Variation of discrete morphological characters in *Cytheridea* (Crustacea: Ostracoda).

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The analysis of the discretely varying characters: frequencies of anterior spines (of which there is a maximum of 6), the presence or absence of a posterior spine and the type of lateral ornament (a dichotomous character) in the Miocene ostracod *Cytheridea acuminata* Bosquet *caumontensis* Carbonnel shows that time-dependent shifts occur. The results were obtained by means of standard methods of statistical analysis. It is suggested that the multivariate statistical analysis of discrete variables of the ostracod carapace can be a useful aid in biostratigraphy.
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Introduction

In studying quantitative variation in ostracods, it is usual to consider characters that measure changes in the size and shape of the carapace. Such variables are said to be continuous in the statistical sense; for example, length, height, and breadth of the carapace, location of the adductor muscle field, the width of the marginal zones, etc.

Other kinds of characters have not been given the same attention, as it may not always be obvious what should be measured or how to treat measures that have been made. We refer here to discontinuously varying attributes, such as the numbers of posterior and anterior spines, the nature of the lateral ornament (cf. Reyment 1963, 1965, and Reyment & Van Valen 1969) and the shape of the posterior part of the carapace.

Cytheridea acuminata Bosquet *caumontensis* Carbonnel is, in many respects, well fitted for a study of changes in the frequencies of discrete characters over time. We also examined a second species from the same Miocene sequence in France, a species of *Haplocytheridea*, but it was determined to be far less suitable for our purposes.

If changes in discontinuously varying characters can be shown to have a time-correlated pattern, it is natural to enquire whether such changes can be utilized in the solution of certain stratigraphical problems. Normally, biostratigraphical methodology leans heavily on the marker concept, the appearance and disappearance of zone fossils, as well as, to a certain degree, shifts in the compositions of associations of fossils.

This way or working relies on the existence of easily recognizable faunistic changes in the stratigraphical column. The possibility of being able to use changes below the taxonomical level of the species in establishing stratigraphical profiles would appear to be worth examination.

The analysis of continuously varying attributes has already been considered by Reyment (1963, 1971). In this paper, we take up the study of variables that vary discontinuously in the statistical sense.

The characters analyzed in this report are:
The frequencies of the six anterior spines
The frequencies of the posterior spine
The frequencies of the lateral ornamental types.

We shall begin by asking:

whether there is a significant pattern of association within the anterior spines;
whether significant association occurs between the anterior and posterior spines;
whether significant trend exists in these characters.

We shall continue by taking up an analysis of the lateral ornamental variation. Finally, we shall examine the relationships between all variables taken simultaneously, using standard methods of

multivariate statistical analysis (cf. Blackith & Reyment 1971).

Provenance of the material studied

The samples studied were obtained from six sites in the Rhone basin in southeastern France. All are of Miocene age (Carbonnel 1969). The localities denoted A, B and C in the text are:

    A = La Lauze No. 32
    B = Caumont-Picabrier No. 314
    C = Caumont-Picabrier No. 10 and La Fongoline No. 94.

These belong to the early Miocene (the zone A of Carbonnel 1969). Locality D = Grignan-Nord No. 113 is stratigraphically higher than the foregoing and is dated as Early or Middle ? Miocene (the zone B of Carbonnel 1969).

    The levels denoted as E and F in the text are:

    E = Cucuron No. 183
    F = La Déboulière No. 442.

These localities are datable as being Late Miocene in age and they are part of the zone C of Carbonnel (1969).

We note that the relationships between the ostracod zones and those based on the planktic foraminifers have yet to be established.

The generic characters of *Cytheridea*

The genus *Cytheridea* Bosquet (1852) occurs from Early Eocene (*C. appendiculata* Ducasse (1967)) until Recent, according to van Morkhoven (1963). Its roots in the Cretaceous are still not known with certainty. As far as we are aware, no recent study of the taxonomical relationships of the group, nor of the evolution of its principal characters, has been made.

According to van Morkhoven (1963), the average outline of the genus is trapezoidal. The posterodorsal angle is fairly marked to well marked. The hinge is composed of two crenulated bars, anteriorly and posteriorly located in the right valve and a crenulated bar in the left valve (the amphidont hinge category). The anterior and posterior margins may be provided with spines which occur in variable number within species; the spines may be pierced by continuations of the marginal canals. The marginal spines are often ignored in descriptions of species. More attention has been paid the ornament: there are smooth forms, others with a finely punctate surface (*C. fallens* Oertli), some
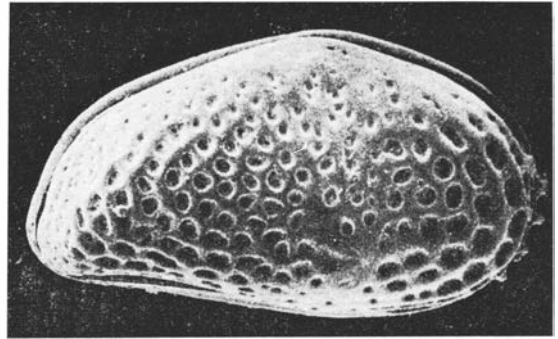

Fig. 1. Example of a regularly ornamented individual. Specimen from level E, Cucuron (4 anterior spines, lacking a posterior spine).

with a pitted surface (*C. acuminata* Bosquet) and still others with ribs.

The genus rarely displays forms with "phenotypic" tubercles, although such are well known in *Hemicyprideis* Malz and *Cyprideis* Jones. An exception is provided by *Cytheridea plagosa* Eagar.

Ecologically, species of the genus are generally considered as preferring a near-shore environment and there is probably a tendency to euryhalinity. The widest development of the genus took place in the Oligo-Miocene and at the present time, it is in a phase of geographical regression.

The characters analyzed

C. acuminata caumontensis (possibly synonymous with *C. ottnagensis*) possesses anterior and posterior spines. The ornament is not a constant feature from specimen to specimen. There are maximally six anterior spines; these constitute variables x_1 to x_6 inclusively. The observations on these variables were made by noting the presence or absence
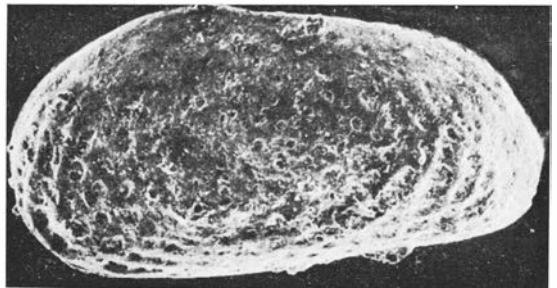

Fig. 2. Example of an irregularly ornamented individual. Specimen from level E, Cucuron (5 anterior spines, no posterior spine).

of the spine at each of the six locations. There are two posterior spines, but the occurrence of the second spine is so rare that for the analyses, only one spine was scored; this is variable x_7. The length of each carapace was recorded, this being variable x_8, used in some of the exploratory calculations. The ornamental varieties were scored dichotomously, the categories being regular ornament (cf. Fig. 1), that is ornament resembling the sculpture of final larval carapaces, and irregular ornament (cf. Fig. 2). This is variable x_9. Only adult carapaces were used for making our observations.

Methods

Each specimen studied was first photographed under the Stereoscan microscope and the data on the discrete variables obtained from examination of the resulting pictures. Stereoscan photographs offer the best way of studying small-scale variations in the ostracod carapace, as some of the features of decisive interest cannot be observed consistently under the light microscope.

The study material comprising 224 specimens from six stratigraphical levels was analyzed using computer programs developed in the Department of Historical Geology and Palaeontology at the University of Uppsala.

Plan of analysis

The first question we shall consider is whether there is association in the anterior spines; that is, whether they are significantly correlated in their mode of occurrence. We shall also ask whether there are variations in the strengths of such associations should they exist.

The second question we shall ask concerns the relationship between the anterior and posterior spinosities. It is not unreasonable to suppose that spinosity is a unified response in the carapace and that the occurrences of anterior and posterior spines are to a certain degree related.

The third problem we take up is that of the variations in the ornamental frequencies. In connexion herewith, the inter-relationships between all variables will be examined.

Fourthly, we shall examine the data for significant trend.

Variation in the anterior spines

The development of anterior spines at six loci on the anterior margin of the valve appears at first

Table 1. Array of chi-squares between pairs of spines for sample B (N = 51).

	spine 1	spine 2	spine 3	spine 4	spine 5	spine 6
spine 1						
spine 2	13,02**					
spine 3	6,80**	14,83**				
spine 4	9,82**	17,07**	12,67**			
spine 5	1,83	2,09	0,01	5,76*		
spine 6	1,30	1,15	1,15	0,03	10,45**	

Ho: Independence of spine-producing potential between every pair of spine loci.
* null hypothesis rejected at the 95 % level.
** null hypothesis rejected at the 99 % level.
All significant associations are positive $(ad - bc > 0)$.

sight to be a polymorphic character, marked by the presence or absence of a spine at each site and the presence or absence of combinations of spines. Were this variation polymorphic, 2^6 phenotypes are theoretically possible. Although it is unlikely that the development of each spine is controlled by independent genetical factors, it should be noted that about 60 of the possible combinations have actually been identified in our rather small sample.

For our purposes, we think it more reasonable to analyze this variation by assuming that there is a "potential to produce spines", which is approximately normally distributed and which is controlled by multi-factors such as polygenes. To a certain extent, this approach is tangential to Rendel's (1969) concept of "Make".

The analysis of the relationships between the

Table 2. Array of chi-squares between pairs of spines for sample C (N = 45).

	spine 1	spine 2	spine 3	spine 4	spine 5	spine 6
spine 1						
spine 2	19,06**					
spine 3	8,52**	15,79**				
spine 4	5,13*	2,80	8,39**			
spine 5	1,19	1,63	3,59	25,64**		
spine 6	0,05	0,04	0,04	2,00	5,27*	

Ho: Independence of spine-producing potential between every pair of spine loci.
* null hypothesis rejected at the 95 % level.
** null hypothesis rejected at the 99 % level.
All significant associations are positive.

Table 3. Array of chi-squares between pairs of spines for sample E (N = 58).

	spine 1	spine 2	spine 3	spine 4	spine 5	spine 6
spine 1						
spine 2	11,75**					
spine 3	0,99	15,63**				
spine 4	0,06	6,07*	7,38**			
spine 5	1,59	9,28**	11,44**	18,04**		
spine 6	0,02	3,25	2,02	2,04	10,08**	

Ho: Independence of spine-producing potential between every pair of spine loci.
  * null hypothesis rejected at the 95 % level.
  ** null hypothesis rejected at the 99 % level.
All significant associations are positive.

Fig. 3. Histograms for proportions of anterior spines; these are unimodal with peaks located around spines 3 and 4.

anterior spines can be suitably initiated by examining the associations between pairs of spines. If we take as our null hypothesis that there is complete independence between every pair of spine loci, this can be tested by computing chi-squares. For the purposes of these calculations, we have confined ourselves to the three largest samples, namely, those deriving from levels B, C and E. The chi-squares between all possible combinations of the six spines, grouped pairwise, are listed in Tables 1, 2 and 3. These results indicate that the null hypothesis for the independent development of spines can be rejected for many of the combinations. There is a clear indication that the development of spines is closely and positively associated between neighbouring loci. The values of chi-squared between neighbouring loci are in all cases significant. As indicated in Fig. 3, the histograms for the frequencies of the six spines are unimodal for each of the samples. A possible interpretation of this situation is that the middle spines are apt to be better developed than the external ones; this suggestion seems to be supported by direct observation. We return to this question further on.

As demonstrated by Fig. 4, there does not appear to be a general pattern of agreement in the histograms for the number of anterior spines. The empirical distributions are either strongly skewed or pronouncedly flattened and, on the basis of our admittedly small samples, they seem to deviate from what would be expected for the normal distribution.

The chi-squared analysis would appear to suggest that the six anterior spines were not independent of each other in *C. acuminata caumontensis*. The
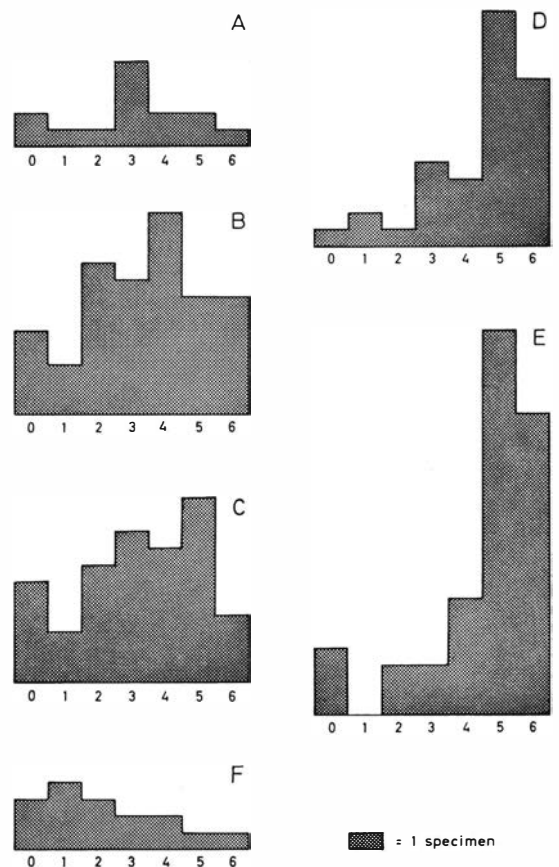
association between spines seems to be always positive and to be very strong between neighbouring loci.

In the genus *Cytheridea*, it is known that the anterior and posterior spines tend to be produced at the outer openings of marginal pore canals (thus forming an extension of a particular canal), although only a few of the canals will continue into spines. Although the genetical background of the spinosity is not very clear, we presume that it is polygenic and pleiotropic: the development of each spine is controlled by many genes and a single major gene may give a phenotypic effect to more than two loci of spines, particularly neighbouring ones. Moreover, some kind of spectral threshold can be expected to operate in the development of spines, such as has been assumed by Reyment & Van Valen (1969) to occur in *Buntonia olokundudui*.
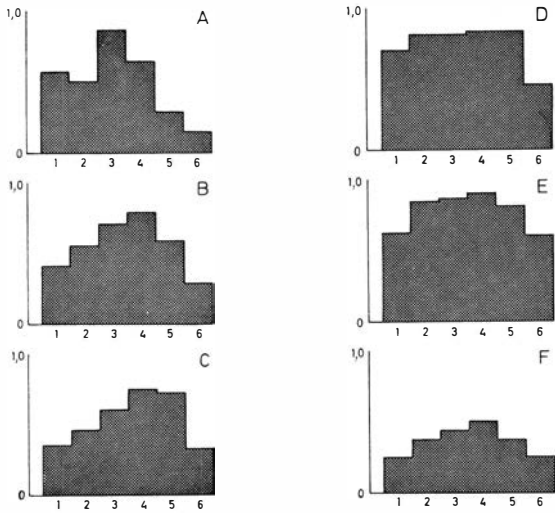
Fig. 4. Histograms for numbers of anterior spines.

Fig. 5. Frequencies in the nine variables for the six levels.

Spine-making potential

We have in the foregoing made brief mention of "spine-making potential". The analysis of associations between adjacent spines promotes the suggestion that it might be possible to express the average potential for making spines at each level sampled as the sum of the frequencies for each spine. The sums for all samples are listed in Table 4. It will be seen that the spine-making potential tends to increase gradually, on the average, from level A to level E, only to fall off strongly for level F. As will be demonstrated later, sample F often shows a tendency to overlap with samples A, B and C in the analyses. This apparent agreement in spine morphology may be largely ascribable to a drop in spine-making potential suffered by the species at time F.

Secular variation in spine frequencies

As will become apparent from inspection of Fig. 5, the change in spinosity with time displays ob-

vious correlations between different loci. It is, however, not possible to infer evolutionary shifts in the species from such local samples.

For levels A and B, the agreement in variation is not remarkable, but it is striking for levels C to F. This situation is, of course, neatly reflected in the values for the spine-making potential of Table 4, but these do not tell us anything about the structure underlying this variation; this is, however, available from the elements of the mean vectors.

The grand mean for all samples summarizes the differences in frequencies for the six spines. Spines 3 and 4 have the highest scores, while the frequencies for spines 2 and 5 are less and those for spines 1 and 6 less again. This suggests that there is a preference for spines to develop in the central area of the anterior margin. There are nevertheless combinations in which the popular loci 3 and 4 are not represented.

The information on the means for all levels is summarized in Table 5.

In the samples from levels D and E, there is little to choose between the frequencies for spines 2, 3, 4 and 5. In general, spinosity is greatly reduced in the sample from level F (cf. Table 4).

The confidence intervals for the means (these are not set out in Fig. 5) are all relatively broad and this should be kept in mind when interpreting the oscillations in the mean frequencies for the spines. We note that in a few cases, in sample A for spines 3 and 6, and in sample B for spines 1 and 5 and spines 3 and 6, the frequencies are complementary; that is, they sum exactly to one. It might possibly be significant that these relationships occur in outer spines and that in two cases, spines 3 and 6 are involved; this might indicate that these loci are complementary in at least some of the material.

The confidence intervals (see above) show that apart from the smallest samples, those of levels A

Table 4. Spine-making potentials.

Level	Potential
A	3,00
B	3,33
C	3,23
D	4,46
E	4,64
F	2,19

Table 5.   Means and confidence intervals for the anterior spines.

Level	N	spine 1	spine 2	spine 3	spine 4	spine 5	spine 6
A	14	$0,571 \pm 0,259$	$0,500 \pm 0,262$	$0,857 \pm 0,183$	$0,643 \pm 0,251$	$0,286 \pm 0,237$	$0,143 \pm 0,183$
B	51	$0,412 \pm 0,135$	$0,549 \pm 0,137$	$0,706 \pm 0,125$	$0,784 \pm 0,113$	$0,588 \pm 0,135$	$0,294 \pm 0,125$
C	48	$0,354 \pm 0,135$	$0,458 \pm 0,141$	$0,604 \pm 0,140$	$0,750 \pm 0,123$	$0,729 \pm 0,126$	$0,333 \pm 0,135$
D	37	$0,703 \pm 0,147$	$0,811 \pm 0,126$	$0,811 \pm 0,126$	$0,838 \pm 0,119$	$0,838 \pm 0,119$	$0,456 \pm 0,161$
E	58	$0,621 \pm 0,125$	$0,845 \pm 0,093$	$0,862 \pm 0,090$	$0,897 \pm 0,078$	$0,810 \pm 0,101$	$0,603 \pm 0,126$
F	16	$0,250 \pm 0,212$	$0,375 \pm 0,237$	$0,438 \pm 0,243$	$0,500 \pm 0,245$	$0,375 \pm 0,237$	$0,250 \pm 0,212$
Grand mean	224	0,500	0,634	0,732	0,786	0,643	0,397
Frequencies		112	142	164	178	144	89

and B, the chronological trends persist. That is, within the limits of the 95 percent interval of confidence, pronouncedly correlated trends occur in the frequencies of the spines.

The hypothesis of homogeneity in spine-making potential was tested by chi-squared. This showed that this hypothesis must be rejected for all six spines. There is, therefore, a significant shift in spine-making potential over time for all spines in the material studied.

In Table 6, a summarizing analysis of the associations between spines is given for samples B, C and E ($N = 157$). Here, again, the closeness in association between neighbouring spines is brought out.

A simple matching coefficient was computed between spines of the same pooled sample. It indicates that when the results are plotted as a

dendrogram, there is a distinct suggestion that the interior four spines are more intimately related than they are with the outermost pairs (1—2 and 5—6).

Multivariate study of the anterior spines

The multivariate statistical method of canonical variates analysis provides a suitable vehicle for examining the changes in the 6 spines, considered simultaneously. We have thus to analyze six variables at six levels, the sampling points A through F.

The main results of the calculations are presented in Table 7. These were performed using an updated version of the program in Blackith and Reyment (1971). There are two statistically significant eigenvectors. The discriminant function associated with the first of these eigenvalues can be expressed as a function in terms of four of the spines, to wit:

$$z_1 = 0,25x_1 + 1,35x_2 + 0,83x_5 + 0,80x_6.$$

The examination of the plots of the transformed canonical observational vectors for the first two canonical variates, in conjunction with the pre-
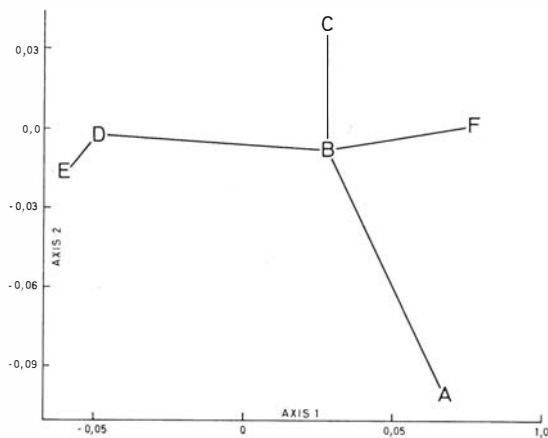

Fig. 6. Plot of canonical means for the first two axes. The lines joining the centroids are the Prim (1957) network of minimum linkage spans.

Table 6.   Chi-square array for anterior spines (without Yates' correction). Combined sample (Samples B+C+E) $N = 157$.

	spine 1	spine 2	spine 3	spine 4	spine 5	spine 6
spine 1						
spine 2	49,88**					
spine 3	19,78**	52,97**				
spine 4	12,76**	27,41**	32,35**			
spine 5	6,50*	11,75**	7,70**	44,63**		
spine 6	2,51	6.12*	0.94	4,68*	28,45**	

G•(010110)

BCDF(10)•(000110)

BC(4)•(000011)

E•(010111)   BCDEF(7)•(000111)

F•(100010)

0,13 —

C(2)•(110110)

C•(011010)
D•(100011)

BE(2)•(110111)   BCDE(8)•(011110)

C•(100111)

BCE(7)•(001110)

BCDEF(17)•(0111111)   CDF(4)•(010000)

0,03 —   D•(111010)   CDEF(5)•(001111)   ABCDEF(19)•(000000)

ABCDE(33)•(111110)

AB(3)•(101110)   BEF(3)•(000100)

E
•(111011)   C•(000001)

ABCDEF(41)•(111111)   ACD(3)•(101111)   B•(000101)   E•(100000)

-0,09 —

A•(011000)

ABDE(8)•   ABC(5)•(001000)

BCEF(6)•(001100)

A•(011000)

-0,19 —   CDEF(4)•(111000)   AC(3)•(101010)

ABCDEF(11)•(111100)

E•(111001)

E•(111101)

-0,29 —

CDEF(4)(111000)

FIRST CANONICAL AXIS

SECOND CANONICAL AXIS

0,24
0,13
0,03
-0,09
-0,19
-0,29

-0,15   -0,08   -0,01   0,06   0,13   0,20

DECREASING SPINE-MAKING POTENTIAL

Fig. 7. Plot of the transformed observations for the first two canonical variate axes. The compositions of the phenotypes are denoted as [i i i i i i], where i takes the values 0 or 1.

ceding univariate analysis, agrees with what is being expressed by this linear function. That is, that the main stratigraphical differences (thence variability over time) occur in the frequencies of the outer spines $(1, 2, 5, 6)$.

If we now consider the structure coefficients, we observe that all spines are approximately equally important in the variation. It is, however, apparent that, as we have noted in the earlier analyses, spines 3 and 4 form a natural pair, thereafter 2 and 5 and, finally, spines 1 and 6. We have then an expression for average variability in spine frequencies. There is no clear indication of what

the second vector of structure coefficients can mean.

The canonical means are plotted in Fig. 6. The sample points show ordering in the stratigraphical sense, with the exception of sample F, which lies in a position located oppositely to samples D and E. This figure also shows the Prim (1957) minimum linkage spanning tree. This tree further brings out the fact that the specimens of sample F are not "identical throwbacks" to the morphology of the oldest individuals in the sequence. The reason for the divergent position occupied by individuals of sample F lies with their greatly

Table 7.   Summary of the canonical variates calculations for the anterior spines (the first two variates).

Canonical variate vectors	spine 1	spine 2	spine 3	spine 4	spine 5	spine 6
Vector 1 (eigenvalue $= 0{,}2414$)	0,247	1,347	0,064	$-0{,}087$	0,832	0,803
Vector 2 (eigenvalue $= 0{,}0948$)	$-0{,}833$	0,103	$-1{,}313$	$-0{,}236$	2,106	$-0{,}476$
Structure coefficients						
Vector 1	0,526	0,771	0,461	0,477	0,664	0,592
Vector 2	$-0{,}429$	$-0{,}302$	$-0{,}566$	0,030	0,588	0,097

decreased spinosity in comparison with all older samples.

The transformed observational vectors are plotted in Fig. 7 for the first two canonical axes. There is much overlap in the plot as individuals with the same combination of spines will coincide in the graph. The letters in Fig. 7 denote the sampling levels at which a particular combination of anterior spines occurs. The number of specimens coinciding at this point is given in round brackets; the combination of spines for this point is placed within square brackets. Thus, ACD (3) [101111] means that three specimens from samples, A, C and D have the combination of spines "first, third, fourth, fifth, sixth". Unusual combinations can easily be picked out in Fig. 7 as there will only be one or two letters alongside the unusual combination. Thus, D [111010] says that there was only one specimen in the entire material that had the

combination "first, second, third, fourth" and it came from level D. A significant feature of Fig. 7 is that the canonical axes ordinate the specimens according to their degree of spinosity, with the most spinose combinations to the left of the graph and the least spinose ones to the right. It is thus a good example of the ordinating power of the method of canonical variates while, at the same time, the plot is easy to reify. There is also a stratigraphical effect in the figure, which derives from the trend towards decreasing average spinosity displayed by our material. Consequently, there is only one individual from level F in the extreme left of the figure.

Can the levels be discriminated?

It is natural to enquire whether the canonical analysis is also successful in discriminating between

Table 8.   Generalized distances and probabilities for the anterior spines.

	A	B	C	D	E	F
			D²			
			Samples			
		1,01	1,93	2,27	2,40	1,45
	0,097 NS		0,26	0,78	0,83	0,66
	0,003	0,404 NS		0,81	1,09	0,77
	0,002	0,015	0,013		0,25	1,95
	0,000	0,002	0,000	0,523 NS		2,11
	0,107 NS	0,252 NS	0,177 NS	0,002	0,000	

Probability that D² is significant

NS = not significant

Table 9. Table of D^2 for anterior spines, posterior spines, length, and ornament (levels A, B, C, D, E).

	A	B	C	D	E
A		22,52	22,35	14,09	20,67
B			0,38	3,43	2,84
C				2,91	2,86
D					5,00
E					

the six samples. It is well to remember that R. A. Fisher originally developed the concept of the discriminant function in answer to a problem of multi-level identification and it can be expected that at least some of the variation in spinosity will be relatable to time. The efficiency of the discriminant function formed from each canonical vector of Table 7 can be measured usefully by utilizing it for finding the generalized distances between each of the samples, and then ascertaining the significances. The array given in Table 8 presents the values of the generalized distance, D^2, in the upper triangle and the probabilities for each distance differing from zero in the lower triangle. It will be seen that samples D and E are most alike, then samples B and C, followed by the pairs B-F, C-F, B-D, C-D, B-E, A-B, C-E, A-F, A-C, D-F, in order of increasing unlikeness, up to sample pairs E and F, A and D, and, finally, the most unlike pair of all, samples A and E. These results are not unexpected in the light of the earlier analyses.

The discriminant analysis indicates that it is possible to discriminate between samples from levels A and C, A and D, A and E, B and D, B and E, C and D, C and E, D and F and E and F with reasonable accuracy.

Anterior spine combinations in order of frequency

The four most popular combinations of anterior spines are:

　　　　1 1 1 1 1 1 (proportion = 0,186)
　　　　1 1 1 1 1 0 (proportion = 0,149)
　　　　0 0 0 0 0 0 (proportion = 0,086)
　　　　0 1 1 1 1 1 (proportion = 0,077)

These combinations dominate in our material, indicating that the strongest tendency is for all the spines to develop followed by five contiguous spines. As an alternative to these anterior pheno-types, we have, as the third most popular state, no anterior spines developed. Although the above four combinations together account for 49,8 %

of the pooled sample, virtually all other possible combinations of spines were identified. The most unpopular combinations are those in which the central spines do not appear. Such combinations seem to occur most frequently in sample E, although they can be found in all of the samples.

We conclude, tentatively, the following:
1) there is some kind of coherence in the spinosity
2) spines develop preferentially in the middle of the anterior margin.

Shifts in spine development

There seems to be a tendency in the material to shift towards a greater average spinosity. The number of specimens observed at higher levels (apart from F) without anterior spines is relatively less than at the lower levels.

The probits for the cumulative proportions of each spine are plotted against stratigraphical position in Fig. 8. These plots expose the differences in spine-making potential; thus, spines 1 and 6 are similar in potential, but not identical. Spines 2 and 5 occur next and, finally, spines 3 and 4. The plot of the probits arranges the spines in order of probability of occurrence, from left to right. These plots disclose that threshold values do not occur as far as can be ascertained from the samples available.

The slopes of the lines from level to level indicate the relative frequencies of individuals bearing the spines. It is clear, that an increase in the angle of
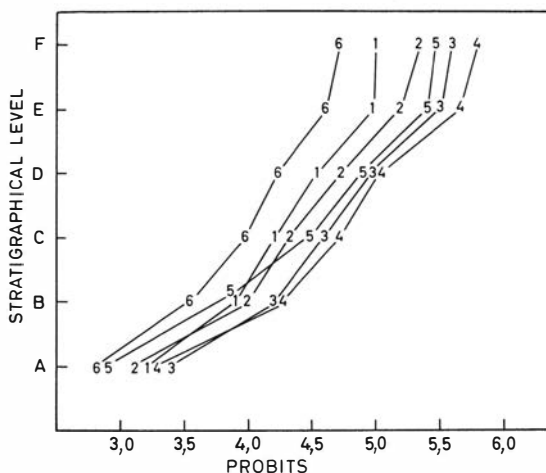

Fig. 8. Plot of the probits for each of the anterior spines for the six levels.

slope between the probits for two successive levels indicates a fall in the frequency of a spine. We note that the plot for spine 5 differs somewhat in pattern from those of the other spines in that it is located "too far to the left" for levels A and B. It conforms, however, with the patterns of the other spines for levels C through F.

Variation in the posterior spines

Although there are two posterior spines in the species, the second of these occurs vary rarely in the material at hand. There is some variation in the locations of the two spines and so it was not possible to distinguish between the 01 and 10 types. As far as we could ascertain from chi-squared tests on suitable samples, the two spines do not seem to occur independently of each other. The correlations between anterior and posterior spines indicate that significant associations between the two categories are unusual.

Variation in the lateral ornament

A significant feature of *C. acuminata caumontensis* is the existence of two kinds of lateral ornament at all six levels. One of these categories, here denoted as "irregular", is composed of an irregular pattern of pits, disposed in an unordered fashion over the shell surface. An example of this ornamental category is illustrated in Fig. 2. The alternate type, here denoted "regular", consists of regularly aligned pits. It bears a close similarity to the ornament borne by the latest instars of the species.

Before recounting the results of the statistical analysis of the ornamental frequencies, we note that the frequency of regularly ornamented individuals increases rapidly from level D to level E. There seems to be a trend for the frequency of regularly ornamented individuals to increase with time.

A log-likelihood ratio test for the 2×6 contingency table for all six sampling levels gave the highly significant $\chi_5^2 = 85$. Excluding the two smallest samples, the result is still highly significant and it would appear highly probable that a significant shift in the frequencies of the ornamental categories has occurred over the period under consideration.

It seems possible that the ornamental variation in *C. acuminata caumontensis* (and other ostracods) belongs to the class of threshold characters, that is, such as display quasicontinuous variation (Fal-
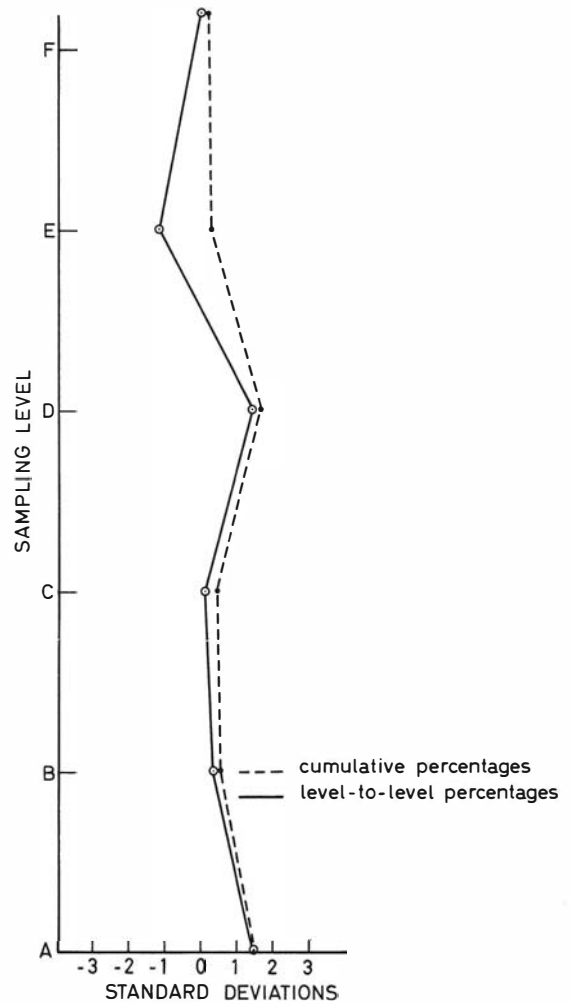

Fig. 9. Shifts in threshold and the cumulative proportions on a probit scale for the ornamental variants.

coner 1970, p. 301). In such organisms, the phenotypic values are discontinuous while the mode of inheritance is continuous. The threshold connects the underlying and continuous variation (genetic and environmental) with the visible and discontinuous variation at the point of discontinuity.

It is convenient to analyze thresholds by the probit transformation of the points of discontinuity, which permits one to express the mean phenotype in terms of its standard deviation. The pertinent values for the six levels are listed in Table 10. The shifts in threshold and the cumulative proportions on a probit scale are illustrated in Fig. 9. There is a slight though definite shift over time.

The analysis of the ornamental variation also

Table 10. Proportions, probits, and deviations of thresholds from the mean for the ornamental variations.

	Proportion of irregular phenotypes	Probit	Mean to theshold distance
A	0,929	6,468	1,460
B	0,647	5,377	0,370
C	0,625	5,319	0,320
D	0,919	6,398	1,400
E	0,121	3,830	−1,170
F	0,500	5,000	0

shows that the seeming similarity between samples A and F is superficial, as already hinted at by the previously reported observations, for the ornamental frequencies for the two are very different.

Analysis of all characters simultaneously

We shall now consider all of the characters simultaneously in a suite of multivariate statistical analyses. Owing to the fact that some of the characters are poorly correlated with each other, there are, for some of the methods used, problems occasioned by the amount of near-random variation in the material.

The methods we have used for studying all characters are those of principal coordinates analysis, canonical variates analysis, discriminant functions and generalized distances and canonical correlation.

Although all of these analyses disclosed something of interest about the structure in the data, we confine our discussion to only the most significant results obtained.

The canonical variates analysis of the six samples shows that the resulting linear discriminant function is moderately efficient in correctly locating individuals. Using this function, all specimens from level A are correctly allocated, less than half of the specimens from samples B and C, 68 percent of D, and 72 percent of sample E are correctly identified. The biggest lack of success was noted for level F of which most individuals were wrongly identified. The BMD07M standard program of Dixon et al. (1973) was used for doing the calculations.

Analysing levels A through E in an updated version of Reyment's and Ramdén's (1969) program for canonical variates analysis showed that there are three significant eigenvalues. We note that all of the generalized distances, except that between samples B and C, are highly significant (cf. Table 9). The addition of further variables

over the six anterior spines of the previous canonical variate analysis has yielded a greatly improved discriminator, it would seem. The first eigenvector of canonical variates shows, however, that almost all of the discrimination is being done by variables 7, 8, and 9, the posterior spine, the length, and the ornament.

The sample from level A shows up now as being the most divergent. This is brought out in the canonical variate plots in which the specimens of A always form a tight cluster. The plot of the canonical variate means forms a straight stratigraphical relationship, with the samples ordered by increasing youngness. This is most expressively shown by the plot for the first and third canonical axes. As only to be expected, the plots of the transformed observations show much overlap between samples.

We also applied the method of correspondence analysis (cf. Jöreskog et al. 1976) to the pooled sample for all nine variables. This gave the following main results: Although the first axis could not be reified, the second axis could be determined as representing variability in the posterior spine and the ornament. Three major constellations of points were formed by these axes, notably:

1. A group constituted by most individuals of sample D and a few of samples A, B, and C, and only a few specimens from sample E.

2. Most of the specimens of A, B and C, and a few from E, and a very few from samples D and F.

3. A group comprising most of the specimens of E and F, a few from B and C and a single specimen from A.

These results tend to confirm what we have already learnt from the other analyses, namely, that there appears to be a shift in frequencies of the ornamental features over time.

Anterior spines balanced against the other features

We have established the fact that there are persuasive reasons for regarding the anterior spines of our species as a homogeneous variational unit. The posterior spinosity does not appear to be correlated with the anterior spines and the ornamental variation seems to be free from variation in either or both of these characters. Although there is undeniably some logical difficulty involved in justifying the next step, we decided to balance the homogeneously linked anterior spines against a set composed of the ornamental variants and
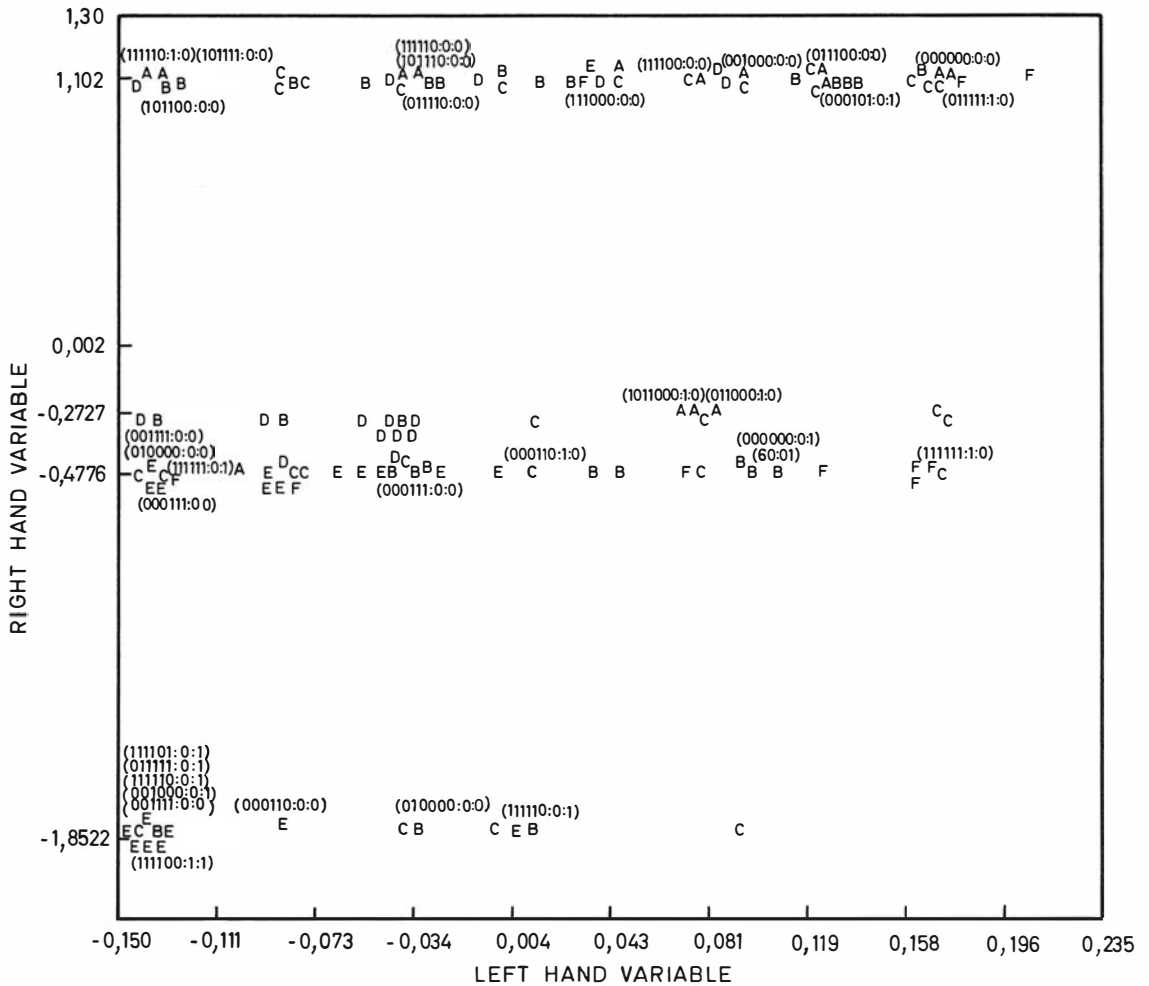
Fig. 10. Plot of scores for the first canonical correlation (eight discrete variables); the transformed observations for the first canonical vector are plotted against those for the second.

the posterior spine frequencies. We have thus eight dichotomous variables to consider. The multivariate statistical method of canonical correlations was employed to examine the structure of the correlations between the two sets.

Only the first canonical correlation is statistically significant ($\chi^2_{12} = 19,1$). Instead of considering the canonical loadings for the two sets of variables, we took the structure coefficients (Cooley & Lohnes 1971 p. 170); these indicate that for the first canonical correlation there is a significant and positive relationship in all the variables. The most interesting single result arising out of this analysis is the plot of the scores for the first canonical correlation which gives evidence of the existence of "quantal structure" in the data.

Fig. 10 demonstrates that the specimens of the six samples pooled fall neatly into four discrete classes. This "quantal" arrangement of the individuals is stratigraphically ordered. The topmost group contains most of the individuals of sampling level A, specimens from levels B, C and D, one ostracod from level E and two from level F. The second group lacks individuals of F. It has a few specimens from level A, most of those from D, a few from C and none from level E. The third group is devoid of specimens from level A and is composed of individuals from all other levels. The fourth group lacks individuals from levels A, F, and D; it contains mainly ostracods from level E and there are a few specimens from levels B and C.

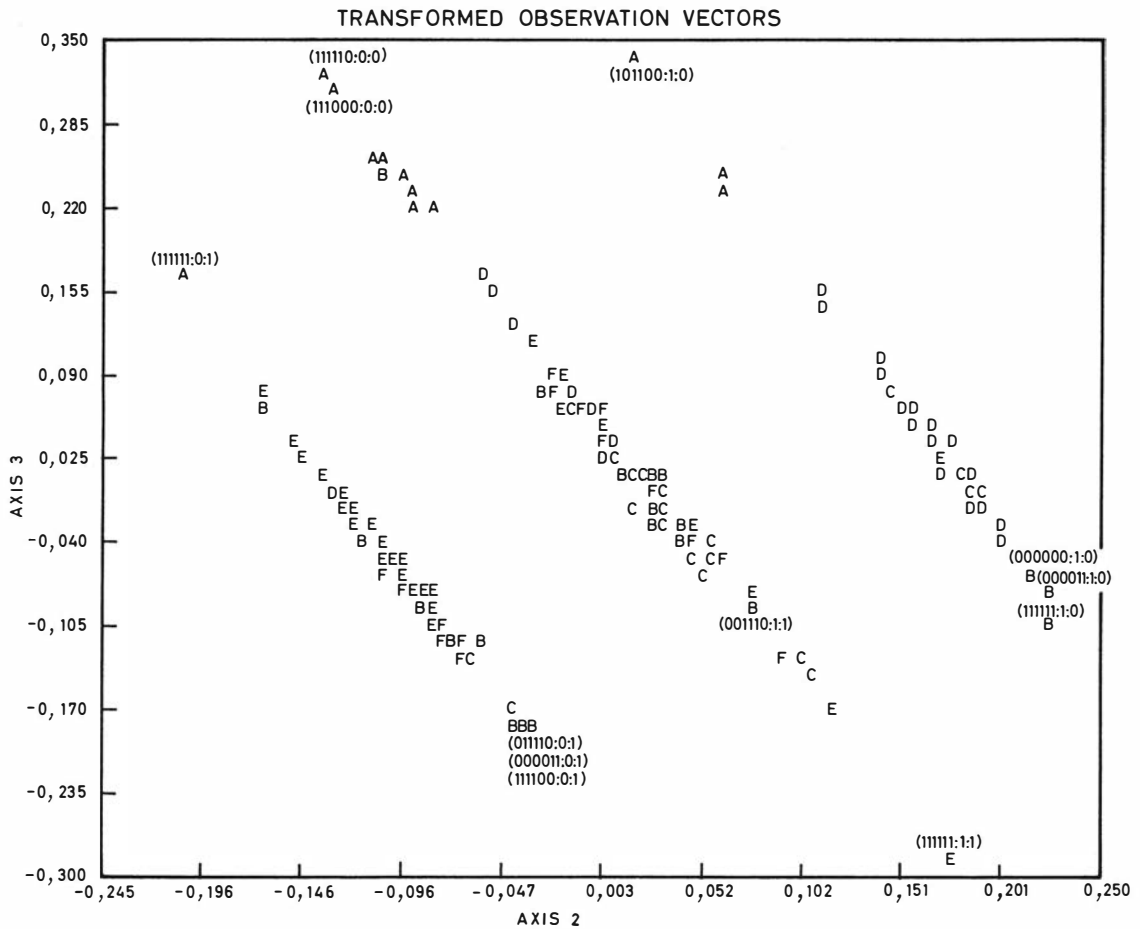We note that the distribution of the groups in the plot is symmetrical. The breakdown of the

TRANSFORMED OBSERVATION VECTORS

Fig. 11. Plot of the transformed observational vectors for the second and third eigenvectors of the correlation matrix for the nine variables.

material into the distinct groups of Fig. 10 is a reflection of the major phenotypes and the shift in their relative proportions that appears to have taken place over the period of time represented by the six sampling levels. The ordering into the elongated groups of Fig. 10 is the result of representing as a bivariate plot a situation in which all variation lies with the first axis, with respect to phenotype. The second axis lacks variational spread and the linear combination of variables associated with it appears to be selecting four dominant morphological types occurring in the material.

The foregoing aspect of the analysis can be further illuminated from another standpoint by considering the plots of transformed observations obtained from the eigenvectors of the correlation matrix for all variables, including length of carapace.

The most informative pair of axes turned out to be the second and the third. The first eigenvector of the correlation matrix was found to express equal variation in the frequencies of the six anterior spines on their own. The second eigenvector is entirely concerned with covariation in size, the frequency of the posterior spines, and the ornamental variation. The third eigenvector shows variation in size and ornamental frequencies alone.

The plot of the transformed observations for vectors two and three yields a stratigraphically significant result (Fig. 11). The 224 observations fall into three greatly elongated and entirely disjunct fields. These fields can be identified in the following terms: To the left of the graph we find that most of the individuals from the younger levels have been congregated and these are regularly ornamented. In the middle group, there is a con-

centration of specimens from all levels which either lack a posterior spine and are irregularly ornamented, or possess a posterior spine and are regularly ornamented. The third constellation of points, located in the right part of the plot, consists of individuals from all sampling levels, except F, which bear a posterior spine and are irregularly ornamented.

We note that for all three groups, the specimens from sampling level A lie in the uppermost part of the figure. The phenotypes of younger individuals tend to lie in the lower part of the plot for the left hand constellation of points. The two eigenvectors are attempting to range the points in order of time; this is being most effectively done for the regularly ornamented specimens.

As a consequence of the foregoing observation, if one were interested in improving the efficiency of the quantitative biostratigraphical analysis, and sufficient material were available, one could confine the treatment to the regularly ornamented individuals.

Concluding remarks

1. The analysis shows that there is a shift over time in the phenotype of the species.

2. All characters studied are useful for tracing this shift in phenotype; their individual usefulness is enhanced when analyzed simultaneously.

3. Multivariate methods permit the identification and evaluation of relative similarity in phenotypes.

4. Although it is not possible to identify the point in time with which a single specimen is connected, a sample can be placed with fair accuracy, on the basis of the eight dichotomous variables. This is a practical quantitative biostratigraphical result of the study.

5. For the anterior spines, we found that there is a greater preference for them to develop in the middle of the margin.

6. The statistical analysis fits in well with the chronological information, as represented by the field evidence, with the exception of level F. The sample from this level breaks with the trends established for sample A through E.

REFERENCES

Blackith, R. E. & Reyment, R. A. 1971: *Multivariate morphometrics.* 412 pp. Academic Press, London.

Carbonnel, G. 1969: Les ostracodes du Miocène rhodanien. Systématique, biostratigraphie écologique, paléobiologie. Thèse. *Doc. Lab. Géol. Fac. Sci. Lyon 32.* 469 pp.

Dixon, W. J. (ed.). 1973: BMD biomedical computer programs. 3rd ed. 773 pp. University of California Press, Los Angeles.

Falconer, D. S. 1970: *Introduction to quantitative genetics.* 365 pp. Oliver and Boyd, Edinburgh.

Jöreskog, K. G., Klovan, J. E., Reyment, R. A. 1976: *Geological factor analysis.* 188 pp. Elsevier, Amsterdam.

Prim, R. C. 1957: Shortest connection networks and some generalizations. *Bell System Tech. J. 36*, 1389 —1401.

Rendel, J. M. 1967: *Canalisation and gene control.* 166 pp. Academic Press, London.

Reyment, R. A. 1963: Studies on Nigerian Upper Cretaceous and Lower Tertiary Ostracoda. Part 2, Danian, Paleocene and Eocene Ostracoda. *Stockh. Contrib. Geol. 10*, 1—286.

Reyment, R. A. 1966: Studies on Nigerian Upper Cretaceous and Lower Tertiary Ostracoda. Part 3, Stratigraphical, paleoecological and biometrical conclusions. *Stockh. Contrib. Geol. 14*, 1—151.

Reyment, R. A. 1971: Multivariate normality and morphometric analysis. *Math. Geol. 3*, 357—368.

Reyment, R. A. and Ramdén, H. Å. 1970: Fortran IV program for canonical variates analysis for the CDE 3600 computer. *Comput. Contrib. Geol. Surv. Kansas 39*, 1—39.

Reyment, R. A. and Van Valen, L. 1969: *Buntonia olokundudui* sp. nov. (Ostracoda, Crustacea): a study of meristic variation in Paleocene and Recent ostracods. *Bull. Geol. Inst. Univ. Uppsala*, N. S. 1, 83—94.

Van Morkhoven, F. P. C. M. 1963: *Post-Paleozoic Ostracoda.* Vol. 2. 478 pp. Elsevier, Amsterdam.