A CRITIQUE OF SEROLOGICAL AND ELECTROPHORETIC STUDIES ON THE INDIAN OIL SARDINE AND MACKEREL

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A method found to have been applied of late for fish population studies is the use of electrophoretic, biochemical and serological techniques in the identification of genetic stock as the natural units of fish resources (deLigny 1969, 1971, Eckroat 1973, Iwata 1973, Mangaly and Jamieson 1978 and Grant and Utter 1980). The common goal of these investigations is to describe the genetic composition of the sample population in terms of observed gene frequencies or genotype proportions. A standard procedure followed in all these studies involves detection of one or more suitable gene-marker in the form of electrophoretic variants or serological polymorphs, calculation of gene-frequencies or genotype proportions in the tested samples containing statistically sufficient number of specimens, comparison of the observed distribution pattern of different phenotypes with that expected pattern calculated, on the basis of a proposed genetic model, and interpretation of significant gene-frequency differences or zygotic proportions at one or more loci as indicative of heterogeniety of populations tested. Jamieson (1974) has focussed high-lights on these points.

A critical examination of some reports on electrophoretic and serological studies on the Indian oil sardine, *Sardinella longiceps,* val and the mackerel, *Rastrelliger Kanagurta* (Cuvier), reveals that the authors (Dhulkhed and Nagesh 1976, Drulkhed and Rao 1976, Menezes 1980 and Rao and Dhulkhed 1976) have ignored the basic procedures mentioned above and consequently arrived at fundamentally wrong conclusions. As erroneous conclusions are bound to influence and mislead persons unfamiliar with biochemical genetic data, it is necessary to critically evaluate such reports.

Rao and Dhulked (1976) described two electrophoretic patterns of eye-lens proteins in both oil sardine and mackerel. The number of eye-lens studied in both species is only 10 each. In both the cases three-banded and four-banded types were reported. However, the number of each type was not given. Similarly, Menezes (1980) also described two electrophoretic patterns of eye-lens proteins in oil sardine collected from Panaji, Goa; pattern A with 7 fractions and pattern B with 9 fractions. The author has mentioned neither the total number of specimens tested nor the number of specimens under each pattern. Dhulkhed

and Rao (1976) have also reported electrophoretic patterns of serum proteins in both oil sardine and mackerel. The three patterns described in oil sardine consisted of A with 4, B with 5 and C with 6 components. The pattern in the mackerel was made of 4 and 5 components.

Rao and Dhulkhed (1976), Dhulkhed and Rao (1976) and Menezes (1980) had interpreted the observed differences in the total number of electrophoretic fractions as genetic variations and, therefore, concluded that the populations from which tested specimens were drawn were genetically different. The inferences made on the basis of unqualified pattern variations were "that the population of oil sardine and mackerel is heterogeneous. The presence of extra component among these fishes could reasonably be attributed to genetic differences" (Rao and Dhulkhed 1976, page 14). At the same time they considered that a detailed study was necessary "to establish the intraspecific differences" (Rao and Dhulkhed 1976 p. 15). Both these statements are selfcontradictory. A similar inference was made by Dhulkhed and Rao (1976) who stated that "those with four components differ grossly from those which have more", page 18. "The high uniformity in the patterns A and B ..... and the pattern heterogeneity having a genetic basis. This suggests that the specimens of *S. longiceps* used in this study would have probably come from two different breeding populations" (Menezes 1980 p. 185).

Rao and Dhulkhed (1976), Dhulkhed and Rao (1976) and Menezes (1980) drew fundamentally wrong conclusions from mere observations of simple electrophoretic variations in a few individual specimens tested. Because, apparent variation in total number of electrophoretic fractions need not imply or establish genetic variation in individuals as genetic heterogeniety at population level is an entirely different proposition. It is well established that when two electrophoretic variants are described as genetic, the product of such variants should produce one heterozygotic and two homozygotic patterns. No such products were either described or present in the reports examined here. Individual variation is natural in animal population and the phenomenon is know as polymorphism. However, all these variations cannot be easily explained in genetic terms. When such variations, like certain electrophoretic variants, can be explained by simple genetic model, the phenomenon qualifies to be called genetic polymorphism. Naturally, individuals of a species must have different genotypes at a particular locus and these genotypes will have definite proportions within the population (Hardy-Wienberg law). Therefore, it is unscientific to interpret all electropheretic variations in a few specimens as genetic without presenting required basic data to explain the observed variations. On the other hand, even if the above-examined electrophoretic variants are proved to be genetic, it is again basically wrong to tag these different variants or genotypes as representatives of genetically different populations. As mentioned

earlier, differentiation of two populations must arise from the significant differences in the proportions of such genotypes or gene-frequencies between two populations.

In genetic stock analysis, incorporation of densitometric data along with electrophoretic data has no special implication. Though differences in the total number of electrophoretic fractions and densitometric readings are sufficient to identify and seperate two species or subspecies with similar and confusing morphometric cahartceristics, these values have no immediate application in differentiating genetic populations or stocks within a species.

It is rather confusing to note that Rao and Dhulkhed (1976) reported 3-4 eye-lens protein fractions in oil sardine, whereas Menezes (1980) found 7-9 fractions. On the contrary, Menezes (1980) mentions that no such variation was observed in her similar previous studies. Cellogel was used as the supporting electrophoretic medium by both the authors. A standard electrophoretic investigation of a large number of oil sardine of all available sizes at different maturity stages alone will give some clue to the nature of this protein, as to whether the observed variations are due to ontogenic or non-genetic factors.

Dhulkhed and Nagesh (1976) also reported results of serological studies on oil sardine and mackerel. Three blood types, A, AB and O were detected in both the species and the total number of specimens under each group was also given. Here again, the authors' conclusion that "the blood groups A, AB and O indicated the existence of genetically different groups of oil sardine and mackerel" (Dhulkhed and Nagesh 1976, page 9) is no less ambiguous than that of other reports examined here. The authors have apparently mistaken individual blood types for population differences. It was wrongly mentioned that "oil sardine belonging to groups A and B" were more in Mangalore area. (The correct groups to be indicated were A and AB, because B type was not detected in the test). The authors have endeavoured to justify their conclusion saying that authors like Vrooman (1964) have differentiated three Pacific sardine subpopulations on the basis of distribution of antigen "frequencies". It is noticeable that Vrooman (1964) tested no less than 2844 sardine from 25 samples, each of which contained about 100 specimens, and calculated that mean frequency for C-positive blood group alone was highly significant between two populations. Moreover, results of previous conventional studies on the species had already established the existence of more than one sub-population. It may not be proper to explain our data in the same line as that of Vrooman (1964). Besides, a few questions naturally arise here in the light of the facts mentioned on page 10 of Dhulkhed and Nagesh (1976), namely, "weak and doubtful agglutinations were not taken into considerations". It is important to know that how many specimens were excluded or not considered on account of weak and doubtful

agglutinations. If all the excluded specimens in an apparently small sample were able to be typed, how far it would have affected the values reported? When about 17 per cent of total number of different antigens reported was B, why B blood group was not present in both the species? It is known that in blood typing, particularly in fishes, faulty techniques or poor test sera can cause unrealistic classification of blood groups (Marr and Sprague 1963 and Ridgeway 1971), or, in some species, ontogenetic and ecological effect on blood types also cannot be ruled out (deLigny 1972).

In conclusion, the electrophoretic and serological data examined here have been wrongly interpreted by the authors and hence their suggestive conclusions are erroneous and misleading.

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