

Biochemical genetic divergence in three carangids from the Andaman Sea

Maria R. Menezes

National Institute of Oceanography, Dona Paula, Goa 403 004, India

Genetic divergence and phylogenetic relationships among *Decapterus russelli*, *Selaroides leptolepis* and *Selar crumenophthalmus* were investigated by examining the electrophoretic patterns of seven enzymes, sarcoplasmic proteins and haemoglobins. The allele frequencies of 15 loci were estimated to calculate the genetic distances (*D*). The three carangid species were clearly divided into two groups at a *D* value of 1.72.

THE technique of starch gel electrophoresis of enzymes with specific staining for the activity in the gel, the so-called zymogram method developed by Hunter and Markert¹, is useful in many research applications. Screening studies and comparing a relatively large number of enzymes among a variety of tissues and organisms, are finding increasing use in research on population genetics, taxonomy, etc.² Such molecular variation is known to be genetically controlled and codominant³ and on the whole only slightly affected by environmental disturbances⁴.

Cumulative comparisons among loci between taxonomic groups can be summarized by a variety of methods into indices of similarity or conversely, genetic distance⁵. Genetic distance (*D*) is a measure of the number of allelic substitutions per locus that have occurred during separate evolution of two populations or species (which is detected roughly as differences in electrophoretic mobility of the corresponding enzymes). The basis of the measure is a so-called 'normalized identity'⁶ which expresses the probability that a

randomly chosen allele from each of two different populations will be identical relative to the probability that two randomly chosen alleles from the same population will be identical.

This paper describes an electrophoretic investigation into species relationships and levels of genetic distance in three members of the carangid family. The carangid fishes are widely distributed in the temperate and tropical seas around the world and occur along the east and west coasts of India supporting fisheries almost round the year⁷.

Methods

Three carangid species—*Decapterus russelli* (Ruppell), *Selaroides leptolepis* (Cuv) and *Selar crumenophthalmus* (Bloch)—were collected from Aberdeen jetty, Port Blair, Andaman, during the 41st cruise of *ORV Sagar Kanya* in April/May 1988. The specimens were kept frozen until tested.

The skeletal muscles, heart and eyes were dissected from individual specimens. The cell-lysate obtained by freezing and thawing was subjected to electrophoresis for phenotypic analysis. Starch gel electrophoresis was carried out by the procedure reported earlier^{5,8}. Two buffer systems were used: Tris-citric acid (T-C) pH 8.0 and citric acid-aminopropylmorpholine (C-APM) pH 6.0 (ref. 9).

The list of enzymes, tissue-specificity and loci are given in Table 1. When multiple loci was coded for an

Table 1. List of enzymes examined, loci identified and tissue assayed.

Enzyme	Locus	Buffer	Tissue assayed
α -Glycerophosphate dehydrogenase (α -Gpd)	α -Gpd	C-APM	Skeletal muscle
Lactate dehydrogenase (LDH)	Ldh-1	C-APM	Eye
	Ldh-2	C-APM	Heart
	Ldh-3	C-APM	Skeletal muscle
Malate dehydrogenase (MDH)	Mdh-1	C-APM	Skeletal muscle
	Mdh-2	C-APM	Heart, skeletal muscle
Fumarate hydratase (FM)	Fm	T-C	Skeletal muscle
Isocitrate dehydrogenase (IDH)	Idh	T-C	Skeletal muscle, heart
Malic enzyme (ME)	Me-1	T-C	Skeletal muscle
	Me-2	T-C	Heart, skeletal muscle
Creatine kinase (CK)	Ck	C-APM	Skeletal muscle
Sarcoplasmic protein (SP)	Sp-1	C-APM	Skeletal muscle
	Sp-2	C-APM	Skeletal muscle
Haemoglobin (Hem)	Hem-1	T-C	Heart
	Hem-2	T-C	Heart

enzyme, the locus with the most anodal migration was designated one, the next as two, and so on. Allele frequencies at each locus (i.e. the banding position of isozymes on the same gel) in the three species were calculated by a simple gene counting method assuming that a protein with the same mobility was controlled by the same allele even in different species, and that variations within species were controlled by two or more codominant alleles¹⁰. From the gene frequency data thus obtained, the genetic similarity (I) and genetic distance (D) along with standard error (SE) were calculated between every pair of the species compared. The Nei measures¹¹ ($D = -\log_e I$, and $SE = [(1-I)/(ns)]^{1/2}$ where ns is the number of proteins examined. Nei defined the genetic similarity (I) or the normalized identity of genes between the j th and k th taxa, I_{jk} as:

$$I_{jk} = \frac{\sum_i q_{ij}q_{ik}}{(\sum_i q_{ij}^2 \cdot \sum_i q_{ik}^2)^{1/2}}$$

where q_{ij} and q_{ik} are the frequencies of the i th allele at a locus in the j th and k th taxa, respectively, and the averages are over all the gene loci examined. Nei¹² proposed a formula for estimating the phylogenetic divergence time (t years) from D i.e.: $t = 5 \times 10^6 D$. The above estimations of D and t are based on the assumption that the amino-acid substitution rate is constant among different proteins. The dendrogram was drawn by the unweighted pair-group method (UPGMA)¹³.

Results

Figure 1 shows a comparison of the electrophorograms of isozymes among *D. russelli* (nos. 1, 3, 4, 6, 7, 9 and 10); *S. leptolepis* (nos. 2 and 8) and *S. crumenophthalmus* (no. 5). The patterns clearly showed similarities and differences among the genera in the positions of the homopolymeric band for each isozyme locus. The electrophoretic pattern of seven enzymes, sarcoplasmic proteins and haemoglobins enabled identification of 15 separate loci (Table 1). These stable and clear isozymes, in all the three species, were used as genetic markers. The system of nomenclature to designate the electrophoretically-detected loci and alleles was adopted from Allendorf and Utter². Table 2 shows the alleles and allele frequencies at each of the 15 loci for the three carangid species. Allelic variants were designated according to their relative electrophoretic mobility. The most common allele was designated 100 and the others were given numbers that indicate their mobility relative to that of the common allele. Cathodal systems were similarly designated but given a negative sign. Almost

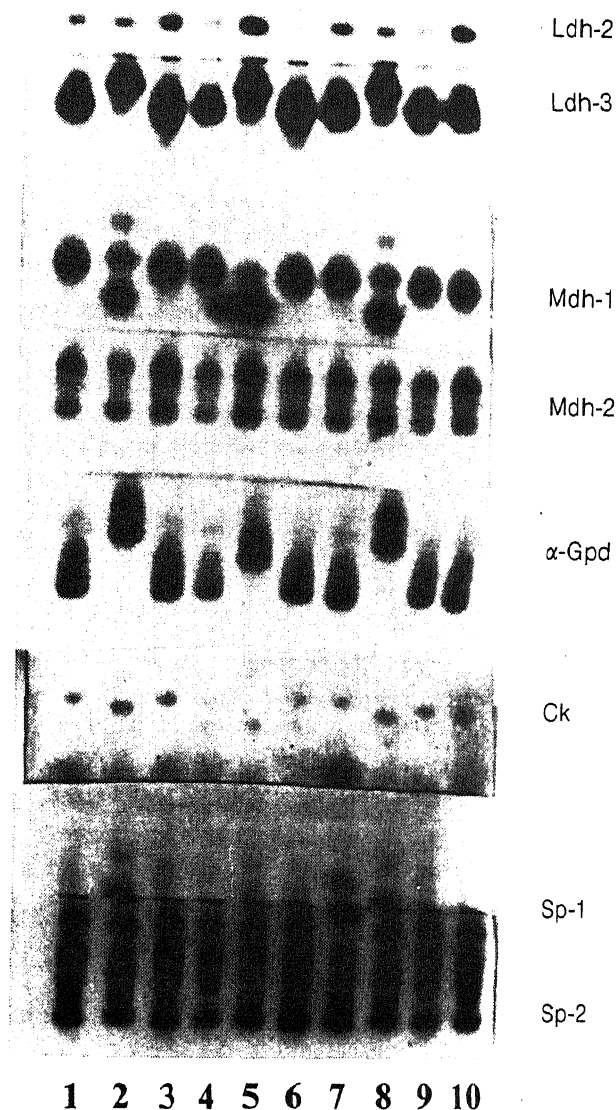


Figure 1. A comparison of isozymes of the carangid species. Nos. 1, 3, 4, 6, 7, 9 and 10—*D. russelli*; nos. 2 and 8—*S. leptolepis*; No. 5—*S. crumenophthalmus*.

all the loci were monomorphic and fixed in an allele. When the allele frequencies were compared between every two species, a large number of divergent loci were found in more distantly related species pairs.

To estimate the degree of genetic divergence among the three species, the value of D along with SE was calculated¹¹ between every pair of species using the allele frequencies shown in Table 2. The D value became zero when the allele frequencies of the two species compared were completely identical, and infinite when the allele frequencies of the two species compared were completely divergent, (i.e. they do not share any common allele¹¹). Matrices of Nei's genetic distance between pairs of the three species given in Table 3 show that the amount of genetic divergence between *S. leptolepis* and *S. crumenophthalmus* is about 27% [$= 0.470/(1/2)(2.066 + 1.373)$] of that between these two species and *D. russelli* using the genetic distance values. These distances can be converted into the divergence time (Table 3). Nei's genetic distance measurements

Table 2. Allele frequencies at 15 loci in three carangid species.

Locus	Allele	Species		
		Dr(22)	Sl(6)	Sc(2)
α -Gpd	185	0.0	1.0	0.0
	154	0.0	0.0	1.0
	100	1.0	0.0	0.0
Ldh-1	124	1.0	0.0	0.0
	100	0.0	1.0	1.0
Ldh-2	100	1.0	1.0	1.0
Ldh-3	-60	1.0	0.0	0.0
	-100	0.0	1.0	1.0
Mdh-1	160	0.0	1.0	0.0
	100	1.0	0.0	1.0
Mdh-2	-100	1.0	1.0	1.0
Ck	150	1.0	0.0	0.0
	125	0.0	1.0	0.0
	100	0.0	0.0	1.0
Fm	120	0.0	1.0	0.0
	180	0.0	0.0	1.0
	100	1.0	0.0	0.0
Idh	135	0.0	1.0	0.0
	100	1.0	0.0	1.0
Me-1	100	1.0	0.0	0.0
	90	0.0	1.0	1.0
Me-2	100	1.0	0.0	0.0
	77	0.0	1.0	1.0
Sp-1	-40	0.318	0.0	0.0
	-80	0.682	0.0	0.0
	-100	0.0	1.0	1.0
Sp-2	-100	0.0	1.0	1.0
	-105	1.0	0.0	0.0
Hem-1	141	1.0	0.0	0.0
	100	0.0	1.0	1.0
Hem-2	100	0.0	1.0	1.0
	-75	1.0	0.0	0.0

Figures in parentheses show numbers of individuals examined. Dr, *Decapterus russelli*; Sl, *Selaroides leptolepis*; Se, *Selar crumenophthalmus*.

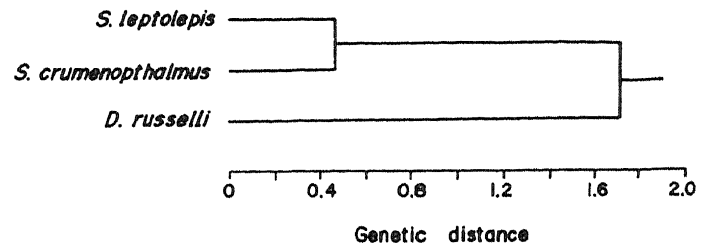
Table 3. Estimates of genetic distance (D) along with standard error between three carangid species. Values above the diagonal are estimates of D , those below are estimates of divergence time (in million years).

Species	1	2	3
<i>Selaroides leptolepis</i>	—	0.470 \pm 0.258	2.066 \pm 0.874
<i>Selar crumenophthalmus</i>	2.35 \pm 1.29	—	1.373 \pm 0.573
<i>Decapterus russelli</i>	10.33 \pm 4.37	6.87 \pm 2.86	—

have the advantage that they vary linearly with the phylogenetic divergence time¹⁰.

To estimate the relationships among the three genera, a dendrogram was drawn based on the genetic distances (Figure 2). From this dendrogram, the three species were clearly divisible into two major groups at a distance of 1.72. One of the groups had *S. leptolepis* and *S. crumenophthalmus* while the other group consisted of *D. russelli*.

Biochemical diversity measured as genetic distance was reported among species in many organisms¹⁴. Nei¹⁵ estimated that for local races of a species, D

**Figure 2.** Dendrogram showing the relationship among the 3 carangid species based on values of genetic distance.

ranges from nearly 0 to 0.05; for subspecies $D=0.02$ to 0.2. For full species, $D=0.10$ to 2.0; sibling species, which are morphologically nearly identical, generally have D values two to three times smaller than the D s between morphologically distinct species. D is usually greater than 1.0 for genera. In Carangidae, collected from Okinawa and Kochi, Japan, Kijima *et al.*¹⁶, using 6 enzymes and 9 loci coding for them, indicated the average genetic distance as 2.20 between *D. russelli* and *S. leptolepis*; 1.64 between *D. russelli* and *S. crumenophthalmus* and 0.79 between *S. leptolepis* and *S. crumenophthalmus*. In sparid fish, the average genetic distance was reported as 0.002 between conspecific subpopulations, 0.115 between congeneric species, 0.842 between consubfamilial genera and 1.273 between subfamilies¹⁷. In Sciaenidae⁵, the average genetic distance varied between 0.0017 and 0.039 for conspecific subpopulations, 0.092 between two species of *Nibeia* and 1.212 between consubfamilial genera. Relationships between standard genetic distances observed for various taxonomic categories are about as expected. Higher categories are on the average more different than lower ones. The present estimates of genetic distance are more or less similar to that of Kijima *et al.*¹⁶ and show that the structural gene difference between *Selaroides leptolepis* and *Selar crumenophthalmus* is quite small. In estimating genetic distance a large number of loci rather than a large number of individuals per locus should be used when the total number of genes to be examined is fixed¹⁸. Ideally, more than 50 loci should be used to obtain reliable estimate¹⁸. In practice, technical difficulties often limit the number of loci studied. In fact, less than 30 loci have been studied in most protein surveys. A large number of individuals must be examined if one wants to study the allele frequency distribution for each locus.

The rapidly expanding application of multilocus electrophoretic techniques to the analysis of systematic and evolutionary problems is transforming the taxonomic work. Building up of a systematic arrangement more consistent with the genetic relationships of organisms is expected as a major result of this new approach¹⁹.

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Effect of Japanese encephalitis virus-induced suppressor cells on Fc-receptor function of mouse macrophages

Shashi Rawat, Asha Mathur, R. Kulshreshtha and U. C. Chaturvedi

Postgraduate Department of Microbiology, K. G. Medical College, Lucknow 226 003, India

During Japanese encephalitis virus (JEV) infection Fc-receptor-mediated attachment of opsonized sheep erythrocytes (EA) by macrophages was significantly depressed. The capacity of macrophages to attach EA reached the lowest point on day 18 following intraperitoneal inoculation of JEV and then recovered gradually, but remained less than the controls. We have observed earlier that T lymphocytes of JEV infected mouse spleen produce a suppressor factor on the 18th day p.i. The present study shows that JEV-induced suppressor cells have a variable effect on the activity of macrophages. The second generation of the suppressor T cells (Ts_2) significantly depressed the Fc-receptor-mediated attachment of EA. Further simultaneous administration of JEV and suppressor factor (SF) resulted in early depression in macrophage Fc-receptor-mediated attachment of EA compared to JEV alone. Thus SF appears to protect the cells by inhibiting the entry of virus into macrophages.

MACROPHAGES play an important role in the immune response to an antigen. The various functions of macrophages are regulated by extracellular signals that operate via different types of receptors present on the surface of macrophages. Mouse macrophages have four types of receptors for the Fc portion of immunoglobulin

and are controlled by two genes^{1,2}. The extracellular signals can increase the Fc-receptor-mediated activity, as has been demonstrated with lymphokines³ and gamma interferon⁴, or decrease them as has been observed with prostaglandins and macroglobulin protease complexes⁵.

We have earlier reported that JEV induces generation of suppressor cells for the humoral and cell-mediated immune responses during the 3rd week of infection which mediate suppression through suppressor factor^{6,7}. The live macrophages are obligatory for the transmission of the suppressor signal⁸. In the present study we have studied the Fc-receptor-mediated function of macrophages in JEV-infected mice and the effect of suppressor cells/suppressor factor on them. The findings show that during JEV infection the Fc-receptor-mediated functions are depressed due to the generation of suppressor pathway.

Methods

Preparation of suppressor cells (Ts_1 population). Two-to-three months old inbred Swiss albino mice from the