

ORNAMENTAL AND SHAPE VARIATION IN *Hemicytherura fulva* MCKENZIE, REYMENT AND REYMENT (OSTRACODA; EOCENE, AUSTRALIA)

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ABSTRACT

Hemicytherura fulva displays ornamental variation of two kinds. One of these (termed the morph *LACE*) is vaguely linked to shape and is generally differently distributed from the rest of the material; this morph may possess intrinsic evolutionary significance. The other categories are probably ecophenotypic in nature. Methods of distance-based multivariate morphometry and geometric morphometry were used for the shape study.

Keywords: Multivariate morphometry, geometric morphometry, Ostracoda, polymorphism, shape ecophenotypy.

RESUMEN

La especie *Hemicytherura fulva* McKenzie, Reyment y Reyment muestra unas variaciones ornamentales de dos tipos. Una de ellos (morfotipo "LACE"= "encaje") esta en cierto modo relacionado con la forma del caparazón y, desde un punto de vista estadístico, se diferencia claramente del resto del material; lo que podría tener una importancia evolutiva intrínseca. La otra categoría podría ser de naturaleza ecofenotípica, de hecho no puede ser separada del conjunto principal. Para el estudio de la forma se utilizan los métodos usuales de morfometría multivariable y geométrica.

Palabras clave: Morfometría multivariable, morfometría geométrica, ostracodos, polimorfismo, ecofenotipia de forma.

INTRODUCTION

Covariation in shape and ornament in species of an ostracod genus was investigated by Reyment (1985). It was found that in Eocene species of *Echinocythereis* from northern Spain, shape modifications were correlated with irreversible changes in ornament. That study could, however, only point to the possibility that there was a genetic relationship between the two categories, but could not prove that any genuine pleiotropic connection really existed. More recently, Irizuki and Sasaki (1993) at the Eleventh International Symposium on Ostracoda, Warrnambool, Australia interpreted morphological variation in recent species of ostracods in terms of heterochrony, although without providing a biometrical basis for their conclusions.

The preliminary scanning of all of the material available of *Hemicytherura fulva* McKenzie, Reyment and Reyment, of Eocene age, suggested the possibility of that species being polymorphic with respect to shape. It was also observed that there was ornamental polymorphism in the material that could perhaps in part be linked to the suspected variability in shape.

A suitable means of testing hypotheses about morphological variations in shape, with and without the intervention of size, is provided by the tensor-biometrical methods of Bookstein (1991), supported, where relevant, by standard methods of multivariate statistical analysis (Reyment *et al.*, 1984); that is, multivariate analyses made on the usual distance measures - lengths, heights,

breadths etc., of quantitative taxonomic work. Current concepts of multivariate morphometrics contrast with the original scope defined by Blackith and Reyment (1971); the new synthesis may be regarded as a pragmatic fusion of geometric morphometry and "phenometry". It is important that this distinction be kept in mind in what follows.

Complications occasioned by sexual dimorphism had to be taken into account in the analysis. It was assumed that where sexual-dimorphic differences did exist, the morphological differentiation would show up when size was allowed to remain in the computations. Reasonably well supported identifications of males are indicated in the figures.

As always in studies involving ornamental variability in the ostracod carapace, it is necessary to be aware of the possibility that quite marked differences in ornamental patterns can be caused by a deficiency or a superfluity of calcium ions during moulting and secreting a new shell (McKenzie and Peypouquet, 1984). Hence, the "lacelike ornament" reported in the following is like what results from a deficiency of calcium carbonate, and the thickly ribbed variant resembles a common product of supersaturation with calcium. I am inclined to dismiss the likelihood of the concentration of calcium ions as being the motor of ornamental variation in the present case because of the rarity of the two variants of interest and the fact that I can see no external evidence likely to support the supersaturation model in the sedimentary properties of the samples I have had at my disposal.

The quantitative analysis was made at two levels. The total evaluation of all landmarks (as defined below) on all specimens by the procedure termed "relative warps" on samples of shells (hence directed towards covariances), and on sample mean landmarks by the method of "principal warps". Further information was obtained by the method of "shape-coordinates" applied to each landmark referred individually to a chosen baseline (here, the length axis of the shell). There is no hiding the fact that tensor-biometry is a highly technical subject, well beyond the usual level of multivariate statistical analysis confronting the biologist, and I must perforce refer the reader to Bookstein (1991) for the necessary elaboration of the fundamentals. Two examples are worked in my palaeobiological text (Reyment, 1991). The geometric morphometric calculations were carried out using the public domain programs *TPSPLINE* and *TPSRW* by F. James Rohlf (Stony Brook, U.S.A.), distributed with the proceedings of the Michigan Morphometrics Workshop (Rohlf and Bookstein, 1990), and subsequently updated.

The hierarchy of methods used in shape analysis here may seem to be confusing, hence the need for a brief orientation in **geometric morphometry** and what this new field of analysis promises. The change in shape of an organism can be helpfully viewed as the deformation (i.e. warping) of a thin metal plate. Viewed globally, the effects of these deformations will not be mechanically great in that the force needed to make one form fit the other (by Procrustean superposition, for example) will be relatively slight. Passing to the opposite end of the scale, we have the situation where the force required to displace adjacent landmarks (buckle the plate) will be large. This situation may seem paradoxical on first encounter, but the logic involved will soon make itself apparent if you think yourself into the properties of the plate-deformational model.

The interpretational strategy is primarily graphical, and only in part statistical, although this latter aspect is currently being given more attention. I mention this fact so as to eliminate a source of misconception at an early stage in my report. We shall not then be concerned with testing statistical hypotheses, but rather with expressing, in pictures, relationships between objectively defined shapes. This can be done at several levels. The appropriate procedure for assessing shape variability with respect to a set of landmarks within a single sample has already been mentioned under the name of *relative warps*, which is in many respects an analogue of principal component analysis. Mean landmark configurations can be conveniently compared by the procedure of *principal warps* (Bookstein (1991) for references), whereby the deformational effect of mapping one configuration into a second one can be scrutinized.

The method of relative warps consists of fitting an interpolating function (i.e. the thin-plate spline) to the pairs of coordinates of the landmarks, which are diagnostic features located on the organism for each specimen of a sample. The parameters of the fitted functions can be used to portray variation among specimens which is expressed in relation to a figurative *bending energy matrix* (from the analogy of the deformation of a thin metal plate). This matrix is constructed from the coordinates of the landmarks of a reference configuration,

usually an average of values. The relative warps are the principal component vectors in this space; the non-uniform shape variation signified by each eigenvector is then interpreted graphically in terms of the magnitudes of the associated eigenvalues. Thus, the smallest eigenvalue expresses global change, that is, change that affects the entire surface under consideration (because little force is needed for a deformation of the entire plate to make one form fit the other by, for example, Procrustean superposition), whereas, at the other end of the scale, the eigenvector associated with the greatest eigenvalue is connected with local changes in shape, i.e. local "deformations", that is, relative movements of landmarks located near to each other (because more energy is needed to buckle a local section of the plate). The rationale for this interpretation lies with the model of shape-change on which geometric morphometry is based.

THE CHOICE OF LANDMARKS

The landmarks selected for analysis are indicated in Fig. 1. The baseline used in all computations is denoted by the axis 1-14 (anterior mid-rounding to posterior tip). Other landmarks are located at ornamental intersections, the dorsal end of the adductor muscle-scar field, and at features on ribs. All sites could be determined with satisfactory accuracy, = 0.1 mm or better. In the analyses of the entire material, only 11 of the 14 landmarks of Fig. 1 could be used, owing to the fact that three intersections made by ribs do not occur on the morph LACE. The seven distance traits employed in the usual multivariate statistical analyses are also shown on Fig. 1.

In the original sense of the term, landmarks were supposed to be located at homologous sites on the organism, such as at intersections, structures and at ornamental prominences. It has been found useful to expand this concept so as to encompass "pseudolandmarks", which are centres of rounding of the outline of the organism. As only to be anticipated, the accuracy of such landmarks is not always as good as available from true landmarks.

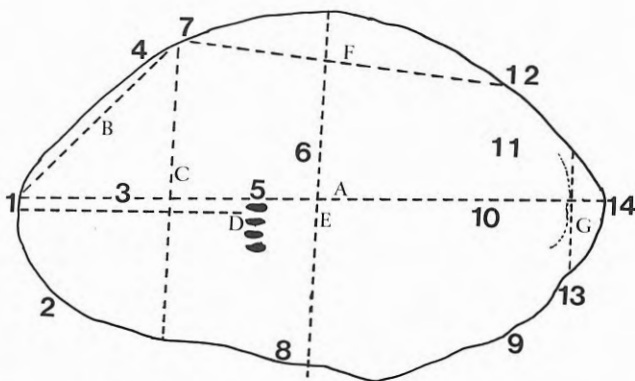


Figure 1. Locations of the landmarks selected for shape analysis denoted 1 through 14, and the distance measures for multivariate analysis, denoted A through G.

THE POLYMORPHIC STATES

The following polymorphic states were identified under the scanning electron microscope in the specimens available for study. Naturally, the relatively small size of the sample available must be kept in mind in that one should expect transitional shapes to exist for some of the categories identified for which the main variational influence was environmental.

1. An *ovoid* shape in the sense that the posterior is more broadly rounded than the anterior (one specimen).

2. The presence or absence of a caudal extension on the posterior process, designated **CAUDAL** (14 specimens - in relation to 21 specimens that lack the posterior extension).

3. Divergent ornament of two kinds. Firstly, the type here designated **EFFACED** in which the surface ornament is greatly subdued and the median rib much broadened, though feeble (8 specimens). Secondly, strongly divergent ornament with shape differences, here designated as the morph **LACE**. There are four specimens. The ornament consists of a fine network of diffuse riblets, pierced by coarse pores. There is variation in the distribution of these features. The question we ask is whether this variability is accompanied by shape innovations (see Reyment 1985 for examples of polymorphism in marine ostracods, and references).

MULTIVARIATE ANALYSIS OF DISTANCE MEASURES

The formal results of these analyses are summarized in Tables 1- 3. The "distance-traits" measured are indicated in Fig. 1. All measures are of the usual kind, to wit, length (A), anterior arch of the shell (B), height of the shell at the anterodorsal angle (C), position of the top of the adductor muscle field determined from the anterior margin (D), maximum height of the shell (E), the length of the dorsal arch (F), and the width of the caudal extension (G). The fourth trait D, the location of the adductor muscle group, is of more subtle significance than the others. This character unites features of the hard-parts with the anatomy of the soft parts. Whether or not a caudal process is developed on a specimen, there is always a base for it; trait G is the width of that base.

Raw observations; covariance matrix

One variable, height of the carapace (E), deviates from the normal distribution in that it is skew multivariate. This may be due to sexual dimorphic differences in maximum height. The first three eigenvalues together account for 82.46% of the trace. We shall now consider these. The results are summarized in Table 1.

The first latent vector has significant loadings for (A), (C) and (F), with a minor contribution from (E), which, in the usual interpretation of such principal component analyses, would be seen as representing variation in size of the carapaces. The second eigenvector

Table 1. Largest and smallest eigenvectors for the covariance matrices (raw, upper row, and logarithmic, lower row) of *Hemicytherura* (variables as indicated in Fig. 1).

Variable	Eigenvectors				
	I	II	III	VI	VII
A	0.446 0.023	0.203 0.043	0.851 -0.101	0.181 0.511	-0.038 0.480
B	0.096 0.052	-0.405 0.790	-0.005 0.596	0.190 -0.090	0.013 0.077
C	0.465 -0.014	-0.765 0.592	-0.061 -0.776	0.017 0.019	-0.131 0.012
D	0.081 0.044	0.111 0.091	0.009 0.040	-0.521 0.560	-0.818 -0.033
E	0.213 0.046	-0.024 0.101	0.088 -0.028	-0.765 0.208	0.558 -0.871
F	0.722 0.050	0.402 0.057	-0.506 -0.168	0.119 -0.612	0.032 0.062
G	0.051 0.995	0.186 -0.045	-0.088 -0.032	0.243 0.010	0.016 0.024
%	48.96	21.99	11.51	1.87	1.12
trace	67.61	20.27	7.27	0.59	0.40

can be interpreted in general terms of the confrontation of the anterior configuration (B and C) and dorsal length. This is, in part, a reflection of the commonly occurring condition in some groups of crustaceans of variability in the posterior configuration (Gilchrist, 1960). The maximum height does not enter into this relationship, but the height registered at the anterodorsal angle does, and quite strongly. The third eigenvector unites the length of the dorsal margin F with total length in an inverse association. This seems also to stem from the factor identified by Gilchrist (1960). The eventual functional significance of this posterior variability in ostracods is not apparent.

There are two traits that do not enter strongly into any of the principal components hitherto discussed. These are (D), the location of the adductor muscle field, and (E), the maximum height of the carapace. However, these two characters dominate the two smallest principal components, which represent virtually invariant relationships in the material (because the corresponding eigenvalues denote axial lengths close to zero). The second last vector, the sixth principal component (1.87% of the trace) can be seen as expressing the presence of forms marked by an invariant relationship between height and adductor site. The seventh and smallest principal component (1.12% of trace) expresses an inverse invariant connexion between (D) and (E). The smallest principal component is often said to be an error term, but there are reasons for considering it to represent an invariant relationship in the variables (Gower, 1967).

The Principal Component Scores

The plot of the scores for the first two principal components is shown in Fig. 2. This conveys an impression of the existence of distinct morphs in the material

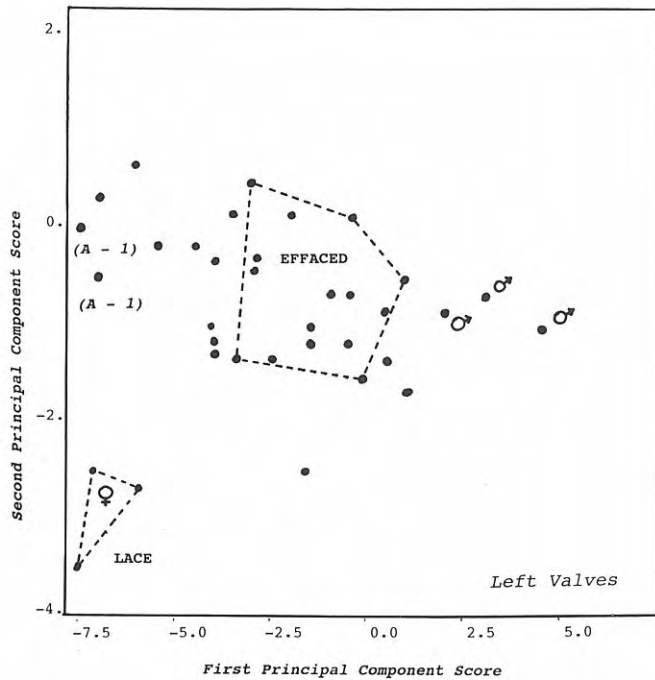


Figure 2. Graph of the scores for the first two principal components of untransformed data. Three specimens identified as males are shown as well as two larval shells. The morphs "effaced" and "lace" are provided with convex hulls.

and possibly also of sexual dimorphism, always a difficult property to evaluate in *Hemicytherura* (recall the earlier remark concerning skewness in the height distribution). There are several things worth noting. Firstly, two final instars (denoted *A-I*) segregate to the left. The morph LACE forms a discrete group, well separated from all other specimens. The shells of the morph EFFACED are nested within the field of the normally ornamented individuals. Presumed **males** group to the right (N.B. size is not the criterion deciding this ordination).

Logarithms of observations; covariance matrix

The effects of size differences can be greatly diminished by analyzing the logarithms of the observations. These results are displayed in Table 1. The first eigen-vector is entirely dominated by the width of the caudal process (G) (67.6% of trace). The second component (20.3% of the trace) manifests covariation in the anterodorsal arch (B) and the anterodorsal height (C). The third, small component (7.3% of trace) is also a relationship between these two traits, but inversely.

There are few right valves in our material, but as far as could be ascertained, these behave identically with the left valves forming the main part of the analysis; the two valves are of almost equal size and contour.

Cross-validation treatment of principal components

A useful method of analysis of data-sets that are suspected of harbouring atypicalities has been devised by Krzanowski (1987) - see palaeontological example

Table 2. The effect of deleting variables in the cross-validation principal component analysis. (PC = principal component, asterisks denote important residuals).

Variable deleted	Residual Sums of Squares		
	PC1	PC1 + PC2	PC1+PC2+PC3
A	4.4364	4.3930	12.2368*
B	0.9665	10.8194*	17.4259*
C	4.5552	8.8173*	9.7593
D	4.3828	6.2042	6.5159
E	4.6786	4.4075	4.4677
F	4.8775	4.5467	5.7849
G	0.9053	7.1512*	27.4571*

Table 3. Canonical discriminant analysis of three morphs

Variable	Eigenvalues	4.070	0.204
	First canonical Vector	Second canonical Vector	
A	1.1100		0.0094
B	-0.2237		0.6085
C	-0.1974		-0.4615
D	-0.1132		0.0756
E	-0.9788		0.0608
F	0.0988		0.0174
G	0.3467		0.2395

Generalized distances between morphs

lace - effaced	D = 5.507***
lace - normal	D = 5.586***
normal - effaced	D = 0.965

worked in Reyment (1991). All 29 adult specimens were analyzed by cross-validation principal component analysis. Table 2 presents the results of a form of redundancy analysis, whereby the residual sums of squares after deleting each variable in turn from the computations can be assessed. Large residuals indicate that the deleted variables are important. Little increase in the value of a residual, after deletion of a variable, suggests that variable is not essential. It will be seen from Table 3, that variables D and E are not influential, whereas variables A, B, and G are important, despite the mediocre performance of B and G in the first principal component.

Cross validation has its main morphometrical application for identifying atypical specimens. The technique it relies on is to examine residuals, as before, for the deletion of each specimen in turn and assessing the effects of this on variance and correlation. In both cases, one "normal" specimen was pointed out. It turns out to be the morphological type denoted "ovoid" earlier on. Two specimens of "lace", one of "effaced", and two of "normal" were separated out as deviating from the distributional pattern for variance of the entire sample.

Discriminant function analysis

The three main morphological categories, "lace", "effaced" and "normal", were tested in a discriminant function analysis, using a program written in GENS-

TAT. The results, summarized in Table 3, indicate that with respect to the generalized statistical distances, the lace-morph carapaces differ significantly from the other two which, in turn, do not differ significantly from each other.

GEOMETRIC MORPHOMETRY

Shape Coordinates

The most interesting landmarks were selected for shape analysis, one at a time, in relation to a baseline given by the length of the carapace (from (1) to (14) in Fig. 1). The formula for doing the actual calculations for producing the new coordinates is simple to use. The interested reader is referred to Bookstein (1991, p. 130) or Reyment (1991, p. 126). In this manner, sensitive sites on the carapace can be isolated and which can be of special value for interpreting the indications yielded by the warping studies. The following salient features were deduced with the help of the shape-triangles (Bookstein, 1991).

1. There seems to be a significant connexion between shape and ornament in the morph LACE (Fig. 3). This indication is given by the tight configuration of the relevant points.

2. Some landmarks are shape-sensitive. The results for Landmark 6 (not shown) group the morph LACE and the morph EFFACED. Another, more clearly expressed example is illustrated in Fig. 4 for landmark 8 (posteroventral intercept) in which the morph LACE

separates out discretely and morph EFFACED is also well segregated, although with some overlap. The separation achieved for the posteroventral rib intercept (landmark 9) yields two groupings of the morph EFFACED and the complete differentiation of the morph LACE. All other combinations of landmarks with the baseline gave some degree of separation, but the cases cited are the most informative ones.

RELATIVE WARPS

The ordination yielded by the first two relative warps produces a certain degree of morphological separation, but this is not as conclusive as the results obtained by the shape-coordinates. It is in my opinion always advisable to precede a complete analysis of landmark data by a suite of appraisals using shape coordinates.

The graph of the first relative warp against centroid size (not shown) separates out the morph LACE, but a more decisive result is given by the plot of the third relative warp against size (Fig. 5); all of the LACE morphs are neatly separated from the rest of the material and the specimens assigned to the morph EFFACE are also clustered together. The specimens, shown as tracings of the outline, are spread across the graph in relation to size in one direction and shape in the other. As indicated by the discriminant analysis, lace-morphs separate neatly from the rest of the material with respect to size. The notable contribution of the geometric analysis is that there is an equally strong separation arising from the shape constituent. There is therefore

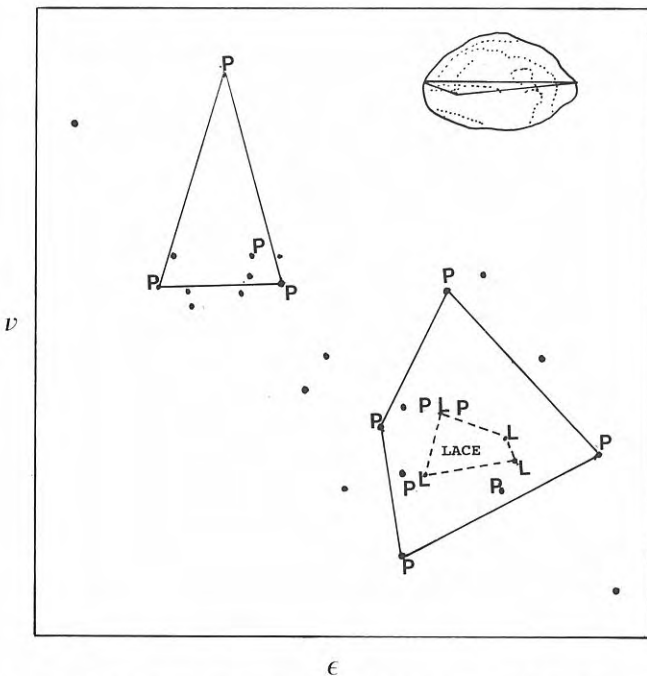


Figure 3. Shape coordinate graph for landmark "3" in relation to a baseline formed by points "1" and "14". *L* denotes the morph "lace", *P* denotes specimens with a posterior process. The outlines are the convex hulls for the two clusterings of *P*. The inset sketch shows the location of the landmark 3.

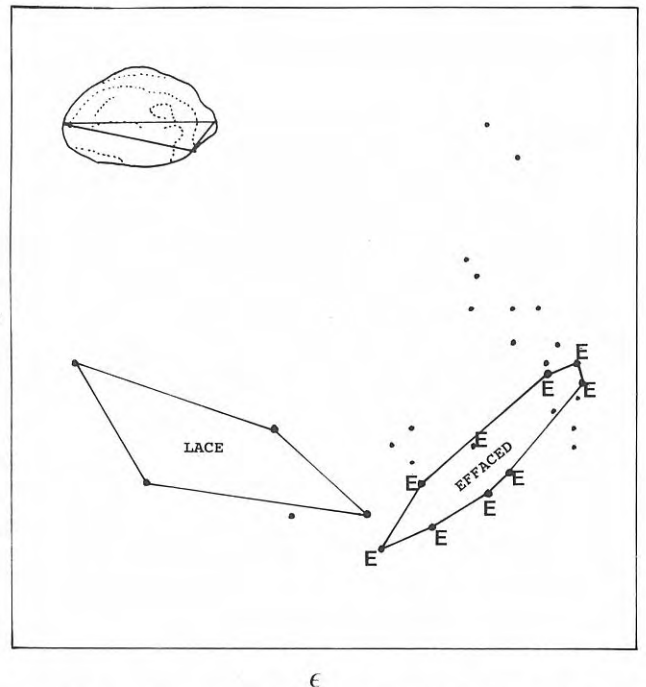


Figure 4. Separation on shape coordinates for landmark 8. *E* denotes specimens of the morph "effaced" around which there is the appropriate convex hull. The specimens of "lace" are likewise provided with a convex hull. The inset indicates the location of landmark 8.

reasonable evidence for accepting that the lace-morph is differentiated not only with respect to size and ornamental features, but also shape.

COMPARISON OF AVERAGE MORPHS BY THE THIN-PLATE SPLINE

1. The average normal morph compared with average LACE carapaces

Fig. 6 shows the compression of the anteriorly situated landmarks (1, 2, 3, 4) and the dilation in the posterior third of the carapace. Fig. 7, the total non-affine transformation, displays this tendency even more clearly. The anterior set of landmarks is being compressed while the features of the posterior group are being forced apart. Hence, it may be suggested that the two morphs differ in their anterior and posterior configurations with respect to variability in shape.

2. Average Normal morph compared with a more coarsely ornamented LACE individual

The morph LACE is not absolutely homogeneous in that one of the specimens presents a more coarsely

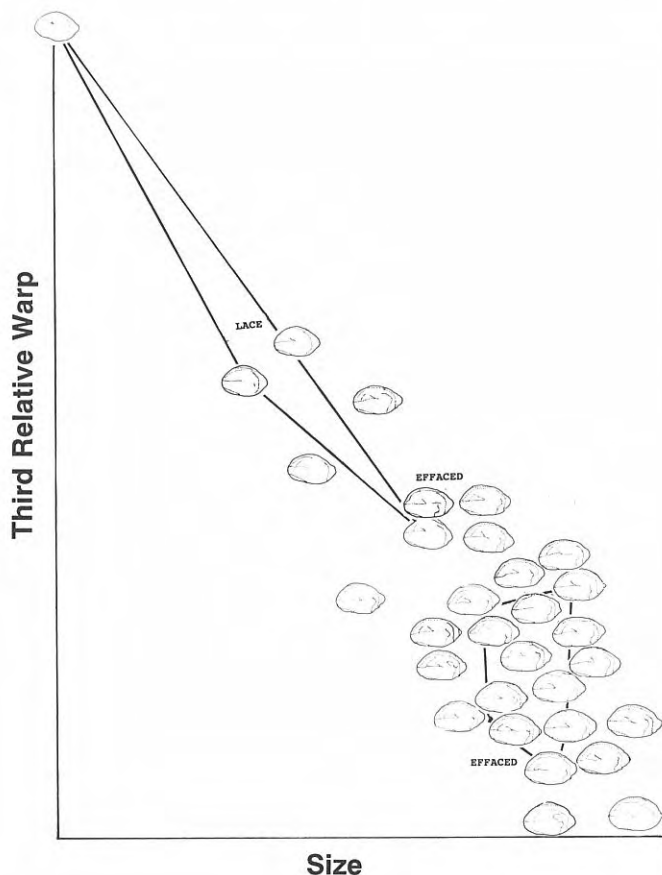


Figure 5. Ordination of specimens by the third relative warp plotted against centroid size. Outline drawings of the individual specimens are shown.

networked pattern. The deformational pattern, shown in Fig. 8, is almost identical with the foregoing (Fig. 6), but with some minor differences in the posterior area and the sharper anterior displacement of landmark 8. The LACE category can thus be seen to differ in shape variation from the principal morphological category represented in the sample, but the two variants of that morph are almost identical in their non-affine variational pattern.

CONCLUDING REMARKS

Hemicytherura fulva, a species typical of the genus *Hemicytherura*, has been shown to encompass a range of ornamental and morphometrical variability. One of the morphs, here termed LACE, can be reasonably regarded as a discretely manifested variant, delineated with respect to a distinctive ornamental pattern, size and shape. The second, more common, morph, here termed EFFACED, is not so clearly differentiated from the main variational pattern present in the species and could, therefore, be an expression of ecophenotypy. A similar situation was described by Abe *et al.* (1988). There is also a suggestion of the existence of a third, ovoid morph, but it is too rare to permit a definite statement.

Compared with other polymorphic ostracod species, the conditions exposed here are not unexpected (cf. Abe *et al.*, (1988) and Reyment (1988) and references therein). It must, however, be emphasized that we are only at the beginning of charting the nature, origin and extent of polymorphism in ostracods. The new information provided by the present analysis is that discrete ornamental subgroups tend to maintain a stable shape relationship (the LACE variant) and that posterior differentiation occurs not only in relation to size but also to shape. Finally, the current study indicates that benefits can be expected to accrue from a rounded approach to morphometric analysis, encompassing not only geometrical procedures but also the multivariate analysis of the usual distance characters of quantitative systematics.

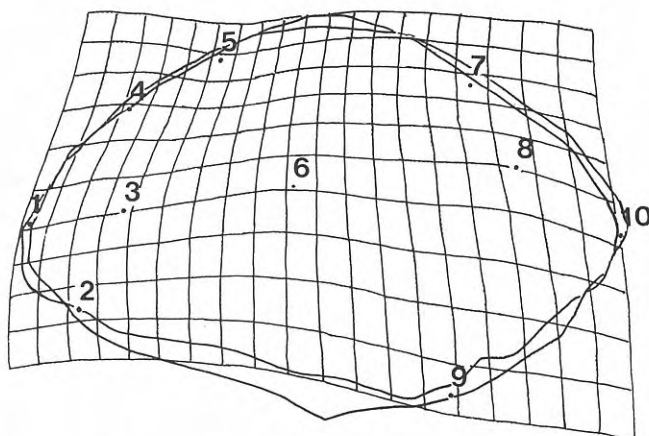


Figure 6. Principal warp comparison of the average morph "lace" with the average morph "normal".

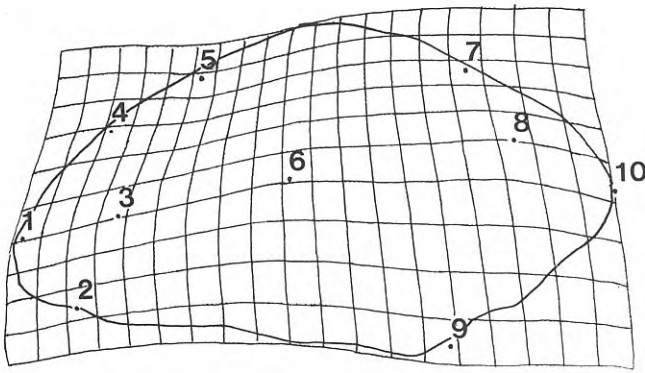


Figure 7. The total non-affine deformation for the principal warp comparison "lace" / "normal morph".

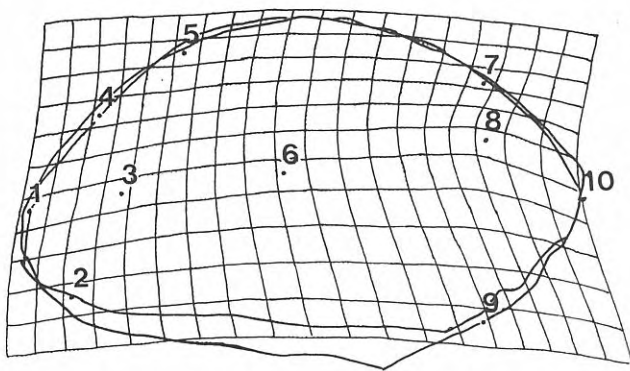


Figure 8. The principal warp comparison for a sub-variety specimen of "lace" and the average normal morph.

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