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Review

Stock structure of the world's istiophorid billfishes: a genetic perspective

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Abstract. Istiophorid billfishes are highly migratory species that inhabit the tropical and subtropical, epipelagic waters of the world's oceans, a large, relatively homogeneous environment that lacks significant physical barriers. Based on these observations alone, one would not expect marlins, sailfish and spearfishes to exhibit substantial stock structure. This assumption has been evaluated with a variety of techniques, including analyses of morphological characters, adult distribution, tag and recapture data, the spatial and temporal distribution of spawning and, recently, molecular genetic characters. This paper focuses on inferences of istiophorid billfish stock structure derived from investigation of several different classes of molecular markers, and reviews our current understanding of the genetic basis of stock structure of striped marlin, white marlin, blue marlin, sailfish and black marlin. Significant genetic differences exist between Atlantic and Indo-Pacific populations of blue marlin and sailfish, and the presence of distinct mitochondrial DNA lineages suggests that ocean populations were isolated in the past. However, the occurrence of identical genotypes in both oceans is evidence of recent genetic contact. The genetic data do not support recognition of separate Atlantic and Indo-Pacific species of blue marlin or sailfish. White and striped marlin are separated by about the same level of genetic divergence as Atlantic and Indo-Pacific populations of blue marlin and sailfish, but preliminary analysis of the mitochondrial DNA control region suggests that, unlike Atlantic and Indo-Pacific populations of blue marlin and sailfish, white marlin and striped marlin represent independent evolutionary units. If white and striped marlin are valid species, they are of very recent origin. Significant intraspecific genetic heterogeneity was found among collections of striped marlin and sailfish within the Indo-Pacific; both species exhibited a clear spatial partitioning of genetic variation among geographically distant collection locations. There was no genetic evidence for within-ocean population structuring for other istiophorids examined. Inferences of billfish stock structure derived from studies of molecular markers complement those obtained using other methods of analysis, and together these studies demonstrate substantial differences in the level of population structuring among istiophorid billfishes, information critical for effective management of these highly migratory species.

Introduction

The development of stock structure within a species is promoted by several factors, including low dispersal ability, a heterogeneous or fragmented environment and temporally or spatially discrete spawning grounds. Billfishes of the family Istiophoridae have the capacity to disperse widely (thousands of kilometres); they inhabit tropical and subtropical epipelagic waters of the world's oceans, a large, relatively homogeneous habitat that lacks significant physical barriers; and most species spawn over broad geographic regions during a protracted season (Nakamura 1985). It is therefore reasonable to assume that istiophorid billfishes would exhibit little intraspecific population structuring. However, it is critical that this assumption be evaluated, as even low levels of stock structure can have important implications for fisheries management (Allendorf *et al.* 1987), and significant differences may exist in the level of population structuring among billfish species.

A variety of techniques have been used to infer stock structure of billfish species. Skillman (1989) reviewed the strengths and limitations of several methods of stock structure analysis that have been applied to billfishes, including studies of adult distribution (catch or catch per unit effort data), spawning times and areas (based on the spatial and temporal occurrence of ripe adults or larvae and early juveniles), population parameters (growth, recruitment and mortality), parasites, tagging, and morphological characters (morphometrics and meristics). As one might expect, different techniques were found to provide differing insights into the stock structure of the billfishes. Skillman (1989) opted to summarize stock structure based on analyses of distributional and tagging data, noting that there was considerable uncertainty

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about the degree of population structuring of some species.

Uncertainty is not restricted to interpretations of billfish stock structure. Currently, there are several areas of confusion regarding the alpha (species level) taxonomy of istiophorid billfishes. In fact, there are problems within each of the three istiophorid genera. Atlantic and Indo-Pacific populations of blue marlin and sailfish have been described both as separate species, and as conspecific populations. Most recently, Nakamura (1985) recognized Atlantic and Indo-Pacific blue marlin as distinct species (*Makaira nigricans* and *Makaira mazara* respectively) based on differences in lateral line morphology. Nakamura (1985) also recognized separate species of Atlantic sailfish (*Istiophorus albicans*) and Indo-Pacific sailfish (*Istiophorus platypterus*) based on the relative length of pectoral fins and differences in scale shape and growth. Within the genus *Tetrapturus*, there is uncertainty regarding the number of species of spearfish, the taxonomic status of the 'hatchet marlin', and the specific relationship of white marlin (*Tetrapturus albidus*) and striped marlin (*Tetrapturus audax*).

Genetic analyses can provide important insights into problems of inter- and intraspecific population structuring (Avise 1994). In his review of billfish stock structure for the Second International Billfish Symposium, Skillman (1989) noted a lack of genetic studies of billfish stock structure to complement investigations using other techniques, and suggested genetic analysis as one avenue for future research. Over the past 10 years, our laboratory has investigated genetic variation of several different types of molecular markers to better understand the stock structure of istiophorid billfishes. These studies have helped to clarify some inter- and intraspecific relationships, and may have obfuscated others. In the present paper, we review our current understanding of the genetic basis of billfish stock structure, compare the results of genetic analyses with inferences derived using other methods, and ultimately consider the implications of the combined data for billfish management.

Molecular markers and genetic techniques

Over the past 20 years, there has been a dramatic increase in the number of molecular markers available for genetic analyses of population structure. Despite the apparent diversity of molecular markers, most can be assigned to one of three major classes: allozymes, mitochondrial DNA (mtDNA), and nuclear DNA (Avise 1994). Each class of molecular marker has particular advantages and disadvantages relating to its level of expression, mode of inheritance and ease of analysis, and all have been used to study billfish stock structure (Table 1). Allozyme analysis surveys charge and major shape changes of water-soluble proteins encoded in the nuclear genome. In most instances, the proteins are enzymes involved in metabolic pathways. Allozymes are biparentally inherited and were the primary focus of investigations of the genetic basis of stock structure within fishes from the late 1960s through the early 1980s (Allendorf *et al.* 1987). Allozyme analysis has been used extensively because it does not require expensive laboratory equipment and large amounts of data can be generated relatively rapidly. However, allozyme analysis also has several limitations. Sampling is generally invasive, especially if one wishes to survey several enzyme systems. Since proteins are susceptible to protease degradation, tissues must either be used fresh or stored at subzero temperatures until analysis, limiting studies to areas where adequate resources for storage and shipping are available, and precluding the use of archival samples. In addition, only mutations resulting in a change in electrophoretic mobility are surveyed, and much variation at the both amino acid and nucleotide level is undetected. This can result in a low

Table 1. Molecular techniques used to infer stock structure within and between Atlantic and Indo-Pacific samples of istiophorid billfish species

'Yes' indicates significant heterogeneity (stock structure); 'no' indicates that no stock structure was detected; 'NA' indicates that the technique was not used

Species	Nuclear allozymes	RFLP (whole molecule)	mtDNA RFLP (specific region)	Sequencing	Nuclear scnDNA	Microsatellites
White marlin	No	No	NA	NA	No	No
Striped marlin	Yes	Yes	NA	NA	No	Yes
Between Oceans	Yes	Yes	NA	Yes	Yes	Yes
Blue marlin Atlantic	NA	No	No	NA	No	No
Blue marlin Indo-Pacific	NA	No	No	NA	No	No
Between Oceans	Yes	Yes	Yes	NA	Yes	Yes
Sailfish Atlantic	NA	No	No	NA	NA	No
Sailfish Indo-Pacific	NA	Yes	Yes	NA	NA	Yes
Between Oceans	Yes	Yes	Yes	Yes	NA	Yes
Black marlin	NA	NA	No	NA	NA	No

mtDNA, Mitochondrial DNA; RFLP, restriction fragment length polymorphism; scnDNA, single copy nuclear DNA.

number of alleles at polymorphic (variable) loci and finding sufficient variability to adequately test hypotheses is often problematic. Finally, because the historical relationships of the alleles are unknown, some types of population genetic analyses are excluded (reviewed in Hillis *et al.* 1996).

Beginning in the 1980s, investigators increasingly focused their attention on the mtDNA genome. Mitochondrial DNA is a closed circular molecule located in the mitochondrion. In most species, including all teleosts examined, mtDNA encodes 37 functional gene regions comprising 22 tRNAs, 13 mRNAs and two ribosomal RNAs (Lee *et al.* 1995; Saitoh *et al.* 2000). Mitochondrial DNA is haploid, maternally inherited and does not undergo recombination. Because of this, mtDNA has a smaller effective population size than nuclear DNA and is more strongly influenced by evolutionary processes. Furthermore, as mtDNA gene regions evolve more rapidly than most types of nuclear DNA, it allows a higher level of genetic resolution than allozyme analyses. Mitochondrial DNA alleles (genotypes) record the evolutionary history of an organism and, although this allows more sophisticated analyses to be used, the effects of historical phenomena can be confounded with present-day processes. Additionally, the lack of recombination means that the mtDNA molecule is effectively a single locus, so it is prudent to use a nuclear marker(s) to corroborate findings.

Originally, the entire mtDNA genome was isolated and surveyed with restriction fragment length polymorphism (RFLP) analysis, a process in which restriction endonucleases, enzymes that cut the DNA into fragments at specific four, five or six nucleotide base pair recognition sites, are used to reveal variation among individuals (Lansman *et al.* 1981). With the development of the polymerase chain reaction (PCR) (Saiki *et al.* 1988), investigators have been able to amplify specific mtDNA gene regions for either RFLP analysis or, if a higher resolution is desired, direct nucleotide sequence analysis. By selecting a particular mtDNA gene region and method of analysis (RFLP, sequence) one can adjust the level of variation detected to the question at hand. Since only a small quantity of DNA is needed for PCR amplification, samples can be taken non-invasively and stored in appropriate conditions for long periods of time at room temperature, greatly expanding the potential for sampling.

In the 1990s, similar techniques were developed to amplify and detect nuclear variation in both coding and non-coding gene regions (Corte-Real *et al.* 1994), and in anonymous regions of single copy nuclear DNA (Karl and Avise 1993). Since the latter half of the 1990s, analysis of hypervariable, nuclear microsatellite DNA loci has been increasingly used to investigate stock structure within marine fishes. Microsatellites are biparentally transmitted markers that are inherited in Mendelian fashion and they are generally considered to be selectively neutral (Weber and Wong 1993). Microsatellite gene regions contain a series of repeated motifs comprising two, three or four nucleotides, and the number of repeats often shows high levels of variation among individuals (Tautz 1989; Jarne and Lagoda 1996). One shortcoming of microsatellite analysis is the presence of null alleles, defined as alleles that fail to amplify as a result of a mutation(s) in the area(s) critical to amplification. It has been estimated that null alleles occur in approximately 15–25% of microsatellite loci (Jarne and Lagoda 1996). A second, more pervasive problem with microsatellites is homoplasy, which is the presence of alleles that are identical in state (appearance) rather than identical by descent. Homoplasy is especially problematic for microsatellites because they have a rapid mutation rate and because they most often mutate by the gain or loss of one repeat unit. A useful summary of molecular markers, as well as detailed instructions for their use, is presented in Hillis *et al.* (1996).

The data resulting from studies of molecular markers are analysed for levels of genetic variation within samples and divergence among samples. Variation is typically quantified for diploid nuclear markers as the average heterozygosity (H), the proportion of loci for which an individual carries two different alleles. For haploid mitochondrial markers, variation is measured as the nucleon diversity (h), which represents the probability of sampling individuals with different alleles in repeated draws from a sample. There are a variety of methods to test for heterogeneity of allele frequencies among samples, and these include both single locus and multiple locus analyses. The classic measure of heterogeneity is Wright's fixation index (F-statistic, F_{ST}), a measure of the decrease in heterozygosity relative to random mating at any level of a population hierarchy as compared with another more inclusive level of the hierarchy (Wright 1921). Theoretical values for F-statistics range from 0 to 1, with 0 representing a single completely randomly mating population and 1 representing subpopulations that share no alleles in common. Wright (1978) suggested the following guidelines for the interpretation of F_{ST} values: 0–0.05, little genetic variation; 0.05–0.15, moderate genetic differentiation; 0.15–0.25, great genetic differentiation; and above 0.25, very great genetic differentiation. Most species of marine fishes fall within the lower end of this range. Ward *et al.* (1994) reviewed the literature and found a median F_{ST} value of 0.02 among populations of marine fishes. The pervasiveness of weak but often statistically significant genetic signals among populations of marine fishes underscores the need for large sample sizes and use of multiple loci to substantiate results (Waples 1987).

F-statistics and their analogues are often evaluated in a hierarchical framework, sometimes called an analysis of molecular variance (Excoffier *et al.* 1992), which is used to estimate the contribution of the variance of gene frequencies sequestered in different levels of structuring to the total variance found in the sample. Population structure is inferred when there is a significant component of variance attributable to differences among samples, and a lack of structure is inferred when essentially all of the variance is accounted

for within samples. These analyses are useful when testing *a priori* hypotheses about stock structure based on other (non-genetic) data.

Genetic analyses are greatly influenced by the sampling design used to obtain specimens. Ideally, studies would sample stocks of billfish during spawning seasons on known spawning grounds; the time when potentially mixed stocks would be segregated. To avoid possible biases owing to age or sex effects, samples would consist of similar proportions of males and females from each age class sampled (Hedgecock *et al.* 1991; Gold *et al.* 1997; Waples 1998). Similarly, samples would be obtained from the same location in different seasons and years, to evaluate the magnitude of temporal variation of allele frequencies. However, because billfish are a relatively rare species and little is known of spawning times and areas, sample designs have been considerably more pragmatic than ideal. Geographically distant collection locations have been selected based on seasonal billfish abundance and logistic support, and the age and sex composition of samples have reflected local availability rather than a predefined sampling strategy. Typically, sample sizes of 20–50 individuals per location have been collected, and some locations have been sampled in multiple years. Although these sample sizes are appropriate for analyses of many molecular markers, they may be not be adequate to estimate allele frequencies of hypervariable molecular markers with reasonable power. These limitations need to be considered when interpreting the results of genetic studies.

In general, most stock structure analyses test the null hypothesis that samples were drawn from the same gene pool. Rejecting the null hypothesis (a finding of significant heterogeneity between samples) does not automatically signify stock structure, but it is a first step in the process. If sources of sampling bias (age and sex composition) are properly accounted for, and the observed genetic differences are stable over time, it is probable that discrete genetic units exist. Alternately, the inability to disprove the null hypothesis (no significant heterogeneity between samples), even for highly variable molecular markers, does not necessarily imply a lack of genetically based stock structure. One must consider the power of the analysis to detect variation (Taylor and Gerodette 1993; Dizon *et al.* 1995). Furthermore, because relatively small amounts of gene flow (on the order of genomes per generation) may be sufficient to counteract the effects of either random drift or small selective differences in large populations, genetic identity can be maintained with limited exchange (Hartl and Clark 1989).

Billfish stock structure

Striped marlin

Striped marlin stock structure has been investigated with allozyme analysis (Morgan 1992), RFLP analysis of whole mtDNA (Graves and McDowell 1994), and a study using four tetra-nucleotide repeat microsatellite loci is underway. Morgan (1992) surveyed 44 allozyme loci within samples of approximately 40–50 striped marlin each from four areas within the Pacific Ocean: Mexico, Ecuador, Hawaii and Australia. Twelve loci were polymorphic at the 0.99 criterion (where the most common allele occurs at a frequency <0.99), but for almost all polymorphic loci, the major allele occurred at frequencies >0.97 and the average heterozygosity over the four samples was only $H = 0.04$. F_{ST} values ranged from 0.00 to 0.36 for the variable loci, with a mean value of 0.018, indicating slight but statistically significant heterogeneity among the four striped marlin collections.

Graves and McDowell (1994) surveyed mtDNA variation within striped marlin from the Pacific Ocean, using the same samples as Morgan (1992). Their RFLP analysis of the whole mitochondrial genome employed 11 restriction enzymes and revealed considerable variation (Table 2). Levels of variation were strikingly similar among the four collections, with an average nucleotide diversity of $h = 0.82$. Because most genotypes were closely related to one another (differing by one or two restriction site changes), the mean net nucleotide sequence divergence between samples was relatively small; however, there were highly significant differences in the distribution of genotypes among collection

Table 2. Distribution of white marlin and striped marlin mitochondrial DNA genotypes

(*a*) White marlin genotypes based on whole molecule restriction fragment length polymorphism analysis using 12 restriction enzymes (Graves and McDowell 2001)

Genotype	US	Caribbean	Brazil	Morocco
1	24	22	39	13
2	12	7	4	1
3	13	0	8	1
4	3	1	2	3
5	1	3	2	2
6	3	1	2	1
7	1	3	1	1
Minor (<3)	18	3	18	14
Total	74	40	76	36

(*b*) Striped marlin mitochondrial DNA genotypes based on whole molecule restriction fragment length polymorphism analysis using 11 restriction enzymes (Graves and McDowell 1994)

Genotype	Mexico	Ecuador	Hawaii	Australia
1	12	20	14	12
2	6	6	7	3
3	8	9	4	1
4	0	2	10	8
5	0	1	2	7
6	0	1	0	11
7	6	0	0	0
Minor (<3)	4	1	6	5
Total	36	40	43	47

locations, and evidence of spatial partitioning of the genetic variation. Overall, the results of the allozyme and mtDNA analyses are consistent with shallow but statistically significant population structuring within striped marlin in the Pacific Ocean.

White marlin

The genetic basis of population structure of the white marlin has been analysed with RFLP analysis of whole molecule mtDNA (Graves and McDowell 2001), and an investigation surveying variation at four tetra-nucleotide microsatellite loci is ongoing. A total of 226 white marlin samples was collected from the US east coast, the Caribbean, Brazil and Morocco. Restriction fragment length polymorphism analysis of mtDNA using 12 enzymes revealed considerable genetic variation (Table 2), with a nucleon diversity of $h = 0.78$. Most genotypes were closely related, differing by the loss or gain of a single restriction site. No significant differences in allele frequencies were found among replicate samples taken from the same location in different years and, unlike striped marlin, white marlin demonstrated no significant heterogeneity of genotype frequencies among the different collection locations. All genotypes represented in more than three individuals in the total sample were found to occur in two or more locations (Table 2). Essentially all of the variance was accounted for within samples; differences between collection locations did not make a significant contribution to the overall variance. In contrast to striped marlin, white marlin do not demonstrate spatial partitioning of genetic variation among geographically distant collection locations, and the null hypothesis that white marlin comprise a single genetic stock cannot be rejected.

The specific relationship of white and striped marlin

White marlin and striped marlin are morphologically quite similar (Nakamura 1985), and appear to have an even greater genetic affinity. Finnerty and Block (1995) investigated the cytochrome *b* nucleotide sequences of white and striped marlin in their analysis of relationships among the Scombroidei. They noted only two nucleotide substitutions in the 590-base pair sequence between white and striped marlin and, on the basis of this observation, questioned the taxonomic status of the species. Their observation has been supported by analyses of several other, mostly unpublished, molecular markers. L. W. Morgan and J. E. Graves (unpublished data) surveyed 36 presumptive gene loci in white and striped marlin. Although they found slight but significant allele frequency differences between the two species at four loci, no fixed allelic differences were detected between the two species. Similarly, comparison of white and striped marlin with a RFLP analysis of whole molecule mtDNA using 10 restriction endonucleases failed to reveal any fixed restriction site differences between the two putative species (Graves 1998). Two genotypes were shared by white and striped marlin, and the most common genotype of each species differed by a single restriction site. Analyses of six anonymous single copy nuclear DNA (ascnDNA) loci revealed no consistent differences between white and striped marlin, although a large allele frequency difference was noted between the species at one locus (K. S. Reece, A. Montagno and J. E. Graves, unpublished data). Analysis of four tetranucleotide microsatellite loci found significant allele frequency differences between white and striped marlin, but there was considerable overlap in allele sizes between species at all four loci (J. R. McDowell, K. S. Reece and J. E. Graves, unpublished data). A potential genetic separation of the two species was finally noted in a sequence analysis of 854 base pairs comprising the variable mtDNA control region for seven striped marlin and nine white marlin. These analyses resulted in a net nucleotide sequence divergence of 2.25% between species and suggest that each species belongs to a distinct evolutionary lineage (Fig. 1; J. R. McDowell, K. S. Reece and J. E. Graves, unpublished data). Based on the allozyme, mtDNA, ascnDNA, microsatellite DNA, and cytochrome *b* and control region sequence data, white and striped marlin are no more genetically differentiated than allopatric (isolated) populations of the same species of many marine fishes. In fact, many conspecific populations exhibit considerably more genetic divergence than white and striped marlin (Graves 1998).

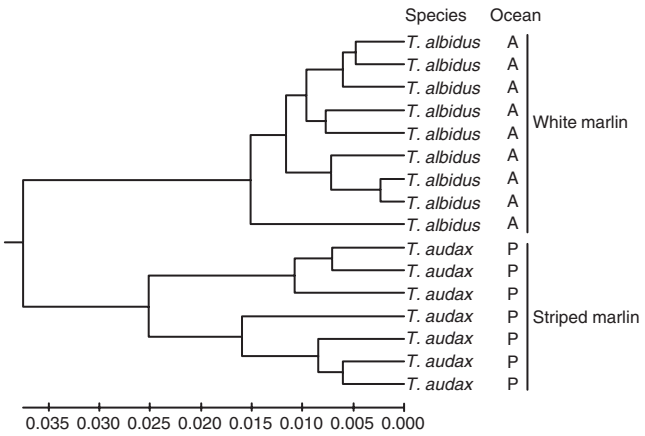

Fig. 1. Neighbour-joining analysis (Tamura and Nei 1993) of DNA sequences of an 854-base pair fragment encompassing the variable mitochondrial DNA control region of white marlin (*Tetrapturus albidus*) and striped marlin (*Tetrapturus audax*) (J. R. McDowell, K. S. Reece and J. E. Graves, unpublished data). Note that at this high level of genetic resolution, each species represents a distinct evolutionary lineage. P = Pacific Ocean; A = Atlantic Ocean.

Blue marlin

The population structure of blue marlin has been well studied with a variety of molecular markers including analyses of allozymes, mtDNA, ascnDNA and microsatellites. Buonaccorsi *et al.* (1999) surveyed variation at 44 allozyme

loci in samples comprising approximately 50 blue marlin each from the Atlantic and Pacific oceans. Although no fixed allelic differences were found between Atlantic and Pacific blue marlin, there was significant heterogeneity in the distribution of allele frequencies between samples from the two oceans. Five of 44 loci were polymorphic (0.99 criterion), and three of those demonstrated significant frequency differences between Atlantic and Pacific samples, resulting in an average F_{ST} of 0.077.

Analysis of mtDNA has also demonstrated significant population structuring between samples of blue marlin from the Atlantic and Pacific oceans. Finnerty and Block (1992) surveyed nucleotide sequence variation in a 612-base pair fragment of the mtDNA cytochrome *b* gene in a total of 26 blue marlin, and reported a significant difference in the distribution of two evolutionarily distinct lineages between Atlantic and Pacific samples. Similarly, RFLP analysis of whole molecule mtDNA using 12 restriction enzymes revealed significant differences between samples comprising about 50 blue marlin from each ocean (Graves and McDowell 1995). Collections of Atlantic blue marlin had considerably more genetic diversity than those from the Pacific. Two genetically distinct mtDNA lineages were present in blue marlin, one of which was apparently restricted to the Atlantic collections. The Atlantic lineage was present in approximately 40% of Atlantic blue marlin, and differed from the widespread (ubiquitous) lineage by a net mean nucleotide sequence divergence of 1.23%. This pattern of lineage distribution between oceans was still evident as new collection locations were added and sample sizes increased to more than 150 individuals from each ocean (Buonaccorsi *et al.* 2001). Sequence analysis of 850 base pairs of the quickly evolving mitochondrial control region for 20 blue marlin resulted in a net mean nucleotide sequence divergence of 5.17% between the two lineages (Fig. 2; J. R. McDowell and J. E Graves, unpublished data). Despite the large sampling effort, there was no evidence of heterogeneity among locations within an ocean. Analysis of four ascnDNA loci revealed levels of variation comparable with that found in allozymes, and demonstrated about the same level of divergence between Atlantic and Pacific samples (Buonaccorsi *et al.* 1999). Investigation of five hypervariable tetranucleotide repeat microsatellite loci showed significant heterogeneity between ocean samples for each locus, some of which displayed differences in the distribution of allele size modes between ocean samples that were characteristic of the mtDNA data (Buonaccorsi *et al.* 2001). None of the microsatellite markers revealed significant heterogeneity among sampling locations within an ocean.

The genetic data resulting from analyses of four classes of molecular markers demonstrates significant allele frequency differences between Atlantic and Indo-Pacific blue marlin. The relatively large divergence between the two lineages of mtDNA genotypes, as well as the differences in modal allele sizes present in some microsatellite loci, suggest that Atlantic and Pacific blue marlin were isolated in the past. The presence of identical alleles in animals from the two oceans is consistent with subsequent gene flow, occurring primarily from the Pacific to the Atlantic. Extensive analysis of samples within the Atlantic Ocean provided no evidence of stock structure within that ocean. Similarly, no significant differences were found among locations within the Pacific Ocean, although sample coverage and sizes were limited.

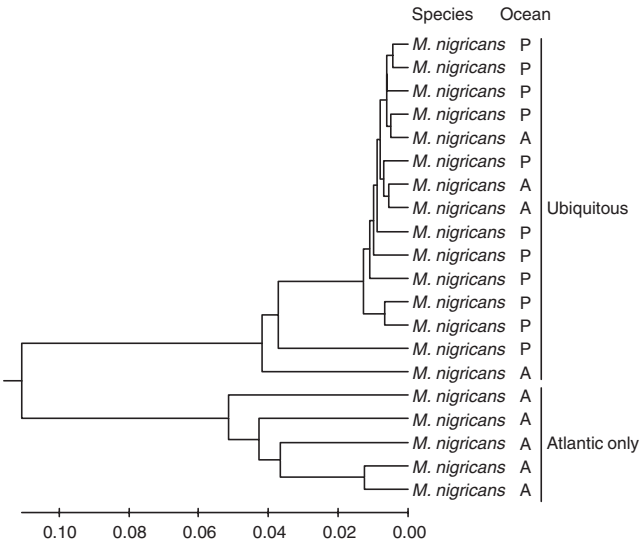

Fig. 2. Neighbour-joining analysis (Tamura and Nei 1993) of DNA sequences of an 850-base pair fragment encompassing the variable mitochondrial DNA control region of blue marlin (*Makaira nigricans*) from the Atlantic and Indo-Pacific oceans (J. R. McDowell and J. E. Graves, unpublished data). The Atlantic evolutionary lineage was restricted to individuals in the Atlantic Ocean, whereas individuals with the ubiquitous lineage were found in all oceans, including the Atlantic. P = Pacific Ocean; A = Atlantic Ocean.

Sailfish

Stock structure of sailfish has been investigated with analyses of allozymes, mitochondrial DNA and nuclear microsatellite loci. Morgan (1992) surveyed 46 loci in samples of 28 Atlantic and 38 Pacific sailfish. Relative to other istiophorids, sailfish exhibited low levels of electrophoretic variation. Only three loci were polymorphic (0.99 criterion), and average heterozygosity was $H = 0.01$. Two of the polymorphic loci exhibited significant allele frequency differences between Atlantic and Pacific samples. The average F_{ST} over the variable loci was 0.023, indicating slight but significant divergence between ocean populations.

Graves and McDowell (1995) surveyed RFLP variation of whole mtDNA using 12 restriction enzymes in collections of 36 Atlantic and 33 Pacific sailfish, and reported a phylogeographic pattern similar to that seen in blue marlin. As with blue marlin, levels of variation were higher in Atlantic sailfish owing to the presence of two evolutionary lineages

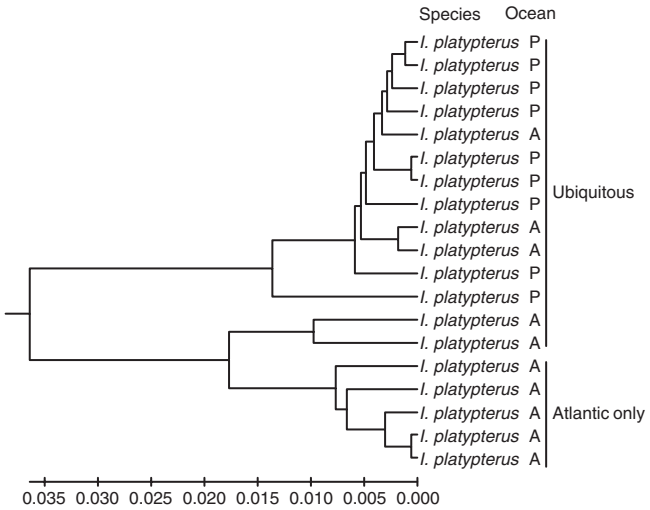

Fig. 3. Neighbour-joining analysis (Tamura and Nei 1993) of nucleotide sequences of an 871-base pair fragment encompassing the mitochondrial DNA control region of sailfish (*Istiophorus platypterus*) from the Atlantic and Indo-Pacific oceans (J. R. McDowell and J. E. Graves, unpublished data). As with blue marlin, one evolutionary lineage was restricted to individuals in the Atlantic Ocean, whereas individuals with the ubiquitous lineage occurred in all oceans. P = Pacific Ocean; A = Atlantic Ocean.

of genotypes, one of which was restricted to Atlantic sailfish. The lineage of unique Atlantic genotypes was present in approximately 80% of Atlantic sailfish, and was differentiated from the other lineage of genotypes by a net mean nucleotide sequence divergence of 0.65%. Several genotypes of the ubiquitous lineage were common to individuals from both oceans.

J. R. McDowell (unpublished data) used five restriction enzymes to survey variation within an 1800-base pair fragment of the mitochondrial genome encompassing the control region. Analysis of approximately 600 sailfish revealed the same two distinct mitochondrial lineages evident in the whole mtDNA analysis, only one of which occurred in Indo-Pacific samples. The unique Atlantic lineage of genotypes was present in more than half of the Atlantic individuals. Sequence analysis of 871 base pairs of the mitochondrial control region resulted in a net nucleotide sequence divergence of 2.6% between the clades (Fig. 3). Significant heterogeneity of allele frequencies was noted among sailfish collections within the Pacific and Indian oceans, but there was no evidence of genetic heterogeneity among Atlantic collections.

J. R. McDowell (unpublished data) also surveyed variation at five tetranucleotide microsatellite loci within the same collections of sailfish. Two of the loci were discarded because the sample sizes were inadequate for the high numbers of alleles (49 and 52). Preliminary analyses of the remaining three loci revealed large differences between the Atlantic and Indo-Pacific sailfish collections. No heterogeneity was evident among Atlantic samples, but as with the mtDNA analysis, significant heterogeneity was noted among collections from the Pacific and Indian oceans.

The evidence from molecular markers suggests that sailfish exhibit significant population structuring both between and within oceans. No fixed allozyme allele differences were reported between Atlantic and Indo-Pacific sailfish, although significant allele frequency differences were noted between samples from the two oceans. As with blue marlin, the presence of two distinct lineages of mtDNA genotypes within Atlantic samples, only one of which occurs in Pacific sailfish, suggests a period of past isolation between ocean populations, with subsequent gene flow from the Pacific to the Atlantic. The genetic divergence between lineages of mtDNA genotypes in Atlantic sailfish is approximately one-half that seen between lineages of mtDNA genotypes in Atlantic blue marlin; however, the unique Atlantic lineage of genotypes occurs at a higher frequency in Atlantic sailfish. There was no evidence of genetic heterogeneity among collections of sailfish within the Atlantic Ocean, but significant heterogeneity was noted among samples from the Pacific and Indian oceans.

Black marlin

Stock structure in black marlin has been investigated with analyses of mtDNA, ascnDNA and microsatellite DNA (Falterman 1999). A preliminary screening of seven ascnDNA loci with 43 restriction enzymes revealed no variation within black marlin, and further analyses with those markers was not pursued. A 1200-base pair region of the mtDNA genome encoding the control region was surveyed with six variable restriction enzymes in 286 black marlin collected throughout the Pacific Ocean (Table 3). There was considerable genetic variation within collections, but no significant heterogeneity was demonstrated among collections from the same location taken in different years, or among collections from geographically distant areas. Analysis of five tetranucleotide repeat microsatellite loci developed for blue marlin (Buonaccorsi and Graves 2000), revealed high levels of variation within black marlin. Two of the loci had levels of variation appropriate for the sample sizes. Analysis of approximately 300 individuals revealed no significant heterogeneity among samples collected at the same location in different years, or among samples from locations throughout the Pacific Ocean. Based on the mtDNA and microsatellite data, the null hypothesis of a single genetic stock of black marlin could not be rejected.

Interspecific relationships

The results of the genetic analyses provide new insights into the three problematic areas of istiophorid taxonomy: the specific status of Atlantic and Indo-Pacific blue marlin, Atlantic and Indo-Pacific sailfish, and white and striped marlin. There is surprisingly little differentiation of allozymes between samples of Atlantic and Indo-Pacific blue marlin and sailfish.

Table 3. Black marlin mitochondrial DNA genotypes based on restriction fragment length polymorphism analysis encompassing the control region using six restriction enzymes (Falterman 1999)
E. Pacific, eastern Pacific (Ecuador, Mexico, Panama)

Genotype	Australia	Vietnam	South Africa	Taiwan	E. Pacific	Total
1	52	4	12	27	12	107
2	31	3	6	18	1	59
3	22	2	2	8	4	38
4	7	2	4	1	1	15
5	6	1	0	1	0	8
6	0	0	0	6	1	7
7	3	0	2	1	1	7
8	6	0	0	0	0	6
9	0	0	0	3	1	4
10	2	0	0	1	0	3
Minor (<3)	17	0	6	5	4	32
Total	146	12	32	71	25	286

No fixed allelic differences were found to distinguish between congeners from the Atlantic and Indo-Pacific oceans, and the unbiased genetic distance (Nei 1978) between each species pair was less than 0.01 (Morgan 1992). This level of genetic divergence is more typical of conspecific populations than congeneric species (Grant 1987; Ward *et al.* 1994).

Analysis of mitochondrial DNA provided additional information with which to consider the status of these putative species. For blue marlin and sailfish, Atlantic samples were characterized by a higher genetic diversity, owing primarily to the presence of two mtDNA lineages, only one of which occurs in Pacific samples. The genetic divergence between the Atlantic and ubiquitous lineages was greater in Atlantic blue marlin than in Atlantic sailfish (1.23% *v*. 0.65%), but the frequency of the unique Atlantic lineage was greater (almost twice as large) in Atlantic sailfish relative to Atlantic blue marlin. Importantly, several genotypes within the ubiquitous lineage were present in both Atlantic and Indo-Pacific samples of blue marlin and sailfish. These data suggest a period of isolation of both Atlantic and Indo-Pacific blue marlin and sailfish in the past and, based on the magnitude of the genetic divergence between the Atlantic and ubiquitous lineages, the separation occurred during the Pleistocene (Graves 1998). It would appear that a barrier to exchange, most likely cooler waters around the Cape of Good Hope (Penrith and Cram 1974), was relaxed in recent times, as evidenced by the occurrence of identical mtDNA genotypes in samples from both oceans. The lack of unique Atlantic lineage genotypes in Pacific individuals, suggests that movement has been primarily from the Pacific to the Atlantic Ocean. These data do not support the existence of separate Atlantic and Indo-Pacific species of blue marlin or sailfish and we recommend that they be referred to as single species.

The overall genetic divergence between white marlin and striped marlin is not significantly different to that observed between Atlantic and Indo-Pacific samples of blue marlin and sailfish. There were no fixed allozyme allelic differences, and the net mean nucleotide sequence divergence between white and striped marlin (0.06%) is quite close to that observed between Atlantic and Pacific blue marlin (0.15%), and Atlantic and Pacific sailfish (0.27%). However, unlike Atlantic samples of blue marlin and sailfish, white marlin do not exhibit greater genetic variation than striped marlin, and there is no evidence of evolutionarily distinct mtDNA lineages within white marlin. This would suggest that either white and striped marlin were not completely isolated during the Pleistocene or, if they were, that subsequent gene flow and genetic drift have resulted in the loss of one lineage of genotypes.

Analysis of mtDNA control region sequences of nine white marlin and seven striped marlin resulted in two distinct evolutionary lineages, one that included all white marlin and one that included all striped marlin (Fig. 2). Unlike the evolutionary lineages found within blue marlin and sailfish, the two lineages within the combined white marlin and striped marlin data set are geographically separate, and there is no evidence of recent gene flow between ocean populations. Although this observation is based on a limited number of individuals and merits further research, the data indicate that at a very high level of genetic resolution, white marlin and striped marlin are independent evolutionary units. There has been considerable debate in the literature about the application of species concepts to situations such as this (Otte and Endler 1989; Howard and Berlocher 1998; Avise 2000), but based on the genetic, distributional and morphological data, it would appear that white and striped marlin would merit being recognized as distinct species using either the biological or phylogenetic species concept. The overall level of genetic divergence between white marlin and striped marlin would suggest that the lineages are of recent origin.

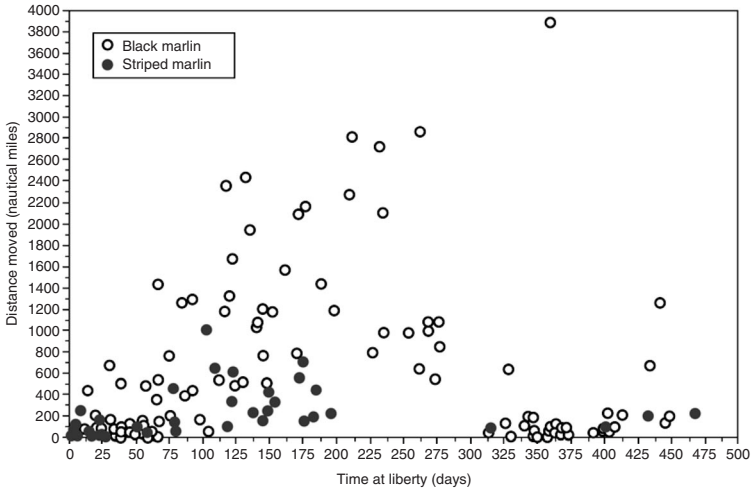

Fig. 4. Net displacement versus days at liberty for striped marlin and black marlin released from eastern Australia (Ortiz *et al.* 2003).

Intraspecific stock structure

Analyses of several different classes of molecular markers have provided new insights into the inter- and intraspecific relationships of istiophorid billfishes. Do they agree with results from other methods of stock structure analysis? Although limitations on space preclude discussion of the stock structures of all istiophorid species, a comparison of inferences derived for a few species using different methodologies is insightful. Within the Pacific Ocean, striped marlin and black marlin represent endpoints of a continuum of genetic stock structure. Striped marlin exhibited significant genetic heterogeneity and a pronounced spatial partitioning of variation among collection sites within the Pacific Ocean. Black marlin, in contrast, demonstrated no significant genetic heterogeneity or geographic localization of genetic variation. Results of tagging studies are consistent with less population structuring in black marlin. Analysis of Australian gamefish tagging data (Ortiz *et al.* 2003), indicates that both striped marlin and black marlin are capable of dispersing large distances. An annual migration pattern is evident in tag returns for both striped marlin and black marlin, with the greatest net displacements occurring about 6 months after the release date, and much smaller net displacements noted for recaptures occurring about 12 months after the release date (Fig. 4). Although a similar pattern is exhibited by both species, black marlin tend to disperse greater distances than striped marlin during the course of the year, and some black marlin do not return to the tagging area on an annual basis. The greater dispersal of black marlin relative to striped marlin is consistent with the genetic observation of significant population structuring in striped marlin, and an apparent lack of structuring within black marlin.

Our understanding of billfish spawning times and areas are limited, and based primarily on anecdotal observations of the occurrence of ripe females and early life history stages that are notoriously difficult to identify (Richards 1974). The incidence of adult black marlin in spawning condition, and the presence of their larvae, suggests that this species may spawn in a relatively restricted area off the Great Barrier Reef during the austral summer (Leis *et al.* 1993). In contrast, striped marlin are reported to spawn over a large region in the Pacific, but reproductive activity appears to be concentrated in restricted areas during specific periods (Squire and Suzuki 1990). The existence of multiple spawning areas for striped marlin in the Pacific Ocean would promote stock structure if individuals demonstrate fidelity to a spawning site. In contrast, the existence of a single spawning time and place would reinforce genetic homogeneity within black marlin. The observation of tag returns of individuals to the same areas in different parts of the Pacific Ocean supports this observation (Squire and Suzuki 1990). In the case of striped marlin and black marlin, it would appear that inferences regarding stock structure derived from analyses of spawning area and times, tag returns and genetic data are highly consistent, suggesting significant population structuring with striped marlin and little or no stock structure within black marlin.

Stock structure and billfish management

The demonstration of significant genetic heterogeneity within a species provides a first step in the delineation of stock structure. Subsequent genetic analyses are needed to demonstrate the temporal stability of the observed allele frequency differences as well as to define the spatial extent of stocks and areas of mixing. These investigations will require large numbers of individuals collected over broad spatial and temporal scales. They will not be inexpensive. In addition, incorporation of data from other methods of analysis will be needed to corroborate genetic inferences.

For istiophorid billfishes, sufficient genetic divergence exists between Atlantic and Indo-Pacific populations of blue marlin, and Atlantic and Indo-Pacific sailfish for these units to be considered distinct genetic stocks. In addition, significant genetic heterogeneity has been demonstrated within Indo-Pacific striped marlin and sailfish. For both species, spatial partitioning of genetic variation is evident, suggesting spatially discrete stocks. As outlined above, additional work is needed to better define the temporal stability and range of the putative stocks, and these should be coupled with tagging studies and investigations of spawning to provide fishery managers with information necessary to determine the extent and interaction of independent fishery management units.

The management significance of genetic analysis is not as clear for those species in which no genetic heterogeneity was found among collection locations within an ocean. Failure to disprove the null hypothesis does not necessarily mean that there is a lack of stock structure, only that it was not observed. As previously mentioned, this could be owing to the markers selected, the presence of sufficient gene flow to prevent the accumulation of significant genetic divergence, or such recent isolation that genetic differences have not had sufficient time to accrue. If one assumes that sufficiently variable markers have been surveyed to demonstrate divergence if it exists, and discounts the possibility of very recent origin, then the observed lack of genetic divergence must result from gene flow. For the purposes of fisheries management, one can have relatively independent fishery management units that are genetically connected. In such cases, it is important to evaluate inferences derived from other methods of stock discrimination to provide insights into stock structure. Management of white marlin and blue marlin within the Atlantic Ocean provide an interesting case history.

Atlantic white marlin and blue marlin are managed by the member nations of the International Commission for the Conservation of Atlantic Tunas (ICCAT). Originally, the ICCAT Standing Committee for Research and Statistics recognized two stock of each species within the Atlantic Ocean, separated at 5°N latitude. The stock boundary was based on the distribution of the catch, the occurrence of seasonally displaced spawning areas north and south of 5°N, and the fact that no fish tagged north of 5°N had been recaptured south of that latitude (and *vice versa*). Subsequently, detailed genetic analyses surveying a variety of molecular markers have revealed no evidence of genetically based stock structure within Atlantic white marlin or blue marlin, and analyses of samples north and south of 5°N do not reveal significant heterogeneity (Graves and McDowell 2001). Based on the genetic results, a re-examination of other lines of evidence for stock structure was undertaken. It was noted that expansion of the longline fishery demonstrated a continuous distribution of white marlin and blue marlin across 5°N in all four quarters of the year. Furthermore, with additional tagging efforts, trans-Atlantic, trans-equatorial and, for the blue marlin, even trans-oceanic tag recoveries were noted (Ortiz *et al.* 2003). Consequently, on the basis of all available data, the ICCAT Standing Committee for Research and Statistics only considered Atlantic-wide stocks of white marlin and blue marlin in the 2000 assessment (ICCAT 2001).

Conclusions

Over the past 10 years, genetic analyses have provided some valuable insights into the inter- and intraspecific relationships of istiophorid billfishes. They have provided information critical to understanding the taxonomic relationships of Atlantic and Indo-Pacific blue marlin and sailfish, as well as white and striped marlin. Analyses of molecular markers have demonstrated differences in the level of genetic heterogeneity among billfishes, and suggested significant stock structuring within striped marlin and sailfish in the Indo-Pacific, although further research is necessary to elucidate the extent and interaction of the stocks. Like any other means of stock structure analysis, the investigation of molecular markers has distinct advantages and disadvantages, and we do not advocate the use of any single technique to infer stock structure. However, by combining inferences from a variety of techniques, one can obtain a good understanding of the level and significance of stock structuring within these highly migratory species. Together with advances in fishery management that incorporate models of stock structures other than simplistic unit stocks or panmictic populations, these techniques will provide the information necessary for the effective management of these global resources.

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