

# Multivariate Morphometrics in Aquaculture: A Case Study of Six Stocks of the Common Carp (*Cyprinus carpio*) from Italy

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Multivariate morphometry was used to investigate the distinctness and interrelationships of six stocks of the common carp (*Cyprinus carpio*). A "size" component was clearly identified by multiple group principal component analysis. Canonical variate analysis computed only on the "shape" components showed that the stocks were morphometrically distinct and that the phenetic relationships based on allozymic and morphometric data are highly congruent. We therefore suggest that multivariate morphometrics could represent an appropriate and convenient tool to detect variation between strains in carp culture.

Des techniques morphométriques à plusieurs variables ont été utilisées afin d'étudier le caractère nettement différent de six stocks de carpes communes (*Cyprinus carpio*) et leurs interrelations. Une composante « taille » a été clairement identifiée par l'analyse en composante principale à plusieurs groupes. L'analyse canonique des variables calculées seulement sur les composantes « forme » a montré que les stocks étaient différents du point de vue morphométrique et que les rapports phénétiques fondés sur des données allozymiques et morphométriques sont très pertinents. D'après nous, les mesures morphométriques à plusieurs variables représenteraient un outil utile et approprié permettant de déceler des variations entre les souches de carpe d'élevage.

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The common carp (*Cyprinus carpio*) is a temperate-water fish which is widespread throughout the Palearctic region. Historically it has been the primary subject of freshwater fish farming in Europe. It is widely reared and several programmes directed towards artificial selection, and the establishment of gene banks have been planned in the last decade (see Jhingran and Pullin 1985).

In Italy, carp from central and eastern Europe have been, and are, continuously transplanted for restocking and angling purposes so that the original populations (Bonaparte 1832–41) are no longer recognizable, and a generally confused picture of the origin of farmed common carps has emerged. In a previous paper (Cataudella et al. 1987), a multidisciplinary approach (i.e. cytogenetic, allozymic, and morphometric) was used to characterize six groups of common carp from different Italian water bodies in order to determine methods useful for stock charac-

terization, interspecific breeding plans, and selection programmes. Multivariate morphometry contributed to stock characterization, but only limited congruences between genetic (allozymic) and morphometric data sets were found.

The present paper attempts to address the following problems: (1) Since the pattern of morphometric relationships can be perturbed by bias in the growth determined size of the individuals from different stocks, is it possible to detect and remove a "size" component from the data? (2) How congruent is the general pattern of morphometric relationships among the stocks with their allozymic affinities? (3) Is it possible to recognize a stock morphometrically with sufficient confidence to make the technique suitable for use in fish farming research programmes?

Multivariate morphometry has been chosen because it represents an appropriate tool to assess distinctness and phenetic relationships between closely related taxa as in the study of

TABLE 1. Stocks, localities, geographic origin, squamation, number of individuals examined, and average total length with standard deviation. Each locality name is followed by its provincial district. The question mark in CCA reflects its uncertain origin. Squamation is a monomorphic trait in all stocks except CCC and CCF.

| Stock | Locality                                    | Origin         | Squamation      | Number of specimens | Total length (cm) |
|-------|---|----------------|-----------------|---------------------|-------------------|
| CCA   | Fish farm (Grosseto)                        | ?              | Complete        | 18                  | 22.5 ± 1.3        |
| CCB   | Alfieri lake (Latina)                       | Eastern Europe | Mirror          | 13                  | 15.5 ± 2.7        |
| CCC   | Valdagri Fish Farm (Matera)                 | Tiber, China   | Complete/mirror | 27                  | 28.4 ± 5.7        |
| CCD   | Land Reclamation Pond (River Reno, Bologna) | Po Plain       | Complete        | 13                  | 43.9 ± 3.9        |
| CCE   | Landi Fish Farm (Bologna)                   | Germany        | Leather         | 5                   | 41.9 ± 20.7       |
| CCF   | Power Plant (Milano)                        | Eastern Europe | Complete/mirror | 16                  | 37.5 ± 15.1       |

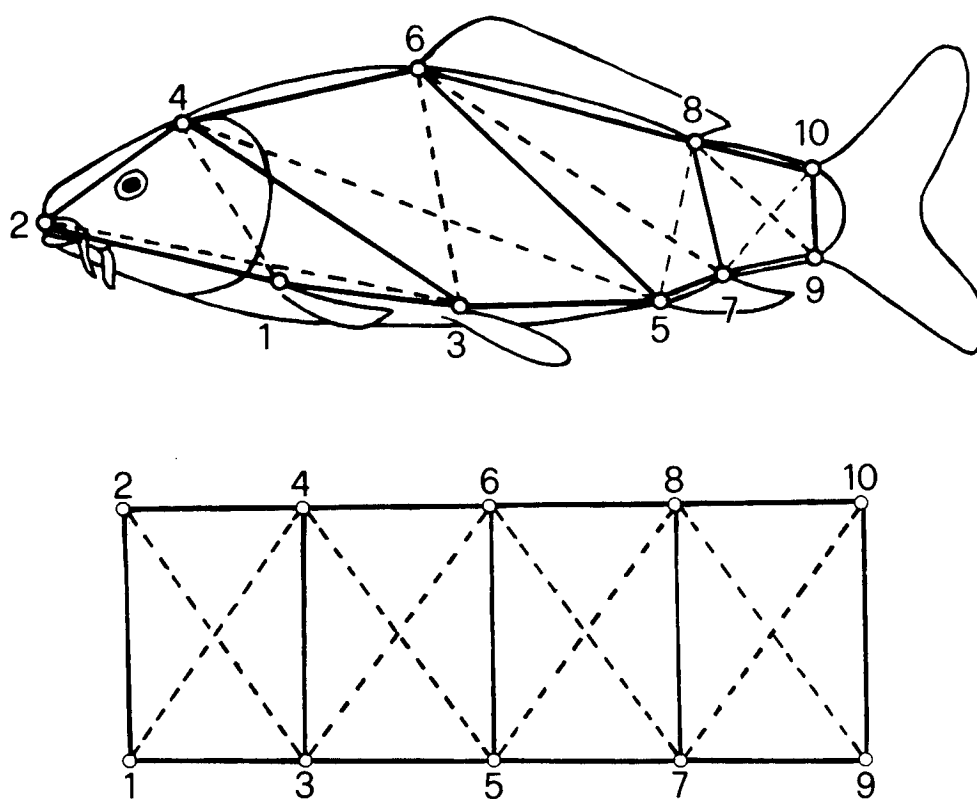


FIG. 1. Data recording procedure. Top: 21 distance characters recorded on X-ray pictures between the following homologous landmarks: 1, upper insertion of pectoral fin; 2, upper end of maxilla; 3, upper insertion of ventral fin; 4, top end of supraoccipital; 5, insertion first ray of anal fin; 6, insertion first ray of dorsal fin; 7, insertion last ray of anal fin; 8, insertion last ray of dorsal fin; 9, lower insertion of caudal fin; 10, upper insertion of caudal fin. Bottom: Geometric representation of the data recording procedure. Each single cell is determined by four sides and two diagonals. Modified from Strauss and Bookstein (1982).

geographic variation and racial affinities (Gould and Johnston 1972; Reyment et al. 1981; Thorpe 1976, 1983a, 1987). Multivariate morphometry has become a widely used approach in ichthyology to detect patterns of phenetic relationships between taxa (Humphries et al. 1981; Johnson et al. 1983; Libosvsky and Kux 1982; Shaklee and Tamaru 1981; Strauss and Book-

stein 1982; Winans 1984), to assess possible and effective hybridization events (Humphries 1984; Neff and Smith 1978), and to describe development in wild and reared larvae (McGurk 1985).

Since we have to contend with relatively slight morphometric differences between similar stocks of the same species, some

TABLE 2. Multiple group principal component analysis giving the 16 eigenvalues extracted from the pooled within-group covariance matrix and the character coefficients of the first normalized eigenvector which expresses 91.398% of the within-group variance. This vector is associated with the largest eigenvalue (No. 1) and all the coefficients are of the same sign and magnitude, so that this vector was considered as representing "size." Coefficients of the other 15 eigenvectors (not represented) all differ in their sign and magnitude and were considered as representing "shape." Only 16 characters were used out of the original 21, as 5 characters were not informative.

| Eigenvalue | Coefficient of first normalized eigenvector | Characters between landmarks |
|------------|---|------------------------------|
| 1          | 0.905                                       | -0.235                       |
| 2          | 0.027                                       | -0.252                       |
| 3          | 0.014                                       | -0.221                       |
| 4          | 0.010                                       | -0.223                       |
| 5          | 0.008                                       | -0.225                       |
| 6          | 0.005                                       | -0.251                       |
| 7          | 0.004                                       | -0.246                       |
| 8          | 0.004                                       | -0.261                       |
| 9          | 0.003                                       | -0.279                       |
| 10         | 0.003                                       | -0.256                       |
| 11         | 0.002                                       | -0.261                       |
| 12         | 0.002                                       | -0.272                       |
| 13         | 0.001                                       | -0.267                       |
| 14         | 0.001                                       | -0.264                       |
| 15         | 0.001                                       | -0.232                       |
| 16         | 0.001                                       | -0.244                       |

care has to be taken to identify size and shape components of variation and to record an appropriate set of characters. Size is a component which can, in continuously growing organisms such as bony fish, perturb an assessment of general racial differentiation (Thorpe 1983a, 1983b). Several approaches have been used in attempts to overcome the problem. Libosvsky and Kux (1982) selected individuals of the same size to analyze morphometric variation between three *Gobio* species. Ratios, where the denominator is standard length, have been used by Johnson et al. (1983) and Shaklee and Tamaru (1981). However, the use of ratios to adjust for size variation has been questioned for many statistical reasons (Atchley et al. 1976; Humphries et al. 1981; Thorpe 1983a). Principal component analysis has been widely used to detect size vectors, usually identified with the first principal component (see Neff and Smith 1978 and Neff and Marcus 1980 for a discussion). Principal component analysis does not require a priori recognition of groups, and if there are several groups, data are pooled irrespective of groups. Humphries et al. (1981) suggested a different multivariate procedure to analyse size and shape, subsequently adopted by Chernoff et al. (1982) and Humphries (1984). Another multivariate technique, multiple group principal component analysis (MGPCA), which can detect a pooled within-group size component, was adopted by Thorpe et al. (1982) and is explained in Thorpe (1983a, 1983b).

Data sets for morphometric purposes in ichthyology have generally been composed of traditional characters, namely total and standard length, total height, etc. The general biases presented by these traditional data sets, namely preponderance of some direction over others, dense coverage measurements in some areas of the body, and paucity elsewhere (Humphries et al. 1981), can be overcome by using the "Truss" network method (Strauss and Bookstein 1982). There is increasing evidence that the Truss method is much more powerful in describing morphological variation between closely related fish taxa

TABLE 3. Pooled within-group correlation coefficients between the 16 multiple group principal components (MGPC) and total length. Component 1 is the "size" vector; components 2-16 are "shape" vectors. Correlation exists only between the "size" vector and total length as measured from the specimens used.

| MGPC | Correlation coefficient |
|------|-------------------------|
| 1    | -0.825                  |
| 2    | 0.103                   |
| 3    | 0.051                   |
| 4    | -0.051                  |
| 5    | -0.037                  |
| 6    | 0.022                   |
| 7    | -0.045                  |
| 8    | 0.072                   |
| 9    | 0.049                   |
| 10   | 0.019                   |
| 11   | -0.043                  |
| 12   | 0.050                   |
| 13   | 0.032                   |
| 14   | -0.054                  |
| 15   | -0.037                  |
| 16   | -0.076                  |

with respect to traditional measurements (Strauss and Bookstein 1982; Winans 1984; Cataudella et al. 1987).

## Materials and Methods

The six stocks used in this study were collected in wild and artificial Italian water bodies managed by private fish farmers. The localities and the origin, together with the squamation, number of individuals, and the average total length for each stock, are reported in Table 1. Stocks have been named with a three-letter code, i.e. from CCA to CCF. The origin of each stock is known except for CCA (Table 1); moreover, CCC, originating from the River Tiber, has undergone frequent and successive introductions.

Twenty-one distance characters have been recorded on X-ray pictures between homologous landmarks (Fig. 1) following the rapid and accurate Truss method adopted from Strauss and Bookstein (1982). The Truss network consists of a series of quadrilateral cells, each one defined by four landmarks among which are four edges and two diagonal distances (Fig. 1). Landmarks are true and unambiguous homologous points from specimen to specimen identified by anatomical features as indicated in Fig. 1. This procedure allows one to detect variation in linear dimensions in horizontal, vertical, and oblique directions throughout the entire body.

In contrast with univariate statistics, rules to test sample size adequacy in multivariate statistics are less known and most of them are asymptotic and inexact (Neff and Marcus 1980). Harris (1975) suggested that if the number of the individuals minus the number of the variables used is greater than 30, then the sample can be considered as large. In our case this criterion gives a value of 71, and so we assume that the number of specimens used in this study (Table 1) is adequate for our purposes. Random resampling, varying sample size and content, is one method of tackling this problem but is beyond the scope of this paper.

There is a bias in the age/size of fish in the various samples, some being predominantly young fish and others being biased towards older, larger fish (Table 1). Consequently, we wished to define a "size/growth" component and remove its influence

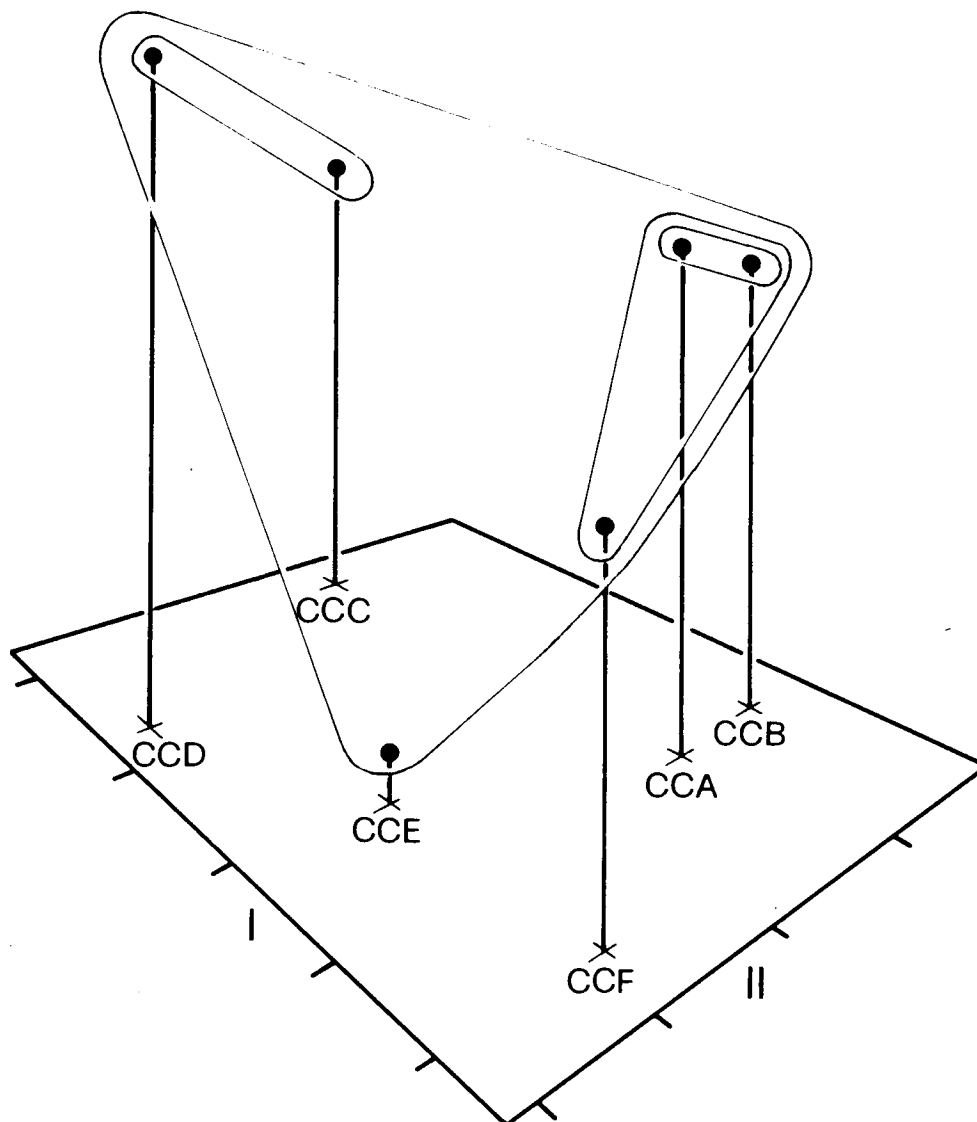


FIG. 2. Canonical analysis of body shape based on "shape" components from MGPCA. In this three-dimensional plot, the third canonical variate is the vertical axis. Units along canonical axes represent pooled within-group standard deviations. Progressive contour clustering between group centroids is determined by UPGMA computed on Mahalanobis distances. The close clustering of stocks with common geographic origin is evident, as is their distinctness from others as a consequence of long-term geographic isolation.

TABLE 4. Eigenvalues, canonical correlations, and percentage of variance of the first three canonical functions (CV) produced by "shape" CVA.

|                       | CV I    | CV II   | CV III  |
|-----------------------|---------|---------|---------|
| Eigenvalue            | 2.63999 | 1.22893 | 0.93123 |
| Canonical correlation | 0.85163 | 0.74253 | 0.69440 |
| % variance            | 45.429  | 21.147  | 16.025  |

from the data set. When there are several groups, as in this case, the use of simple principal component analysis to define and remove size is generally inappropriate because of the confusion of within- and between-group size differences. This is explained geometrically in Thorpe (1976, 1983a, 1983b). To overcome this problem, we used MGPCA, which extracts vectors from the pooled within-group covariance matrix. This gives a pooled within-group size vector when size is a component of the within-group variation due to growth effects. The requirements are as

for canonical variate analysis (CVA; Thorpe 1983a), except that equality of variance along the growth axis is not required (Thorpe 1983a, 1983b).

To assess affinities between carp stocks, we used CVA. CVA orders groups along axes of maximum variation in such a way as to maximize among-group variance relative to within-group variance.

When all the component scores from MGPCA are subjected to CVA, this gives exactly the same results as a CVA on the raw data. When the component scores from the "size" vector are excluded, this gives a size-independent "shape" CVA. A scatter diagram based on the main canonical variates gives a visual portrayal of the relative similarity of the stocks.

## Results

CVAs performed separately on the two sexes did not reveal sexual dimorphism. Consequently, further analyses were performed on data pooled irrespective of sex.

TABLE 5. Above the diagonal: "shape" (upper row) and "size-dependent" (lower row) matrices of Mahalanobis distances between group centroids. Below the diagonal: matrix of *F*-values (upper row) and of Rogers' genetic distances (lower row) between all pairs of the six stocks. The degrees of freedom are 15 and 72; *P* < 0.001 except for the value marked with an asterisk which is not significant.

|     | CCA            | CCB          | CCC           | CCD          | CCE          | CCF          |
|-----|----------------|--------------|---------------|--------------|--------------|--------------|
| CCA | —              | 1.80<br>2.26 | 3.56<br>3.70  | 3.65<br>4.63 | 4.61<br>5.70 | 2.92<br>3.47 |
| CCB | 1.39*<br>0.063 | —            | 3.28<br>4.05  | 4.09<br>5.87 | 4.45<br>6.48 | 3.25<br>4.59 |
| CCC | 7.63<br>0.39   | 5.28<br>0.25 | —             | 3.16<br>3.64 | 4.69<br>5.23 | 4.33<br>4.41 |
| CCD | 5.61<br>0.17   | 6.08<br>0.16 | 4.83<br>0.18  | —            | 4.78<br>4.81 | 4.22<br>4.33 |
| CCE | 4.63<br>0.32   | 3.99<br>0.26 | 5.18<br>0.25  | 4.61<br>0.29 | —            | 5.25<br>4.92 |
| CCF | 4.03<br>0.04   | 4.23<br>0.08 | 10.52<br>0.32 | 7.11<br>0.27 | 4.68<br>0.32 | —            |

TABLE 6. Classification matrix according to "shape" CVA. Note that the major incongruencies concern stock CCC from the River Tiber as a consequence of uncontrolled crosses with different carp stocks (see-text and Table 1 for further details).

| Group | Percent correct | Number of cases classified into group: |     |     |     |     |     |
|-------|-----------------|--|-----|-----|-----|-----|-----|
|       |                 | CCA                                    | CCB | CCC | CCD | CCE | CCF |
| CCA   | 77.8            | 14                                     | 3   | 0   | 1   | 0   | 0   |
| CCB   | 61.5            | 4                                      | 8   | 0   | 0   | 0   | 1   |
| CCC   | 63.6            | 1                                      | 2   | 21  | 3   | 3   | 3   |
| CCD   | 100             | 0                                      | 0   | 0   | 13  | 0   | 0   |
| CCE   | 100             | 0                                      | 0   | 0   | 0   | 5   | 0   |
| CCF   | 87.5            | 2                                      | 0   | 0   | 0   | 0   | 14  |
| Total | 81.73           |  |     |     |     |     |     |

The within-group covariance matrices were computed from the 21 original characters for each stock independently and then pooled to give a pooled within-group covariance matrix. Since the determinant of this matrix equals zero, we carefully checked those characters that contribute least, i.e. characters expressing low variance. When these were excluded (characters between landmarks 2-3, 3-4, 3-5, 6-7, and 6-8; Fig. 1), the resulting pooled within-group covariance matrix was real, symmetric, and positive definite.

The MGPCA run on the 16 selected characters of all six groups produced a clear "size" vector (with coefficients of similar magnitude and sign) associated with the largest eigenvalue (Table 2). The remaining components were taken as representing "shape." To confirm this interpretation the pooled within-group correlation between the 16 multiple group principal components (MGPC) and an independent measure of size, i.e. total length, was computed. This alternative measure of size is highly correlated with the "size" component, i.e. MGPC 1, and is uncorrelated with the "shape" components, i.e. MGPC 2-16 (Table 3), thereby confirming this interpretation of the components.

Discriminant functions based on the score from the MGPCA "shape" components completely distinguish among the six stocks except CCA from CCB.

The separation of the stocks on the group means on the first three canonical variates is given in Fig. 2. The first, second, and third canonical variates absorb 45.429, 21.147, and 16.025% of the total dispersion, respectively, to explain cumulatively 82.601% of the total variation (Table 4). The significance level of the discrimination of the six stocks is shown by the values of the *F*-matrix (Table 5). The classification matrix shows a percent of correct classification varying from 100 to 61.5% (average 81.73%) (Table 6).

It might be argued that since the "size" component alone, as detected by MGPCA, absorbs 91.398% of total variance, it would have been meaningful to perform CVA on "shape" components explaining 8.602% only of total variance. However, it must be stressed that the contribution of the "size" component (as any other within-group component) to between-group discrimination bears no relationship to the percentage of within-group variance it expresses (Thorpe 1983a, 1983b). In this case, a parallel "size-dependent" CVA demonstrates that the "size" component expresses only 25.919% of the between-group discrimination, and in any event, growth bias between samples precludes its inclusion.

To summarize intergroup affinities, Mahalanobis (1936) distances between group centroids were computed (Table 5). These distances test the equality of group means for each pair of groups and can be used as the only realistic measure of multivariate distance (Reyment et al. 1981).

A UPGMA clustering (Sneath and Sokal 1973) computed on Mahalanobis distances between centroids (Fig. 2) has been superimposed on the three-dimensional plot of canonical scores to show the phenetic relationships between stocks (Fig. 2). The pattern of phenetic similarities (Fig. 2) shows a direct relationship with the geographic origin of each stock (Table 1), but not with their squamation (i.e. mirror, leather, or complete). The eastern European stocks (CCB, CCF) cluster first together with the stock of unknown origin (CCA). The isolated position of CCE with respect to the other groups reflects its origin. The stock CCE originated from Germany where carp culture was established long ago with selection and breeding programmes, and it has not been contaminated by later introductions since it was imported into Italy some 40 yr ago. CCC originates from a population of the River Tiber, and it has been subjected to repeated crosses with carps belonging to an Albanian stock originating from China. A genetic constitution with "Chinese" genes of CCD was speculated by some of us (Cataudella et al. 1987) and might account for its clustering with CCC.

## Discussion

A previous paper (Cataudella et al. 1987) reported the multilocus electrophoretic results carried out on the same individuals of the six stocks. Although no fixed different allele was found in 13 enzymatic systems (40 loci), the analysis of allele frequencies at polymorphic loci (Ca-1, Est-3, Est-4, Ldh-1, Pgm) did characterize the stocks to a certain degree. We computed a principal coordinate analysis (PCOA) on Rogers' (1972) genetic distances between the six stocks (Table 5). PCOA is an ordination technique which allows one to compute principal components from a distance matrix (Gower 1966). Figure 3 shows the UPGMA contour clustering, computed on Rogers' genetic distances, superimposed on the PCOA three-dimen-

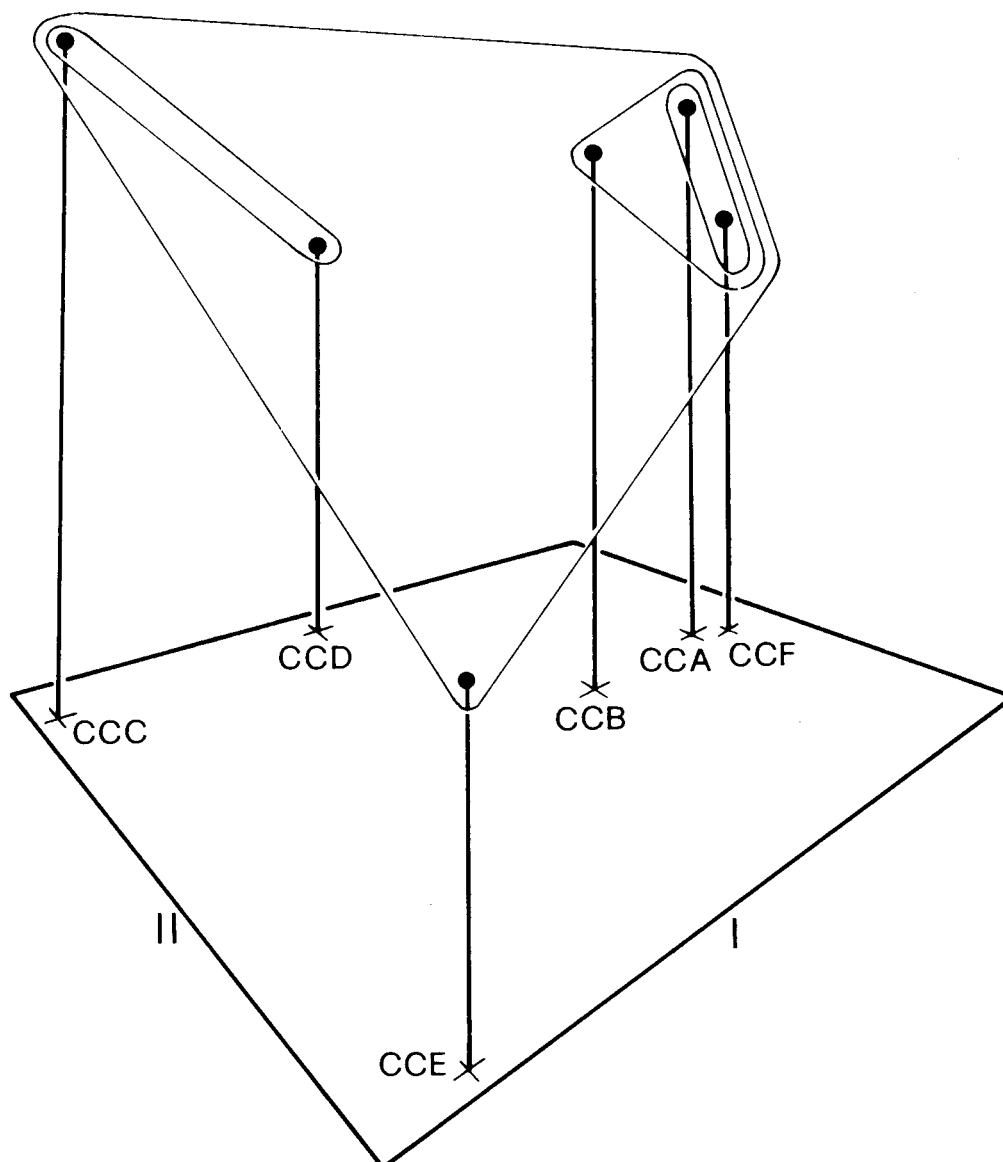


FIG. 3. Principal coordinate analysis computed on Rogers' genetic distances (Table 5). In this three-dimensional plot, the third principal component is the vertical axis. Progressive contour clustering between stocks is determined by UPGMA computed on Rogers' genetic distances. The pattern of genetic similarities between carp stocks is congruent with the one depicted from morphometric analyses (see Fig. 2).

sional scatter diagram. The general congruency in the distinction patterns from the "shape" morphometric (Fig. 2) and genetic (Fig. 3) analyses is evident; therefore, these two analyses corroborate one another. The only discrepancy between the genetic and morphometric relationships is the ordering of the clustering in the CCA, CCB, CCF set. However, the cophenetic correlation between Rogers' genetic distances and "shape" Mahalanobis morphometric distances (Table 5) is highly significant ( $r = 0.704$ ,  $P < 0.005$ ) in contrast with nonsignificant cophenetic correlation ( $r = 0.376$ ) between genetic and "size-dependent" Mahalanobis distances (Table 5) as detected by the parallel CVA performed on "size" plus "shape" components. This evidence suggests that our "shape" analysis reflects a genetic pattern of variation between stocks and that this variation apparently is not strongly influenced by local pond environmental factors.

These findings show that the incongruencies previously found between morphometric and genetic results (Cataudella et al.

1987) are a consequence of the bias in growth within the samples. Although nothing is known of the heritability of these morphometric traits, it is evident that the "shape" character analysis provided by MGPCA depicts a pattern of morphometric variation which is consistent with the "genetic" constitution of the stocks.

Congruence between allozymic and morphometric data sets has been often a source of debate (Lewontin 1984). The frequent lack of congruence is generally attributed to different evolutionary forces operating on the two sorts of characters and to differences in the descriptive power of statistical tests employed in the analysis of morphometric and allozymic data sets. Although a strict congruence is not a priori to be found, our results indicate that the stocks examined are morphometrically distinguishable and that their relationships reflect their geographic origins as well as their genetic constitutions. We suggest that multivariate morphometry represents a powerful and convenient tool for carp stock identification and monitoring in

aquaculture. Aquaculture requires increasing contributions from basic research to accelerate its development on a scientific basis rather than through trial and error.

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