Morphometrics of *Vargula hilgendorfii* (Ostr. Crust.)

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Abstract: Two demes, sampled at Misaki and Tateyama (Tokyo Bay) of the bioluminescent myodocopid species *Vargula hilgendorfii* (Müller) are analysed by methods of geometric morphometry. At the intrapopulational level, indications of shape polymorphism occur, as yet insufficiently characterized to permit precise conclusions. A consistent, and pronounced, difference in centroid size, and a less pronounced though significant difference in shape, could be demonstrated between populations. Granted that Misaki and Tateyama are not far from each other, and that the analysis was confined to females, this marked divergence would seem to have its origin in some kind of persistent ecological factor. Many of the results obtained by geometric morphometry could be duplicated by means of standard multivariate morphometrics.

Introduction

The desire to express variation in shape in quantitative terms is as old as cultivated thought itself. Inscriptions on ancient Egyptian monuments provide early evidence of this wish. More widely known are the suggestive designs of Dürer, the German 16th Century artist, who devised a system of systematically deformed "caricatures" of faces. Even other artists have used this approach to vary physiognomies in mass scenes, using basically few faces as a starting point.

Scientific interest in the analysis of shape has a long history in biology, dating back almost 100 years to the days of the early biometricians. Notwithstanding that little real progress was made in those firstling attempts, it is significant that there was a definite desire to come to grips with the question. It was in 1917 that D'Arcy Thompson published his remarkable treatise on growth and form (Thompson, 1917). This book is replete with interesting suggestions, indicative of deep insight into the fundamental concepts of shape variability. His method of depicting change in shape in terms of coordinates is well known and has been cited, with copies of some of his figures, in many textbooks. Significant as these examples may be, they were not constructed by any mathematical method, but produced by freehand. Huxley (1932) was the first to note this, but the point was overlooked by others, as were most of Thompson's ideas. We can note in passing that Thompson lived a further 31 years after the appearance of his book, but he never returned to his theme to develop it further.

Attempts have been made at different times to deduce a valid solution for the shape-coordinate concept, but all have failed to

resolve the fundamental issues involved (Sneath, 1967). Beginning with some exploratory work by Teissier (1938) in France, the algebraic mode of analysis entirely displaced the geometry of Thompson, for a period of 50 years, until the work of Bookstein (1978, 1989, 1991).

Characters that preserve shape information

Coordinates determined at critical locations on an organism can be used to produce a "map" of these points. These point-coordinates can be termed "landmarks", a word borrowed from the terminology of craniometrics and, ultimately, topographical surveying. Landmark data contain information on the relative locations of the chosen points, as well as a complete archive of the usual distances – that is, lengths and breadths commonly measured in biometrical applications (it is a trivial matter to compute distances between coordinate pairs). The obvious advantage the coordinates have over distance measures is that one can easily reconstruct their locations in space and hence the form of the organism on which they were measured.

Thompson's approximate diagrams expressing shape comparisons as deformations of coordinate systems can now be given convenient descriptors that can be utilized in statistical analysis (Bookstein, 1992). The important break-through was achieved when Bookstein realized that the thin-plate spline technique, used in engineering for solutions to problems involving deformation, could be invoked as an accessory for providing a practical solution to the intercalation problem.

The spline technique and crustacean morphometry

The development of parts of an organism are not autonomous functions, but rather coordinated processes; the set of events is sometimes referred to as "developmental integration", a concept well known to vertebrate specialists.

Many developmental factors have been proven to have quite localized effects, for example, bone growth and deposition, by which mineralized tissue is added on surfaces. The same applies for growth in many shell-secreting organisms. Growth in crustaceans is less easy to typify in that the process takes place as a special event at moulting, during which increase in size, change in shape, and re-assigning of features of the ornament occur simultaneously. In contrast with, say, the cranial development of vertebrates, in which the autonomy of growth of "distant" regions may be a valid consideration, the characters enumerated above for crustaceans impose the restriction that these morphogenetic processes cannot be developmentally autonomously (cf. Zelditch et al., 1992). Nonetheless, little, if anything, is known about the number of developmental units, their hierarchical relationships, or the ontogenetic patterns in the integrated units in any group.

The quantification of developmental integration

The methods of analysis used in the present connexion are due to Bookstein (1989, 1991). These methods use superimposable Cartesian deformations to depict variation in shape. The numerical technique of the thin-plate spline allows a "Thompsonian type" deformation to be calculated from means of forms, which can, in turn, be decomposed into *partial warps*, which are rangeable from large-scale to progressively more localized components of the deformation. The representation of partial warps as displacements may, on first sight, appear to be expressed in an arbitrary coordinate system, but they are in fact computed in a way that is *coordinate-free*. These are what can be referred to as *Procrustean normalized displacements* in the original Cartesian system (Bookstein, 1991, p. 324). They are not "displacements in relation to a baseline" as a superficial perusal of the method might suggest.

The thin-plate spline method

The thin-plate spline method is used to model changes in shape as a deformation. What the method does can be explained in physical terms as follows.

Relative displacement of landmark points in the x,y-plane are depicted as though they were transferred to the z-coordinate of an infinite, uniform and infinitely thin metal plate "tacked" at a given "height" (i.e. the **new form**) above the landmark points of an **old form**. The conformation of the surface of this plate is described by a function minimizing physical bending energy. This is a simple function of the bending (actually, the second derivatives) of this artificial z-coordinate.

For graphical simplicity, this "vertical" can be treated as though it were added to one of the original Cartesian coordinates (either x or y). This yields a picture of *deformation*, instead of "bending"; it is a **mapping** of one picture to another, which extends the correspondence to the tissues in between where there are no data. In the vicinity of the mean form, the interpolation function is linear in the "vertical'" coordinate – landmark locations in the "final" form. The "bending energy" is a quadratic form in these same landmark coordinates.

The extraction of the latent roots and vectors of this array yields two *complementary* subspaces for the description of SHAPE CHANGE, to wit:

(1) The one, with no bending energy, describes what can be designated as *homogeneous affine transformations* (parallel lines remain parallel). These are called **uniform** because the same transformation occurs everywhere.

(2) The rest of the shape space is called the **non-uniform** subspace of transformations that are different in different parts of the form.

   Any deformation is the sum of its uniform and non-uniform
components.

Principal Warps

  When there are more than four landmark points, a non-uniform
deformation can be further decomposed to yield a series of components
(= the principal warps) ordered by amount of associated bending
energy. Principal warps associated with small bending energies
describe large-scale features, because little energy is needed to
bring about the bending of the hypothetical metal plate between
widely separated points. In contrast, relatively more energy is
required to bend the plate to the same vertical extent between
closely spaced points. Another way of envisaging the spline
transformation is by imagining one form to be printed on a stiff
transparent plastic sheet. Manipulate this sheet by an appropriate
pattern of bending (i.e. warping) until the shadow of this form takes
on the prescribed positions of the landmarks on the second form.

The method of Relative Warps

  The procedure just outlined in the foregoing section describes
comparisons between means of two samples. The spline technique can
also be applied to individual samples of forms, in a manner analogous
to ordinary principal component analysis, to describe shape
variability in the material, relative to an average shape. It is then
a natural extension of the original principal warp technique of
Bookstein (1989). This method of "relative warps" is a technique
developed by Bookstein (1991) for the analysis of within-population
morphometric variation. A valuable reference for practical
computational aspects of the method is the excellent review article
by Rohlf (1993). The method consists of fitting an interpolating
function, to wit, the thin-plate spline of Duchon (1973), to the x,y-
coordinates of the landmark for each specimen in a sample. Variation
among the specimens within a sample is described in terms of variance
in the parameters of the fitted function. This is expressed relative
to a bending energy matrix, as introduced above, based on the
coordinates of the landmarks of a reference configuration. In the
present analysis, the reference we use is the mean configuration of
landmarks, but other possibilities are open. The relative warps are
no more than the principal component vectors in this space; these are
used to describe the major constituents of shape variation among
specimens within a sample as deformations in form (i.e. non-uniform
shape variation).

  The present analysis makes use of comparisons of shape vectors with
centroid size. Centroid size is defined as the sum of squared
distances between all pairs of landmarks or, equivalently, the sum
of squared distances of each landmark to the barycentre of the
specimen (Bookstein, 1991, pp. 93-94). The graphical presentations
used here are, firstly, the plot of a relative warp as a deformation
of the space of the reference configuration of landmarks. This can

be demonstrated by computing a thin-plate spline for each relative warp and estimating the coefficients for a unit change in a relative warp score from that of the reference object.

The plots of the first relative warp, *RW1,* against *RW2* are for scaled scores; these plots provide a shape-ordination of the specimens. One expects specimens with similar configurations of landmarks (after removal of the "affine component" from them) to be close together in the ordination space. The plot of selected relative warps against centroid size is likewise a useful method for ordinating of the data if a size-dependent factor is involved.

For purposes of statistical analysis, such as arise in the present work, standard methods of multivariate analysis can be applied to the data in suitable size-free form (cf. Reyment et al., 1984). Rohlf (1993, p. 15) discussed various kinds of appropriate "weight matrices". We make use of two of these. One is for deformations alone, thus corresponding to the non-affine shape variation. The other encompasses both kinds of variation, uniform and non-uniform, and is produced by retaining the last three latent vectors of the bending energy matrix (which for graphical purposes in the relative warp analysis are rejected because they are connected to the affine variation that is orthogonal to the principal warps).

Shape variation in Vargula

The specific problem addressed in this contribution concerns the quantification of a "smooth" ostracod in a shape-analytical context. At first sight, the carapace seems to have about as many landmarks as an egg, but closer inspection discloses that there are in actual fact several good landmarks and pseudolandmarks. Visual inspection of the specimens from Misaki and Tateyama did not suggest any major variations in shape to occur and on just plain observational grounds, there would have been slight justification for assuming any form of morphological polymorphism to exist.

Vargula hilgendorfii is a bioluminescent ostracod species that is of common occurrence in the Bay of Tokyo and its immediate surrounding inlets. It is a relatively "large" species (around 5 mm in length). The problem investigated here was suggested by two observations made by KA: (1) that the area in the vicinity of the rostrum seems, possibly, to be more variable than the rest of the organism; (2) visual inspection of individuals collected at Misaki and Tateyama suggest that the two demes differ in size, on the average. The specimens analysed in this paper were collected at Tateyama in February 1993, all on the one night, and at Misaki in October 1990, all on the one night. All individuals are females and all are adults. Abe *et al.* (in prep.) and Vannier and Abe (1993) have recently investigated the biology of *V. hilgendorfii*.

The Landmarks A set of standard measures on the carapace would, no doubt, settle the issue as far as size differences are concerned,

but not the issue of shape variation. We decided, therefore, to
proceed by the methods of geometric morphometry (Bookstein, 1991) and
to select useful landmarks for doing this. Fig. 1 shows the sites
chosen as landmarks, eight of them in all. They are (1) the
anterodorsal angularity, (2) the upper tip of the rostral incision,
(3) the inner extremity of the rostral incision, (4) the lower lip
of the rostral incision, (5) the site of maximum ventral inflation,
(6) the posterior tip, (7) the posterodorsal angularity, (8) the site
of the maximum dorsal inflation. The sites of maximum dorsal and
ventral inflation are more subjective, but with a little care, could
be measured with sufficient statistical accuracy.

Within sample variability

   The plot of RW1 against RW2 (RW signifies Relative Warp). The
sample of left valves from Tateyama shows several subdivisions to
occur. These are outlined on the graph shown in Fig. 2. One group
comprises 7 specimens quite clearly segregated. Other indications are
persuasive, but the sample is not large enough to permit definite
conclusions. RW2 can be seen to be responsible for most of the
ordination. Likewise, the plot of RW1/RW2 for Misaki, which, although
smaller than the Tateyama sample, indicates groupings to exist in the
material.

   The most interesting shape indicator for right valves is RW10.
The deformation expressed by the tenth relative warp, which was found
to be expressive for illustrating shape variability for the present
material, for the pooled sample of specimens from both sites is shown
for right valves, in Fig. 3. Both figures contain analogous shape
information, but the rostrum for left valves is more compressed than
for right valves. The actual distances between landmarks can be
constructive to consider. We have listed some of these in Table 2.
Only the distance between landmarks 2 and 3 is greatly different.
 The two major groupings in the Tateyama collection obtained from the
tenth warp were tested for significant statistical distance using the
weight matrices. The results are listed in Table 1. These results
indicate that most of the differences in shape within the Tateyama
sample derive from the non-affine component. Even other warps
support the polymorphic subdivision.

   There is, therefore, reasonable evidence for the occurrence of
shape polymorphism in *Vargula hilgendorfii*. At the present time,
little of a definite nature can be suggested, but one things seems
certain, namely, that the polymorphic effects touch on different sets
of characters according to the level of proximity of the landmarks
being highlighted by a particular projection. Polymorphism here is
consequently likely to be a complex feature, probably connected to
some hierarchy of integration. The within-sample indications are
further supported by the results obtained for the pooled samples,
analysed below.

Relative warps and centroid size

   The next step in the analysis is to enquire whether there are significant size differences between the two sites. This is not something one would expect to occur, given that both sampling localities are located near to each other, on opposite sides of Tokyo Bay, only females were included in the study, and *Vargula hilgendorfii* is a mobile species with a highly developed optical recognition mechanism.

   First warp: The convex hulls for the plot for left valves is illustrated in Fig. 4A. The points for the two localities are almost completely separated with only slight overlap along the size axis, with one specimen from Tateyama falling with large Misaki individuals. The axis for RW1 distinguishes two large groups. Right valves (Fig. 4B) display likewise slight overlap (the same specimen as for left valves). There is a tendency for points to cluster across groups, the discontinuities being due to ordination along axis *RW1*.

   Fourth warp: There are three overlapped specimens for the convex hulls in the projection for left valves (Fig. 5) and several shape clusters, spread over both sites. Right valves yield an analogous result.

   Tenth warp: The ordination for right valves is illustrated in Fig. 6. There are four discrete shape clusters, two of which encompass both sites. One Misaki individual lies just inside the convex hull for Tateyama.

   Discussion: The graphical analyses indicate that there is a pronounced difference in size between the females from the two localities, manifested in discrimination along the centroid size axis. Secondly, there are hierarchically expressed differences in shape (hierarchical in the sense of the principal component decomposition of the warps). The reason for the strong difference in size, with practically no overlap, remains to be studied in detail, a task for future attention.

Is there any consistent shape difference between sites?

   The same method of multivariate analysis as used before was applied to the weight matrices of left and right valves for both of the samples. The results for the comparisons are displayed in Table 3. In all cases, the generalized distances are significantly different, that is for affine plus non-affine and for affine shape on its own. This somewhat unexpected result fuels further the thought that there are notable dissimilarities between the marine environments at Tateyama and Misaki.

Principal component analysis of distance measures

   Four distances were computed from the landmarks. These are D1: the distance between landmarks 1 and 5; D2: the distance between landmarks 2 and 6; D3: the distance between landmarks 5 and 7; D4: the distance between landmarks 5 and 8.

It was suggested earlier on that a standard multivariate analysis of distance characters could be expected to yield about the same results as the landmark-based procedures as regards differences in size. Four of the many possible distances available for the eight landmarks, selected above, were analysed by principal components. Table 4 lists the latent roots and vectors for the covariance matrices of right and left valves of Misaki and Tateyama, pooled. As to be expected, the elements of the latent vectors correspond closely, as do also the magnitudes of the latent roots. Of particular integrational interest is the fact that the smallest latent vectors are so close, and the percentages of trace of their respective latent roots are identical. The smallest latent vector expresses invariant relationships, which are almost identical for the two valves. This is not unexpected, but rather comforting from the point of view of accuracy of the measurements. The angles between the pairs of latent vectors are listed in Table 4; it will be seen that first and fourth latent vectors are separated by small angles, thus showing good agreement. The second and third latent vectors diverge more, but not excessively so.

The results shown in Fig. 7 for left valves almost exactly duplicate what was obtained by geometric methods. Misaki and Tateyama are well separated in all diagrams.

Concluding Comments

The analysis presented here achieves several purposes. Firstly, it is successful in unveiling shape variability within samples of a mobile species. Secondly, it proves the existence of geographically linked size-differences, a result that is all the more remarkable when the short distance between sampling sites is weighed in the balance. We reiterate that the observed differences are neither sexual-dimorphic nor ontogenetic in nature, given that all material measured consists of adult females. The variability identified in the rostral area could reflect the morphological expression of three factors, to wit, the swimming function in that the second antennae which are protruded there when in use, the secretion of luminescent substance, and the light-sensor ability involved in intraspecific signalling.

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Explanation of the Figures

Fig. 1. The landmarks determined on *Vargula hilgendorfii*.

Fig. 2. Plot of RW1 against RW2 for the Tateyama sample.

Fig. 3. The deformed grid for the tenth relative warp for all right valves.

Fig. 4.
  A. RW1 plotted against centroid size for all left valves. Observe separation on size and groupings on RW1.

B.   RW1 against centroid size for all right valves.
     M = Misaki; T = Tateyama.

Fig. 5. RW4 plotted against centroid size for all left
         valves. Clustering indicated by dotted convex hulls.
         M = Misaki; T = Tateyama; S = centroid size.

Fig. 6. RW10 plotted against centroid size for all right
         valves. Note the complete segregation of sites
         on the size axis and the four "shaoe clusters".
         M = Misaki; T = Tateyama; S = centroid size.

Fig. 7. Plots of the scores for principal component analysis of the
        left valves of the Misaki (M) and Tateyama (T) samples with
        the appropriate convex hulls indicated. Projection on the
        plane of the first (*I*) and second (*II*) latent vectors.
        There is slight overlap along axis one.

Table 1. *Generalized distances of weight matrices* for the Tateyama
sample morphs.

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weight matrix	D^2	F	P
Nonaffine+affine	40.83	$8.88_{12,10}$	>0.001
Nonaffine	23.27	$7.25_{10,12}$	0.001

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 Table 2: Actual distances between landmark deformations (left and
right valves) for the tenth relative warp.

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Between landmarks	left valves	right valves
6 and 7	2.55	2.61
5 and 6	7.50	7.65
7 and 8	6.50	5.40

1 and 8	7.40	6.75
1 and 3	1.80	1.65
2 and 3	1.30	1.95

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Table 3: Generalized statistical distances for comparisons of
          weight matrices for Misaki and Tateyama
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Weight matrix	valve	D_2	F	P
nonaffine+affine	left	29.79	$10.22_{16,22}$	>.01
nonaffine	left	4.74	$3.31_{10,28}$	0.05
nonaffine+affine	right	17.69	$6.80_{16,19}$	>0.01
nonaffine	right	6.19	$3.47_{10,25}$	0.05

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Table 4. Latent roots and vectors for four distance measures on left
and right valves of the Misaki and Tateyama samples
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	Right valves				Left valves			

latent vectors

var.	I	II	III	IV	I	II	III	IV
D1	0.358	0.830	-0.028	0.426	0.394	0.717	0.225	0.529
D2	0.562	-0.109	-0.759	-0.311	0.685	0.097	-0.611	-0.383
D3	0.500	-0.543	0.182	0.649	0.413	-0.671	-0.030	0.615
D4	0.553	0.064	0.624	-0.548	0.452	-0.160	0.758	-0.442

latent roots

	I	II	III	IV	I	II	III	IV
	7.404	0.967	0.521	0.074	5.880	0.896	0.489	0.061

%	82.58	10.79	5.81	0.83	80.26	12.24	6.67	0.83

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Angles between pairs of latent vectors in degrees:

I	10.74		II	20.17
III	22.33		IV	8.08

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