BIOCHEMICAL GENETICS OF PACIFIC BLUE MARLIN, *MAKAIRA NIGRICANS*, FROM HAWAIIAN WATERS[1]

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ABSTRACT

An electrophoretic survey of 35 enzyme-coding gene loci in Pacific blue marlin was accomplished to determine levels of genetic variation and the feasibility of using electrophoresis to study stock structure in this species. Polymorphism ($P_{.99}$) in the marlin was 0.26 and the average heterozygosity (H) was 0.06. Allele frequencies at 11 variable loci were determined for a sample of 95 fish from Kona, Hawaii. The observed levels of polymorphism and heterozygosity suggest that a biochemical genetic analysis of blue marlin stock structure is possible and may reveal stock heterogeneity.

The Pacific blue marlin, *Makaira nigricans*, is the predominant billfish species in the central tropical Pacific. As such, it is an important commercial species and the object of a considerable sport fishery. The average annual catch of this species in the Pacific exceeds 14,000 t (metric tons) (Shomura 1980). The Pacific blue marlin is primarily distributed in equatorial areas, although Japanese longliner catch records indicate that its range extends from lat. 48°N to 48°S. During the Southern Hemisphere summer (December through March) a center of concentration occurs in the western and central South Pacific (between lat. 8°S and 26°S). In the Northern Hemisphere summer (May through October) a center of concentration occurs in the central North Pacific (between lat. 2°N and 24°N). During April and November the fish appear to be concentrated equatorially between lat. 10°N and 10°S (Rivas 1975). There is currently no direct evidence of migration of blue marlin within the Pacific. However, a general movement to the northwestern Pacific during the Northern Hemisphere summer and to the southeastern Pacific during the Southern Hemisphere summer has been postulated by Howard and Ueyanagi (1965) on the basis of the shifting abundance patterns of the fish.

Little is known about spawning, other than that Pacific blue marlin appear to spawn throughout the year in an area 10°-20° on either side of the Equator, and up to 30° on either side of the Equator during the Northern and Southern Hemispheres' respective summer months. In general, the highest spawning densities occur in the western Pacific, with the density decreasing eastward (Strasburg 1970; Matsumoto and Kazama 1974; Rivas 1975). Because of the apparently single equatorial Pacific spawning area, it has been assumed that the species consists of a single unit stock (Yuen and Miyake 1980; Yoshida 1981), yet there has been no direct test of this assumed stock structure. The most recent report available on the condition of the Pacific blue marlin stock considers it to be badly overfished. Yuen and Miyake (1980) calculated that the present fishing effort (commercial longliner effort only, since no data are available on recreational fishing effort) is about twice that suitable for maximum sustainable yield. Because the catch per unit effort of Pacific blue marlin has steadily declined over the past 10 yr, in spite of a fairly constant level of effort, Yuen and Miyake (1980:19) concluded "...that continued fishing at high levels will continue to reduce the abundance of the stock and a recruitment failure will become a distinct possibility."

The importance of being able to define subpopulations or stocks of fishes with respect to the formulation of appropriate fishery management schemes has long been recognized (Marr 1957). This problem is especially acute for species (such as Pacific blue marlin) which are highly migra-

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tory, subjected to an oceanwide multinational fishery, and which, because of relatively low catches, are not well suited to tag-recapture studies. In fact, the pressing need to understand blue marlin stock structure has been recognized for some time (Shomura 1980; Yoshida 1981).

The electrophoretic analysis of protein polymorphisms in natural populations can be a powerful approach for analyzing genetic aspects of population structure in sexually reproducing organisms. For this reason, the technique has been applied to the study of racial or subpopulation differentiation in numerous invertebrates and vertebrates (Ayala 1976). Because of the basic importance of information on subpopulation or stock structure to fisheries management (Berst and Simon 1981), population genetic studies have been conducted for many species of fishes [reviewed by de Ligny (1969) and Allendorf and Utter (1979)]. Most of the fishes investigated to date have been freshwater species or marine forms which are either inshore shallow-water species or demersal species.

Stock heterogeneity for oceanic species has not generally been reported (but see Fujino 1976; Fujino et al. 1981). Although open water, pelagic species *may* be characterized by large panmictic cosmopolitan populations, this pattern has not yet been clearly established. One problem in testing this hypothesis has been the unusually low levels of genetic variability observed to date in several large marine vertebrates such as skipjack tuna (Fujino 1970) and seals (McDermid et al. 1972; Bonnell and Selander 1974). Indeed, Selander and Kaufman (1973) have even suggested that large, mobile vertebrates may generally have low levels of heterozygosity—a characteristic which, if true, would preclude definitive stock analysis using electrophoretic techniques (but see Ryman et al. 1980). The general lack of progress in defining stock structure in oceanic fishes, such as scombroids, using electrophoretic methods, is attributable to several factors. Many of the reports in the literature have been preliminary in nature dealing with small samples of fish and few variable loci. Although such small sample sizes are not unexpected given the remote, far-seas nature of many of the commercial fisheries, they severely limit the subsequent statistical treatment of the data. Similarly, the analysis of only one or two polymorphisms reduces the likelihood of demonstrating any population subdivision which may exist. Finally, the schooling and/or highly migratory nature of many of these fishes makes it difficult to plan and execute adequate sampling programs.

The study described in the present report was designed to determine the suitability of utilizing electrophoretic techniques to study stock structure in the Pacific blue marlin. Three specific questions were addressed:

1) How much and what kind of electrophoretically detectable genetic variation is there in the Pacific blue marlin? Specifically, is there enough genetic variation to allow an electrophoretic analysis of stock structure in this species?
2) What combinations of enzymes, tissues, and buffer systems can be utilized in a study of genetic variation in this species?
3) What allele frequency distributions characterize the population of Pacific blue marlin in Hawaii?

MATERIALS AND METHODS

Muscle, liver, heart, eye, and brain samples were dissected from Pacific blue marlin landed at the Hawaiian International Billfish Tournament held at Kailua-Kona, Hawaii, in August 1980. All tissue samples were taken immediately after each fish had been weighed, and all fish had been dead for at least 1 h but <8 h. The dissected tissues were initially placed on ice and subsequently transferred to a freezer within 12 h. The time delay between fish capture and the freezing of dissected tissues did not seem to adversely affect any of the polymorphic enzymes screened with the possible exception of L-iditol dehydrogenase which could only be scored in 84 of the 95 fish analyzed. Tissues were stored frozen at −20°C until extracted.

Tissue extracts were prepared by homogenization using a loose-fitting, motorized stainless steel pestle in polycarbonate centrifuge tubes. The extraction buffer consisted of 0.1M Tris-HCl pH 7.0 containing 1×10^{-3}M EDTA and 5×10^{-5}M $NADP^+$. After homogenization, the extracts were centrifuged at $25{,}000 \times g$ for at least 30 min. Supernatants were transferred to individually labeled glass vials, capped, and stored at −75°C until the electrophoretic analysis was completed.

The supernatants were subjected to horizontal starch gel electrophoresis (modified from Selander et al. 1971), using some 15 different buffers. The gels were made using Lot 60F-0558 starch

(Sigma Chemical Co., St. Louis, Mo.) at a concentration of 12% w/v. After electrophoretic separations, enzyme patterns were visualized using standard histochemical staining recipes modified from Shaw and Prasad (1970), Selander et al. (1971), and Siciliano and Shaw (1976). All zymograms were photographically recorded.

Patterns of enzyme variation which were consistent with the subunit structure of the enzyme (when known) and simple models of Mendelian inheritance were scored and recorded as genotypes. Names of enzymes and Enzyme Commission numbers follow the recommendations of the Commission on Biochemical Nomenclature (1973). For multilocus enzyme systems, loci were given alphabetic designations when appropriate (e.g., Gpi-A) or were simply assigned a number beginning with 1 for the most anodally migrating isozyme. The most common allele at each locus was designated 100, and all other alleles at that locus were numbered according to their electrophoretic mobility relative to the 100 allele. Negative numbers refer to alleles with cathodal migration. The putative genotype data were summarized as genotype and allele frequency distributions. The genotype distributions were examined for internal consistency with the Mendelian inheritance model by chi-square testing of goodness-of-fit of observed genotype ratios with those expected for a single random mating population in the absence of differential selection among the alleles. The expected ratios were computed from observed allele frequencies using Levene's (1949) unbiased method for small samples. Heterozygosity for each locus (h) was calculated as $h = 1 - \Sigma Xi^2$ where Xi is the frequency of the ith allele. Average heterozygosity (H) was calculated as the mean of h over all loci examined.

RESULTS

Tissue samples from 95 Pacific blue marlin were analyzed. A total of 23 enzyme systems representing 35 gene loci were satisfactorily resolved using extracts of muscle, liver, and eye (Table 1). Heart and brain tissue did not add significantly to this total. Eleven loci exhibited detectable genetic variation in the sample of 95 fish analyzed. The enzymes adenosine deaminase (Ada), mannosephosphate isomerase (Mpi), and phosphoglucomutase (Pgm) all behaved as monomers with two-banded heterozygotes. Aspartate aminotransferase (Aat-1), alcohol dehydrogenase (Adh), glucosephosphate isomerase (Gpi-A), muscle glycerol-3-phosphate dehydrogenase (G-3-Pdh-2), liver isocitrate dehydrogenase (Idh-1), phosphogluconate dehydrogenase (Pgdh), and umbelliferyl esterase (Umb) behaved as dimers exhibiting triple-banded heterozygous patterns. L-iditol dehydrogenase (Iddh), often referred to as sorbitol dehydrogenase in the literature, appeared to be a tetramer as heterozygotes exhibited a five-banded phenotype.

Two of the 11 variable loci were represented by only a single heterozygous individual out of the 95 fish screened. The remaining nine loci were

TABLE 1.—Electrophoretic analysis of *Makaira nigricans* from Hawaii. M = muscle, E = eye, L = liver.

Enzyme			Loci	
Name (Enzyme Commission number)	Abbr.	Tissue	Invariant	Variable
aspartate aminotransferase (2.6.1.1)	Aat	L	1	1
adenosine deaminase (3.5.4.4)	Ada	M	—	1
alcohol dehydrogenase (1.1.1.1)	Adh	L	—	1
creatine kinase (2.7.3.2)	Ck	M+E	2	—
enolase (4.2.1.11)	Eno	M	1	—
esterase (3.1.1.—)	Est	L	2	—
glyceraldehyde-phosphate dehydrogenase (1.2.1.12)	Gapdh	M	2	—
glutamate dehydrogenase (1.4.1.2)	Gdh	L	1	—
glucosephosphate isomerase (5.3.1.9)	Gpi	M	1	1
glycerol-3-phosphate dehydrogenase (1.1.1.8)	G-3-Pdh	M+L	1	1
hexose diphosphatase (3.1.3.11)	Hdp	L	1	—
L-iditol dehydrogenase (1.1.1.14)	Iddh	L	—	1
isocitrate dehydrogenase (1.1.1.42)	Idh	M+L	1	1
lactate dehydrogenase (1.1.1.27)	Ldh	M+E	3	—
malate dehydrogenase (1.1.1.37)	Mdh	M	3	—
malate dehydrogenase (NADP$^+$) (1.1.1.40)	Mdh(Nadp$^+$)	M	1	—
mannosephosphate isomerase (5.3.1.8)	Mpi	M	—	1
peptidase (3.4.11.—)	Pep	M	2	—
phosphogluconate dehydrogenase (1.1.1.44)	Pgdh	M	—	1
phosphoglucomutase (2.7.5.1)	Pgm	M	—	1
superoxide dismutase (1.15.1.1)	Sod	L	1	—
umbelliferyl esterase	Umb	M	—	1
xanthine dehydrogenase (1.2.1.37)	Xdh	L	1	—

polymorphic by the normal criteria (common allele at a frequency of 0.99 or less) with five loci (Ada, Mpi, Pgdh, Pgm, and Umb) having the most common allele at a frequency of between 0.95 and 0.99 and four loci (Aat-1, Adh, G-3-Pdh-2, and Iddh) having the most common allele at a frequency of <0.95 (Table 2). All 11 variable loci exhibited two or three alleles except for Aat-1 which had five different alleles, three of which were reasonably common.

Heterozygosity values for the individual loci ranged from zero for all of the apparently monomorphic loci to 0.494 for G-3-Pdh-2. The average heterozygosity (H) across all 35 loci was 0.0605.

Where possible, the observed genotype distributions were tested for goodness-of-fit to Hardy-Weinberg equilibrium expectations (Gpi-A, Idh-1, Mpi, and Pgm were not tested because of the very small number of observed variants in the sample). Where necessary, rare alleles were pooled prior to the tests. All tests were nonsignificant except that for Adh ($\chi^2 = 6.97$, df = 1; $P<0.01$) where there was a significant deficiency of heterozygotes.

Further analysis of the Adh data was undertaken to attempt to identify the major contributor(s) to this significant chi-square value. Since sex linkage of a locus can result in a deficiency of heterozygotes, the sample of blue marlin was subdivided into males and females. A chi-square test of the Adh genotypes of the 81 male fish also revealed a significant deficiency of heterozygotes ($\chi^2 = 9.36$, df = 1; $P<0.005$). Another possible source of the deficiency of heterozygotes could be the pooling of different year classes which actually had different frequencies of the Adh alleles. Indeed, year class fluctuations of allele frequency have been reported in other fishes (Williams et al. 1973; Mitton and Koehn 1975; Smith et al. 1978; Smith 1979). In the absence of growth data for this species, the only subdivision we could make was on the basis of size. The 95 blue marlin were subdivided into two groups: 1) 100-200 lb total weight and 2) 201-450 lb. There were 74 fish in the 100-200 lb group and the statistical analysis of this group once again revealed a deficiency of heterozygotes ($\chi^2 = 9.95$, df = 1; $P<0.005$). The similarity of the results for small fish and for males is not unexpected since all of the female fish ($N = 13$) were in the large size class (>200 lb). Therefore, the deficiency of heterozygotes seems to characterize the overall sample and cannot be attributed to sexual or gross age (= size) differences.

TABLE 2.—Allele frequencies and heterozygosities for 11 variable loci in *Makaira nigricans* from Hawaii.

Locus[1] (heterozygosity)	Allele	Frequency
Aat-1	250	0.016
(h = 0.4497)	145	0.145
	100	0.720
	27	0.102
	−30	0.016
Ada	107	0.005
(h = 0.0716)	100	0.963
	92	0.032
Adh	−220	0.344
(h = 0.4556)	−100	0.656
Gpi-A	100	0.995
(h = 0.0099)	86	0.005
G-3-Pdh-2	100	0.595
(h = 0.4944)	75	0.389
	60	0.016
Iddh	147	0.006
(h = 0.4219)	100	0.702
	22	0.292
Idh-1	100	0.995
(h = 0.0099)	84	0.005
Mpi	104	0.005
(h = 0.0316)	100	0.984
	90	0.011
Pgdh	155	0.016
(h = 0.0529)	100	0.093
	67	0.011
Pgm	144	0.011
(h = 0.0316)	100	0.984
	33	0.005
Umb	100	0.953
(h = 0.0896)	87	0.047

[1]Sample size = 95 fish for each locus except for Mpi where $N = 94$, Aat-1 and Adh where $N = 93$, and Iddh where $N = 84$.

DISCUSSION

In spite of the low levels of genetic variation reported in the literature for skipjack tuna (Fujino 1970; Fujino et al. 1981; Lewis 1981; Richardson in press) and suggested by Selander (1976:34) that "...levels of variability are unusually low in large marine vertebrates such as tuna fish and porpoises," the above data clearly indicate that the Pacific blue marlin does not have abnormally low levels of genetic variation. The level of polymorphism ($P_{.99}$) observed for marlin in the present study ($P = 0.26$; i.e., 9 out of 35 loci), although slightly lower than the average for fish ($P = 0.31$) reported by Selander (1976), is higher than the averages for reptiles, birds, and mammals (0.23, 0.15, and 0.23, respectively). Furthermore, the average heterozygosity (H) of 0.0605 for the 35 loci screened in the Pacific blue marlin is greater than the average of 0.0494 calculated by Nevo (1978) for 135 species of vertebrates and the average of 0.0478 calculated by Winans (1980) for 82 species of fishes. Although perhaps somewhat un-

expected, the relatively high level of genetic variation reported above for blue marlin is not unique among large marine vertebrates as several other scombroid fishes (e.g., white marlin, southern bluefin tuna, and Spanish mackerel) exhibit similar or even higher levels of variation (Edmunds 1972; Smith and Jamieson 1980; Lewis 1981; Shaklee unpubl. data).

The observed pattern of genetic variation can be used to subdivide the 11 variable loci into two general categories. Four loci (Aat, Adh, G-3-Pdh-2, and Iddh) form one group characterized by high heterozygosities (0.4219-0.4944) due to the presence of at least two relatively common alleles. The second group, which is composed of the remaining seven variable loci (Ada, Gpi-A, Idh-1, Mpi, Pgdh, Pgm, and Umb), is characterized by low heterozygosity per locus (0.0099-0.0896) and the presence of a single common allele at a frequency of at least 0.95. In reality, both groups of loci are of utility in population analyses because the power of the statistical tests for detecting significant differences in allele frequency between pairs of samples actually increases somewhat as the frequencies approach the extremes (i.e., 0 and 1.0) compared with samples having frequency distributions close to 0.5.

The close agreement between observed and expected genotypic frequencies for all but one of the variable loci is consistent with all 95 fish analyzed belonging to a single panmictic population. However, the significant ($P<0.01$) deficiency of heterozygotes observed at the Adh locus is not. Although this observation may be due to selection or simply be an anomaly, another potential explanation is that this heterozygote deficiency is due to the mixing of two or more different stocks of blue marlin which have different frequencies of the two Adh alleles (Wahlund effect). Such potential stock mixing would not be unreasonable given the presumed migratory nature of Pacific blue marlin.

The fundamental significance of the above observed levels of genetic variation is that they are adequate to allow a biochemical genetic analysis of stock structure in Pacific blue marlin. We are now in the process of initiating just such an analysis and are employing an experimental design which should allow us to detect stock heterogeneity which has either a stable geographical basis or a temporally shifting geographic basis. The former basis for stock heterogeneity, namely the localization of two or more stocks in different regions of the species' range is by far the most commonly observed form of population subdivision among organisms. We will be testing for this type of heterogeneity by analyzing samples from different localities (Hawaii, Guam, Samoa, etc.) throughout the range of the Pacific blue marlin. However, this type of analysis is complicated by limited access to marlin caught in many areas of the range, by the shifting patterns of abundance which characterize this species, and by the virtual impossibility of obtaining simultaneous samples of blue marlin from multiple localities throughout the range. Indeed, it is the apparent migratory nature of the species which suggests that the second type of population structuring, that based on both temporal and geographic isolation, may be occurring in this species. Our approach to this problem is to sample continuously in the Hawaiian Islands to look for significant seasonal shifts in allele frequency such as might be expected if different stocks of blue marlin migrate past the Hawaiian Islands at different times of the year. Given the present potentially overfished nature of billfish stocks and the difficulties associated with alternative forms of stock analysis such as tag-recapture studies, this biochemical genetic approach may well represent the only practical means of gathering information on stock structure in a time frame compatible with the urgent need for the formulation of meaningful management programs for this species.

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