Geographic Variation in the Milkfish *Chanos chanos*  
II. Multivariate Morphological Evidence

GARY A. WINANS

Morphological variation in six meristic and 19 morphometric characters was examined in the milkfish, *Chanos chanos*. Samples were collected at 15 locations in the Pacific Ocean, from the Philippine Archipelago in the west, along the equatorial Pacific Ocean to Tahiti and Hawaii in the east. Variation within each sample, estimated as the multivariate generalization of the coefficient of variation, did not vary substantially among the localities. Principal component analyses indicated that multivariate body shape differences existed, but that meristic differences were lacking among the samples. Along a morphometric shape axis, the Hawaiian samples and the equatorial Pacific Island samples generally had larger head features-smaller tails in comparison to the Philippine samples. The data for a repeat sample from the island of Hawaii indicated that the multivariable shape criterion increased with the size of fish and thus provided an insight into the principal component results. The patterns of morphological differences and electrophoretic differentiation (Winans, 1980) were compared graphically. The genetic population structure of milkfish in the Pacific Ocean consists of three distinct groups: the Philippine Archipelago; the equatorial Pacific Ocean, including Tahiti; and the Hawaiian Archipelago.

**Milkfish**, *Chanos chanos* (Forskal), are tropical marine fish of the Indian and Pacific oceans that inhabit shallow, inshore regions such as estuaries and sandy reef flats. On the basis of their availability, general hardiness and rapid growth in pond culture, milkfish are considered one of the best suited species of fish for aquaculture (Bardach et al., 1972). Although the pond culture of milkfish already contributes significantly to human nutrition, especially in Southeast Asia, there are few data concerning their basic biology (Liao et al., 1979; Kuo et al., 1979; and Wainwright, 1982).

An important initial step in a study of the biology of a species is to assess the levels and patterns of variability within and among natural populations of the organism (Gould and Johnston, 1972). Contemporary descriptions of geographic variation are commonly based on electrophoretic and morphological characteristics of populations, as these two sets of traits consist of discrete genetic characters and multigenic morphological characteristics and are differently affected by environmental variation. In this approach then, different portions of an organism's genome are represented and evaluating and contrasting these two data sets may be useful from a systematic, evolutionary and/or
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Fig. 1. Location of milkfish sample sites. P8, San Thomas, Luzon; P7, Mercedes, Luzon; P6, Hamitique, Panay; P5, Ormoc, Leyte; P4, Argao, Cebu; P3, Cagayan de Oro, Mindanao; P2, Zamboanga, Mindanao; and P1, Sulop, Mindanao.

a managerial standpoint (Wright, 1978; Utter, 1981; and Ihssen et al., 1981).

To assess geographic variation in milkfish, samples were collected where possible in the Pacific Ocean, from the Philippine Archipelago eastward to the Hawaiian Archipelago and Tahiti. Specimens were subjected to multivariate electrophoretic and morphological analysis. Electrophoretic variability at 38 loci was reported in Winans (1980). In this paper, patterns of multivariate morphological variation in 25 characters are viewed for the same set of milkfish samples used for the electrophoretic analysis. Data from a Tahiti sample and a repeat sample from the island of Hawaii are also included. Levels of variability in each locale are estimated and tested for equality among samples and patterns of differentiation are assessed by principal component analysis. These results are compared with the electrophoretic results.

MATERIALS AND METHODS

Fish were sampled in 1977, 1978 and 1979 from 15 locations in the Pacific Ocean (Fig. 1). The three major island groups and their acronyms were the Philippines (PHI, eight localities), the equatorial Pacific Islands (EPI; Palau, Tarawa, Fanning and Christmas islands) and the Hawaiian Islands (HI, two localities). The sample from Tahiti was considered separately. Gill, hand, or surround nets were used to capture the fish in coastal ponds or waterways. Following electrophoretic studies, subsamples of each fish collection were used for morphological analyses. Average sample size was 29 fish per locality (Table 1). The sample size in all analyses was 471 fish.

Data were recorded for 19 morphometric characters (plus the standard length) listed in Table 2. On larger fish, the first four measurements in Table 2 were measured with a meter stick accurate to 0.5 mm. Otherwise, the morphometric characters were measured with dial calipers accurate to 0.1 mm. Six meristic counts were also made: dorsal-fin rays, pectoral-fin rays, pelvic-fin rays, anal-fin rays, pored lateral line scales and scales along the base of the dorsal fin. All measurements and counts were made on the left side of freshly thawed specimens.

Morphological variability was estimated in each sample by the multivariate generalization of the coefficient of variation (Van Valen, 1978: 41)

$$CV_p = 100 \sqrt{\frac{\sum S_i^2}{\sum \bar{S}_j^2}}$$

where $\sqrt{S_i^2}$ and $\sqrt{\bar{S}_j^2}$ are the sums of the variance and mean squared, respectively, of the six meristic traits in a sample over j populations (see below).

To examine among-sample differences multivariately, principal component analyses were performed on character variance-covariance matrices using a combination of subroutines.
from the International Mathematical and Statistical Library for numerical software (IMSL, 1977). Meristic data were transformed into square roots and morphometric data into common logarithms to reduce the correlation of the measurement means and variances (Sokal and Rohlf, 1969). Bivariate confidence ellipses were calculated by program A3.13 in Sokal and Rohlf (1969).

To estimate measurement error for the 20 morphometric characters, 10 fish (20–30 cm, fork length) were randomly chosen from three samples and each morphometric character was measured independently on each fish five times. The standard deviation of each distance character over the five replications was estimated for each fish; the average standard deviation of a character over the 30 fish was used as an estimate of measurement error. The average measurement error for the 20 characters was 0.48 mm (±0.36 mm), with values ranging from 0.16 mm for the caudal peduncle depth to 1.64 mm for the standard length. Fork length measurements had a measurement error of 0.93 mm and were therefore used in lieu of standard length in all analyses. As 70% of the characters had a measurement error of less than 0.5 mm, measurement error was considered insignificant for the remaining 19 morphometric characters.

RESULTS

Sample statistics for the fork length are presented in Table 1. Since most of the morphometric characters were correlated with the size of the fish, or fork length (see below), an examination of these data provides a good idea of the range of variation in the morphometric characters.

Variation within samples.—To estimate morphological variability within each sample, only the meristic traits were considered. Within the 16 samples, correlation coefficients of meristic characters with the length of the fish (fork length) were generally small and not statistically significant (i.e., in the 96 comparisons, only 6 were significant). On the other hand, all of the morphometric variables were correlated with the length of the fish (mean correlation coefficient = 0.95). Hence, a good estimate of size-independent morphological variability in a sample was a measure of meristic variability.
Values of $CV_p$ given in Table 1, differed little among the samples. Values ranged from 3.10 (P8) to 5.65 (TAR). A test of equality of the 16 $CV_p$ values was not significant ($P = 0.20$ in Smith's test; from Van Valen 1978:35). When the sample values were compared by island group (i.e., PHIL, EPI and HI), a statistically significant among-group difference was revealed ($0.01 < P < 0.025$; Kruskal-Wallis $H$-test). This result may be explained by larger $CV_p$ values in the EPI group ($\bar{x} = 4.72$) which were significantly greater than the PHIL values ($\bar{x} = 3.52; P = 0.01$, Wilcoxon two-sample test). The PHIL and HI values were not significantly different ($U = 4, P > 0.05$). The inclusion of the Tahiti sample into the EPI group (justified on electrophoretic criteria below) did not alter the above results. Finally, the $CV_p$ values did not vary significantly with latitude ($r = -0.27$) or longitude ($r = 0.47$).

Among sample differences.—In the principal component analysis of the morphometric data, 98% of the total variance was explained along the first two axes (Table 2). Character loadings were approximately equal on principal component I (PC I) and mean fork length per sample increased from left to right along the PC I axis (Fig. 2). Thus, PC I was considered a measure of general size. A preliminary analysis indicated that PC II was influenced to a small degree by within-group size variability, i.e., bivariate ellipses tilted with respect to the PC I axis. PC II (the variable loadings and the multivariate scores) was therefore adjusted or sheared by the algorithm of Humphries et al. (1981) to produce a size-free shape component, shared PC II (SPC II). SPC II contrasted several head characteristics (orbital, snout and snout–premaxillary length) with several tail width characters (caudal depth, body depth and anal-fin base) and was considered a shape indicator (Fig. 3). Generally, the Hawaiian samples and the Equatorial Pacific Island (EPI) samples had large SPC II values (larger head features-smaller tails), while the Philippine samples had smaller SPC II values (smaller head features-larger tails) (Fig. 3). The Philippine sample P6 and the EPI sample from Christmas Island (CHR) were exceptions to this pattern. The mean SPC II value for the Tahiti sample was close to the typical Philippine value.

A principal component analysis of the six meristic characters indicated little among-sample variability. Of the total variance, 84% was explained along the first two axes (Table 3), with subsequent functions explaining less than 6% of the total variance. The scale count on the dorsal-fin base predominated on PC I and the lateral line scale count predominated on PC II. In the PC I–PC II scattergram, 11 diagonal columns appeared along the PC I axis corresponding to the 11 observed values of scale counts on the dorsal-fin base, as illustrated in Fig. 4. A scattergram of the bivariate means along the first two axes is presented in Fig. 5. Despite the relative distinctiveness of the samples from Oahu, Fanning Island and Tarawa long PC II, the most remarkable feature of the results depicted in Fig. 5 is the extensive overlap among the samples.

**Discussion**

Morphological variability within each sample, when estimated as $CV_p$, varied little over the 15
TABLE 3. VARIABLE COEFFICIENTS ON THE FIRST THREE PRINCIPAL COMPONENTS FROM AN ANALYSIS OF SIX MERISTIC CHARACTERS OF Chanos chanos.

<table>
<thead>
<tr>
<th>Meristic character</th>
<th>PC I</th>
<th>PC II</th>
<th>PC III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal-fin rays</td>
<td>0.05</td>
<td>0.03</td>
<td>-0.11</td>
</tr>
<tr>
<td>Pectoral-fin rays</td>
<td>0.04</td>
<td>-0.09</td>
<td>-0.01</td>
</tr>
<tr>
<td>Pelvic-fin rays</td>
<td>0.01</td>
<td>-0.08</td>
<td>-0.36</td>
</tr>
<tr>
<td>Anal-fin rays</td>
<td>0.00</td>
<td>-0.01</td>
<td>-0.09</td>
</tr>
<tr>
<td>Lateral line scales</td>
<td>0.12</td>
<td>-0.98</td>
<td>0.11</td>
</tr>
<tr>
<td>Scales dorsal-fin base</td>
<td>0.99</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>% of total variance</td>
<td>64.1</td>
<td>19.8</td>
<td>5.9</td>
</tr>
</tbody>
</table>

locations in the Pacific Ocean (Table 1). When the 15 CV<sub>p</sub> values were compared, no significant differences were detected. When the data were pooled and examined by island groups, the four samples from the equatorial Pacific Ocean (the EPI group) had significantly greater values than the eight Philippine samples; but no other pairwise group comparisons were significantly different. Two samples in the EPI group accounted for the significant island group differences: Tarawa (CV<sub>p</sub> = 5.65) and Fanning Island (CV<sub>p</sub> = 5.57). Thus, there was no compelling evidence for a consistent trans-equatorial increase in morphological variation for all four EPI samples. Finally, no latitudinal or longitudinal trends in CV<sub>p</sub> were detected; and it is concluded that morphological variability within the samples was not different or only slightly different among the locales sampled here. Similar results were obtained when variability was estimated in each sample as the mean coefficient of variation over the six meristic characters.

These results are somewhat dissimilar from the patterns of electrophoretic variability measured at 38 loci in the same Chanos samples (Winans, 1980). Although the estimates of the average heterozygosity per locus and the percentage of polymorphic loci (P<sub>0.90</sub> level) did not change significantly over the same region, the average number of alleles per locus and the percentage of polymorphic loci (P<sub>0.99</sub> level) decreased significantly from the west to the east (e.g., PHIL > EPI > HI). According to the mutation-drift hypothesis, the highest levels of genetic variability are found in regions of greatest effective population size (Nei, 1975). Whereas Chanos are distributed throughout the tropical, inshore marine habitats of the Pacific and Indian Oceans, the most abundant populations have been noted in a region of Southeast Asia that includes the Philippine Archipelago (Rabanal and Ronquillo, 1975). Therefore, if the mutation-drift hypothesis is applicable to these data, it is not surprising to find the highest levels of electrophoretic variability in the Philippine samples. And, by this reasoning, the paucity of electrophoretic variation observed in the most geographically peripheral samples, the HI and TAH samples (Winans, 1980) may also reflect a historical (founder effect) or a contemporary decrease in the effective population size of milkfish in these areas. The lack of a similar pattern in within-sample morphological variability suggests that relevant environmental or population parameters affecting variability did not vary systematically among the samples.

A principal component analysis of six meristic characters indicated little among-group differences, whereas a like analysis of morphometric characters separated milkfish samples by size on PC I and by shape on SPC I<sub>1</sub> (Fig. 3). Since size differences may reflect only sampling variation, and separation of fish by size can be achieved by eye without statistical analyses, the discrimination among samples by the body shape criterion is essentially the more useful result. In general, the Philippine samples were distinctive along the shape axis.

The repeat sample from the island of Hawaii (HAW2) provides an insight into shape differences along SPC I<sub>1</sub> since its analysis represents the only opportunity in the present study to view, in one location, the degree of between-year morphological variation in Chanos. The
principal component analysis of the morphometric data showed that there were considerable multivariate differences between the two samples along the first two PC axes (Fig. 3). Since the HAW2 fish were considerably larger than the HAW fish (mean fork length: 37 cm and 22 cm, respectively), the separation of HAW2 and HAW along the size-related axis was expected. The degree of separation along SPC II, a multivariate shape axis, was also large, indicating a considerable shape difference. Since primary emphasis is placed on shape discrimination among samples, further consideration of this observation is warranted.

There are several possible explanations for the shape difference between HAW and HAW2. Differences may be due to: 1) genetic differences between the samples, 2) morphological changes associated with yearly environmental changes and/or 3) shape changes associated with size differences. The electrophoretic analysis of these samples indicated that the fish did not come from dissimilar genetic stocks. Allele frequencies at the polymorphic loci were highly similar, as reflected in a small Nei's genetic distance value between the samples (Fig. 6). Thus, there is no reason, based on electrophoretic information, to expect morphological differences between HAW and HAW2. It may be argued that the fish in the two samples experienced different environmental variability during development and in response, have slightly altered morphological characteristics. Unfortunately, no precise data are available with which to assess this possibility directly. It is well established that meristic traits may vary as a function of a population's physical milieu (Hubbs, 1922; Barlow, 1961; Fowler, 1970). Since there were no meristic differences between HAW and HAW2—their confidence ellipses overlapped in Fig. 5—there is no reason to suspect, at least on this basis, that the two yearly samples experienced varying environmental conditions.

The third possibility, that the SPC II differences are predictable shape changes that accompany different size distributions, may be evaluated by considering the third Hawaiian sample OAH, the sample most genetically similar (by electrophoretic criteria) to HAW and HAW2. OAH, which had the smallest sized fish in the HI samples, had the lowest mean value along SPC II with respect to HAW and HAW2. In fact, the three Hawaiian centroids were linearly arranged along SPC II in a pattern corresponding to their mean fish size along PC I: the larger the fish, the greater the SPC II value (Fig. 3). With the available data, therefore, the best explanation for the relationship of HAW and HAW2 along SPC II is that there is a size-related or ontogenetic change in a body shape among the milkfish in Hawaii. Importantly, this finding points to the need for more complete sampling of fish (with respect to size) from each locale to completely understand how ontogenetic shape changes within each locale affect our evaluation of among-locale shape differences.

A comparison of the similarity in patterns of morphological and electrophoretic differentiation is depicted in Fig. 7. The pattern of morphological differentiation is a summary of the variation along the SPC II axis in Fig. 3. The pattern of electrophoretic differentiation is a
Fig. 7. Patterns of geographic variation in the milkfish *Chanos chanos*. Circles refer to the samples described in Fig. 1. Patterns represent: A) morphometric variation along the sheared principal component II axis in Fig. 3 (i.e., either positive or negative SPC II mean sample values) and B) electrophoretic similarity for 38 loci (Fig. 6).

summary of the single locus and multilocus analyses over 38 loci presented in Winans (1980) and here (Fig. 6). The dichotomous summary of the morphometric study basically divides the samples into Philippine and non-Philippine samples. The values for one Philippine sample (P6), Tahiti and Christmas Island, however, produce considerable heterogeneity in this simple model of morphological variation. On the other hand, the pattern of electrophoretic similarity reflects the geographic proximity of the samples, i.e., electrophoretic similarity coincides with archipelago boundaries (for the eight Philippine samples and the two Hawaiian samples) or across adjacent island groups (for the four equatorial Pacific Island samples plus Tahiti). In sum, both data sets indicate that the Philippine samples are different from the other samples; but, only in the electrophoretic data set are all eight Philippine samples one homogeneous group and the Hawaiian Island samples a distinctive group. Since environmental influences on morphological variation may be substantial but not assessable here, further evaluation of the levels and patterns of morphological characters requires repeat sampling, tabulation
and analysis of environmental data and/or breeding studies (Riddell et al., 1981; Todd et al., 1981). Since the expression of electrophoretic variation is virtually unaffected by environmental variation and the patterns of electrophoretic similarity follow patterns of geographic proximity, the electrophoretic results are presumed to reflect best the population level conditions affecting gene flow within and among island groups (Winans, 1980).

That there are both similarities and dissimilarities between the patterns of morphological and electrophoretic variation in these Chanos samples is not an unusual finding. Other work with a variety of vertebrate species has also shown an independence of morphological and molecular evolution (Cherry et al., 1978; Larson, 1980; Shaklee and Tamaru, 1981; Wake, 1981). What this independence of morphological and electrophoretic variation means therefore is that both character sets will be useful in future attempts to understand the evolution of this species and to manage milkfish stocks for aquaculture. Further studies of the relationships of these two character sets in milkfish will involve examining the association of electrophoretic phenotypes and bilateral symmetry of meristic characters with both the individual and population level (Vrijenhoek and Lerman, 1982; Leary et al., 1983).

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