the second criterion is not fulfilled is shown by the principal components scatter diagrams. The inability to detect breaks in the spectrum of variation is not due to the inclusion in the analysis of extraneous characters that are very sensitive to environmental variation. Scatter plots of two of the best diagnostic characters show the same complete spectrum of variation (Fig. 6).

These scatter plots also illustrate the failure to fulfill criterion 3. Variants that do exist are not consistently associated with a level on a saltmarsh.

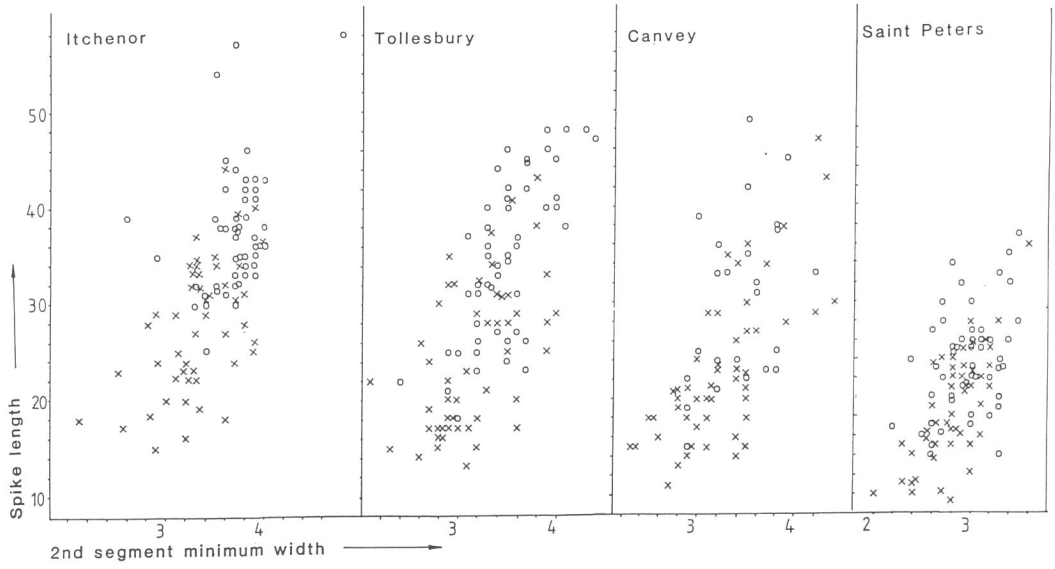


FIGURE 6. Scatter plots of the best cluster diagnostics; spike length and minimum width of 2nd fertile segment (mm). X=upper or middle marsh plants, O=lower marsh plants.

The information presented here provides no evidence for the fourth criterion. No such information is available but the work of Jeffries & Gottlieb (1982) is very suggestive and contradicts our findings. They were able to detect just two electrophoretic morphs which differed consistently in their isozyme pattern for six different enzyme systems. Each 'electromorph' was consistently associated with either lower or upper marsh. More important was the fact that no heterozygotes were detected indicating that the electromorphs do not normally interbreed.

Jeffries & Gottlieb (1982) identify the upper marsh electromorph with *S. ramosissima* and the lower marsh electromorph with *S. europaea*. They further suggest that these two species form, in the British Isles, two distinct homozygous lineages and that the inability to detect any genetic variability within the species throws doubt on previous reports of genetic variants for morphological characters.

If the electrophoretic evidence is taken to its limits all the morphological variation within each species is environmentally induced. The many local variants are then the result of local conditions. Until a proper genetic analysis is carried out this must remain a conjecture. What our work shows is that morphological recognition of the two species is not possible and that it would be better to group them all under *S. europaea* L. The alternative of recognising 'chemical species' is not a practical possibility.

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