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Structure and migration corridors in Pacific populations of the Swordfish *Xiphias gladius*, as inferred through analyses of mitochondrial DNA

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Abstract The swordfish *Xiphias gladius* is a migratory oceanic species distributed in sub-tropical and temperate waters worldwide. Studies utilizing mitochondrial DNA (mtDNA) have demonstrated genetic subdivision between ocean basins, as well as within the Atlantic basin. However, there has been no support of population subdivision within the Pacific. We sequenced 629 base pairs of the control region for 281 swordfish collected in the Pacific. A rate heterogeneity parameter, alpha, was found to be 0.201, indicating substantial variation in mutation rate within the control region of swordfish. Hierarchical analysis of molecular variance supported significant genetic structuring among Pacific populations. Northern and southern populations in the western Pacific were significantly divergent, while populations in the east appeared to be genetically continuous. Regression analysis supported a correlation of genetic differentiation with geographic distance along a U-shaped corridor of gene flow. These results reveal a pelagic biogeographic pattern heretofore unrecognized in the Pacific, and reject the null hypothesis that Pacific populations of swordfish are unstructured and comprise only a single homogeneous stock.

Introduction

The swordfish *Xiphias gladius* has a life history and vagility suggestive of high gene flow and little genetic subdivision among populations. Members of this species are found in all tropical, subtropical and temperate seas,

and provide lucrative commercial fisheries around the world. Swordfish are powerful swimmers, grow to 540 kg, and live for ≥ 9 yr (Palko et al. 1981). These fish elevate temperature in the head region with a specialized eye muscle known as the heater organ (Carey 1982). This heat-generating tissue allows them to traverse steep thermal gradients ranging from 26 to 6 °C, presumably in search of prey (Carey and Robison 1981; Carey 1990). Despite their ability to tolerate a wide range of temperatures, swordfish abundance and distribution is generally associated with surface waters > 18 °C and < 30 °C (Palko et al. 1981).

Swordfish appear to be highly migratory yet solitary animals, rarely pairing except to spawn. Sexual maturity is thought to occur at ~ 5 yr of age, and spawning generally takes place in tropical waters where surface temperatures are > 20 °C (Palko et al. 1981). Identification of spatially or temporally discrete spawning areas has been difficult because of the long spawning season, the widespread area in which larvae are found, and the variety of methods used to survey the ichthyoplankton (Grall et al. 1983). Nonetheless, seasonality in the abundance of larvae and reproductive adults is evident in the northern and southern Pacific, suggesting the possibility of population subdivision (Palko et al. 1981; Grall et al. 1983). Unfortunately, neither intensive analysis of catch statistics nor the limited recapture of tagged fish has provided a clear picture of migratory routes or population structure in the Pacific.

Molecular genetic data can be used to infer population structure by quantifying the degree of genetic relatedness between geographically sampled local populations or demes. Demes found to be undifferentiated through the analysis of F -statistics (Wright 1951; Weir and Cockerham 1984) or lacking geographic partitioning of discrete phylogenetic clades (Avise 1994) are assumed to be linked by gene flow and grouped within the same population. Demes showing a phylogeographic partitioning of discrete clades or a significant degree of genetic difference, as measured by F_{ST} are considered to be separate populations.

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Several genetic studies have demonstrated that swordfish populations are subdivided on an ocean-basin scale (Alvarado-Bremer et al. 1995; Kotoulas et al. 1995; Alvarado-Bremer et al. 1996; Rosel and Block 1996). In these studies, sequence analysis of the 5' end of the mitochondrial DNA (mtDNA) control region has revealed two distinct phylogenetic clades (I and II) that are not equally distributed. Clade I is found in all oceans, and nearly every Clade I fish has a unique mtDNA lineage (haploptype), resulting in very high estimates of haplotypic diversity (99%). Clade II is most abundant in the Mediterranean (ranging from 30 to 50%), and becomes increasingly more difficult to detect in populations sampled from the southern Atlantic and Indian Oceans (~6%). Clade II's haplotypic diversity is also high (82%). Surprisingly, this clade has not been found in the Pacific. The limited distribution of Clade II indicates that swordfish in the Mediterranean, Atlantic and Pacific are structured on a global scale. Statistical analysis of data sets containing both phylogenetic clades rejects the null hypothesis that genetic variance is equally distributed among ocean basins (Alvarado-Bremer et al. 1996; Rosel and Block 1996).

Demonstrating intra-basin population structure in swordfish has been difficult, especially within the Pacific. This is partly because the two-clade structure disappears in the Pacific, leaving a phylogeny of unresolved relationships. Another reason is that nearly every fish in the cosmopolitan Clade I is genetically unique. Thus, it is not possible to group individuals with similar haplotypes for frequency analysis. One way around the latter problem is to assay less genetic variation in the control region using restriction enzymes, and to increase sample sizes so that lineages can be detected multiple times. Two studies utilizing this approach support subdivision within the Atlantic between northern and southern populations (Alvarado-Bremer et al. 1996; Chow et al. 1997). Yet, neither these nor an additional study specifically on northern Pacific populations (Grijalva-Chon et al. 1994) provide evidence of subdivision within the Pacific.

Although recent work now characterizes populations of the highly migratory swordfish as more genetically heterogeneous than originally thought, rejecting the null hypothesis of population homogeneity (panmixia) in the Pacific has been difficult. Under a panmictic scenario, we can assume that gene flow is unrestricted and random among an array of sampled demes. Thus, we would expect no geographic partitioning of haplotypes in a phylogeny. Furthermore, *F*-statistics would fail to support a geographic pattern of genetic subdivision among demes. Alternatively, if populations are subdivided, then there are migration models that can be used to decipher potential corridors of dispersal.

The island model of migration allows for genetic exchange to occur equally among all subpopulations (Wright 1969; Latter 1973). The isolation-by-distance model (Wright 1943, 1951) assumes that migration is unequal and occurs primarily between nearest neigh-

bors. If migration is limited, it becomes possible to detect a correlation between genetic divergence and geographic distance in populations of finite size. This model contrasts with the stepping-stone model (Kimura and Weiss 1964; Maruyama 1969, 1971), in which there is a discrete pattern of dispersal such that some subpopulations do not receive migrants directly from geographic neighbors. In this paper, we present sequences of mitochondrial DNA (mtDNA) from both the 5' and the 3' ends of the hypervariable control region. We show that populations of the highly migratory swordfish are subdivided in the Pacific basin, and that gene flow among them is restricted to a specific corridor.

Materials and methods

Muscle tissue from 281 adult swordfish (*Xiphius gladius*) was collected from eight sites in the Pacific Ocean (Table 1). Total genomic DNA was extracted using the Qiagen Tissue Prep kit (Qiagen Corporation). The DNAs of three Clade II Mediterranean swordfish previously extracted by Rosel and Block (1996) were included in the data set as outgroups for the phylogenetic analysis. Two fragments were amplified from the 5' and 3' variable sections of the control region (310 and 319 base pairs, respectively). Polymerase chain-reaction (PCR) conditions and sequences used for primer design were taken from Rosel and Block. Primer sequences for the 5' fragment are Pro-L: 5'-CTACCCCTAACTCCCAAAGC-3'; XIGL-5: 5'-ACACCAGTCTTGTAACC-3'. Primers for the 3' fragment are: XIGL-6: 5'-AGCGGGTAG-GGGGTTCT-3'; 12sar: 5'-ATAGTGGG-TATCTAATCCAGTT-3'. Each fragment was sequenced using the Prism DNA-sequencing kit and 373A autosequencer, both from Applied Biosystems, Inc. All sequences were deposited in GenBank under Accession Nos. AF199616-AF200183. Sequences were aligned to those previously published by Rosel and Block. Gaps were minimized for insertion/deletion events of multiple bases by removing all but one base. These mutations were conservatively given the weight of a transition in the analysis. All samples (Clades I and II) were used to calculate a mean transition:transversion ratio with the software MEGA (Kumar et al. 1993).

Table 1 *Xiphius gladius*. Collection sites and number sampled (*n*)

Geographic region	(<i>n</i>)	Collection dates
Indian Ocean		
Western Australia ^a	(36)	February 1995
Pacific Ocean		
Eastern Australia ^a	(12)	July 1995
Japan	(46)	November 1991, February 1992
Hawaii	(42)	February 1990, January 1991
Southern California ^b	(30)	October 1994
Mexico ^b	(15)	March through August 1992
Central Equatorial Pacific ^c	(54)	October 1994
Chile	(46)	August through October 1995
Total	(281)	

^{a,b} Samples grouped together as one deme for analysis

^c Central Equatorial Pacific samples collected between 12° N, 165° W and 0° S, 165° W

The vertebrate control region of mtDNA is known to have a heterogeneous rate of nucleotide substitution that can be modeled by a gamma distribution specified by the parameter alpha (Tamura and Nei 1993). Alpha, which can be estimated from the sequence data (Wakeley 1993), was calculated for swordfish using the software package PAUP* 4.0b2 (Swofford 1999). An unrooted neighbor-joining tree (Saitou and Nei 1987) was drawn for both Clade I and Clade II sequences. Distances were calculated using the uncorrected pairwise method to avoid bias in branch lengths. This tree was then used to generate a maximum likelihood estimate of the alpha parameter (Yang and Kumar 1996). Phylogenetic structuring within Clade I was examined by bootstrapping the data set (with permutation) in PAUP* 1000 times. Genetic distances were calculated using the Tamura-Nei algorithm (Tamura and Nei 1993) corrected with the empirically-derived alpha. A neighbor-joining tree was drawn from this distance matrix and rooted with the three Clade II sequences. A homoplasy index for the tree was determined.

A haplotypic correlation measure analogous to F_{ST} (Φ_{ST}) was estimated for all Pacific swordfish DNA sequences using a hierarchical analysis of molecular variance, (AMOVA, Excoffier et al. 1992), available in the software package Arlequin Version 1.1 (Schneider et al. 1997). The significance of this overall Φ_{ST} was determined by a non-parametric permutation procedure described in Excoffier et al. (1992) and available in the Arlequin package. Population Φ_{ST} s were also calculated for each pair of populations and similarly tested for significance. Genetic distance matrices used for these estimates were generated using the alpha-corrected method of Tamura-Nei. They were permuted 10 000 times to produce a null distribution to compare statistically with the observed Φ_{ST} values. The sequential Bonferroni test, as implemented by (Rice 1989), was used to correct for multiple tests of significance.

First-order linear regressions were used to examine whether a geographic pattern of gene flow existed among Pacific swordfish collection sites. Pairwise population Φ_{ST} s generated from the AMOVA were linearized (Slatkin 1991) and plotted against geographic distance between corresponding population pairs. Geographic distances were determined using the Map program in the Macintosh Operating System 7.5, and were calculated as straight-line paths between populations. A regression line was fit to the data, and a Student's *t*-test tested whether the slope of this line was significantly different from the null expectation of zero, which would imply no correlation (i.e. independence) between the two variables.

The Mantel test (Mantel 1976) available in GenePop, Version 3.1b (Raymond and Rousset 1995) was also used to examine whether a correlation existed between Φ_{ST} and geographic distance. In this test, the linearized genetic differentiation and geographic distance matrices were permuted 100 000 times to generate a null distribution of independence between the two variables. A Spearman rank correlation-coefficient was calculated, and a one-tailed test of significance was performed to determine whether this coefficient was statistically outside the null distribution. Because GenePop only calculates conventional *F*-statistics based on frequency differences of haplotypes, a data matrix was created composed of pairwise population Φ_{ST} s from the AMOVA program and pasted into the input file for GenePop's isolation by distance (ISOLDE) program.

Results

Sequence analysis of the control region of *Xiphius gladius* mtDNA (629 base pairs) revealed both an A-T bias in base composition (64.7%) and a bias favoring transition mutations (transition:transversion ratio of 9.8:1.0). The estimated alpha parameter was 0.201, suggesting a large degree of rate heterogeneity. Mean sequence divergence within Pacific swordfish was

0.022 ± 0.011 , and haplotypic diversity was 99.9%. The phylogeny of Pacific swordfish rooted with three Mediterranean Clade II haplotypes is shown in Fig. 1. Branches not supported with bootstrap values $> 50\%$ were collapsed in the tree. Clade II haplotypes were not detected in our collection of Pacific samples, as all individuals phylogenetically belonged to Clade I. The phylogeny provides little support for significant clustering within Clade I. A homoplasy index of 0.699 was observed, indicating a high level of mutational reversions along the branches.

Because sample size was small from both eastern Australia ($n = 12$) and Mexico ($n = 15$), these fish were initially combined with nearby sites. The Australian sample contains fish from both the western (Indian Ocean) and eastern (Pacific Ocean) fishery. Californian and Mexican samples were likewise grouped together, resulting in a total of six Pacific demes that were analyzed. Table 2 lists results of the AMOVA for these six demes. Even though a very large amount of the genetic variation is partitioned within populations (99.1%) and not between them (0.88%), the amount distributed among the six demes (Φ_{ST}) was still highly significant. This outcome rejects the null hypothesis that populations are homogeneous in the Pacific ($p = 0.0047$).

Table 3 is a matrix of Φ_{ST} s and their *p* values showing the level of genetic differentiation between pairs of populations. Population pairs with a *p* value < 0.05 (prior to Bonferroni correction) can be identified in this table, and a pattern of population structuring noted in the western Pacific. Northern Hemisphere samples collected in Hawaii and Japan differed significantly from southern collection sites in Australia and the central equatorial Pacific. The Φ_{ST} between Australia and Japan was highly significant ($p = 0.0007$). One potential problem with this analysis is grouping together two Australian populations that were collected from two different ocean basins. If these samples are analyzed as two separate demes, neither the eastern nor western Australian samples differ significantly from each other ($p = 0.063$). Furthermore, both eastern and western Australia are significantly different from Japan ($p = 0.000$ in each case) as well as Hawaii ($p = 0.000$; $p = 0.007$, respectively). Therefore, grouping of these two sample sites does not bias the observation that Australian swordfish differ from those collected around Japan and Hawaii.

Figure 2 geographically summarizes Table 3, and suggests a biogeographic pattern of population structure and gene flow between Pacific populations. The six sampled demes are located on a map of the Pacific basin, and lines are drawn between demes whose Φ_{ST} s are not significantly different from each other ($p > 0.05$). The resulting pattern of genetic connectivity in Pacific swordfish resembles a U-shape. Northern and southern populations appear to diverge in the western Pacific. However, in the east this differentiation fades, as populations appear to be genetically interconnected across the equator.

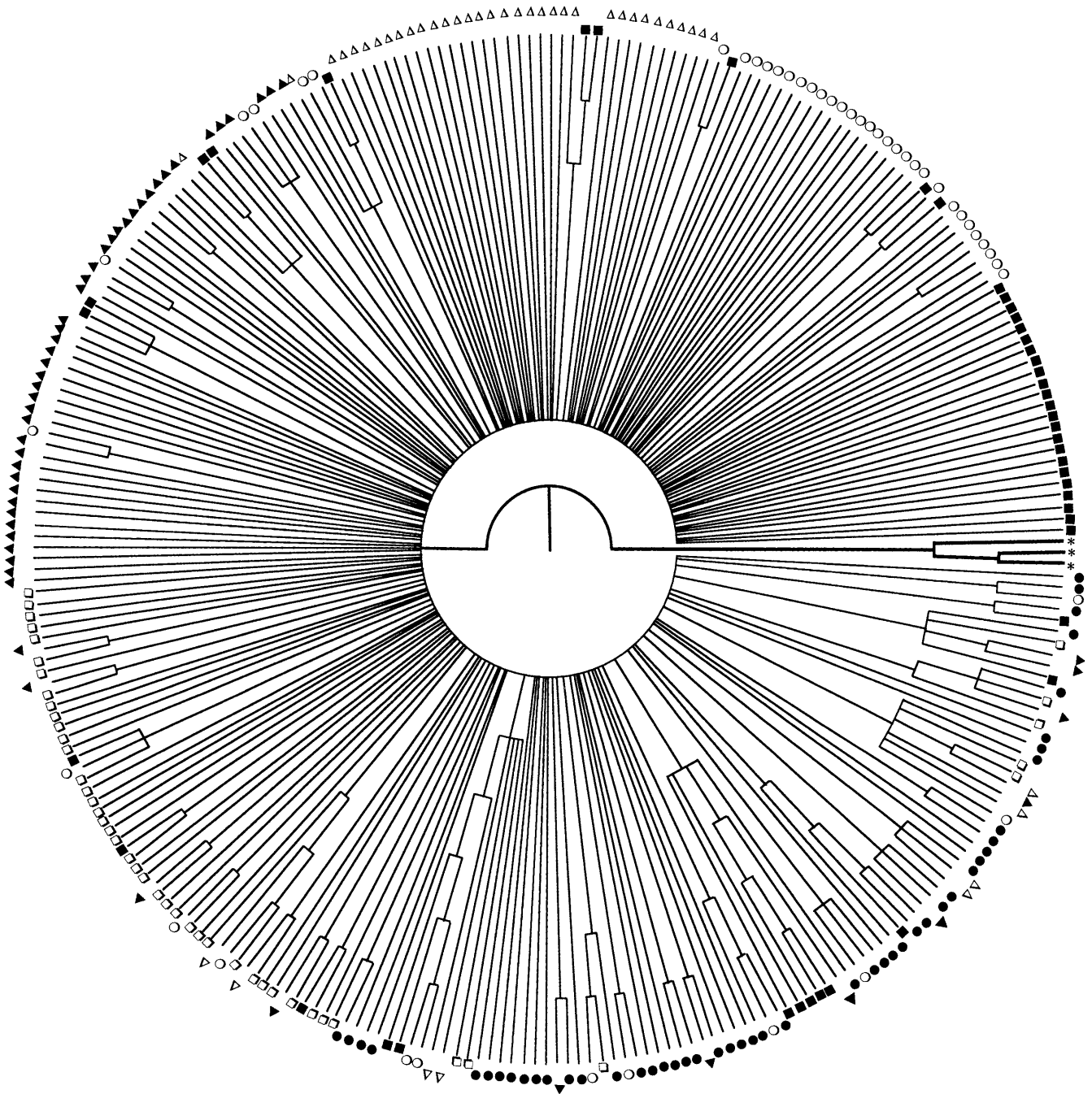


Fig. 1 *Xiphias gladius*. Neighbor-joining tree of Pacific swordfish [open symbols North Pacific demes (○ Japan; △ Hawaii; □ California-Mexico); closed symbols South Pacific demes (● Australia; ▲ Central Equatorial Pacific; ■ Chile)]. Symbols are tiered to enhance readability. Tree is rooted with three Clade II haplotypes from Mediterranean (*). Only nodes supported by bootstrap values > 50% are shown on tree

One factor potentially affecting genetic connectivity in the east may be our grouping of California and Mexico samples into a single deme. If these two collections are analyzed separately, Mexico fails to differ significantly from any Pacific population. However, California differs from Australia ($p = 0.006$), Japan

($p = 0.033$), the central equatorial Pacific ($p = 0.006$), and Chile ($p = 0.012$). California does not differ significantly from Mexico, hence genetic continuity in the eastern Pacific is still observed. Because the U-shaped pattern is derived from multiple p values, an adjustment should be made to control for group-wide Type-I error rates (Rice 1989). When the sequential Bonferroni test (Holm 1979) was applied to the p values in Table 3, only the sample sites of Australia and Japan were significantly different in the Pacific (adjusted $p = 0.019$), consequently eliminating the U-shaped pattern in Fig. 2.

To examine possible corridors of gene flow between the six sampled demes, pairwise Φ_{ST} s were plotted

Table 2 *Xiphias gladius*. Hierarchical analysis of molecular variance (AMOVA) of swordfish mtDNA control region sequences. Test of genetic subdivision among six demes in Pacific Ocean (F_{ST} genetic divergence)

Source of variation	(df)	Variance components	Percentage variation	Fixation index	p -value (10 000 permutations)
Among populations	(5)	0.06143	0.88	F_{ST} 0.009	0.00624 \pm 0.0008 ^b
Within populations	(276)	6.90176	99.12		

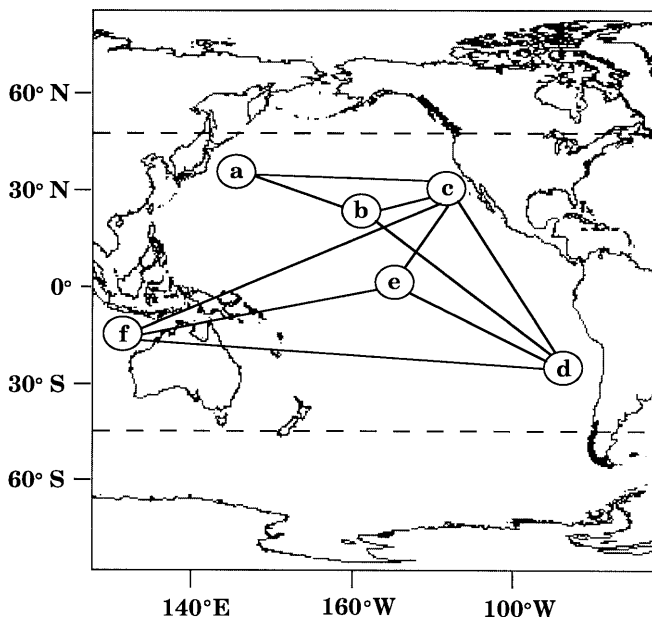
^b $p(\text{random value}) \geq p(\text{observed value})$

Table 3 *Xiphias gladius*. Genetic differentiation matrix of Clade I haplotypes. Φ_{ST} s above diagonal; p values below diagonal; number of permutations = 10 000

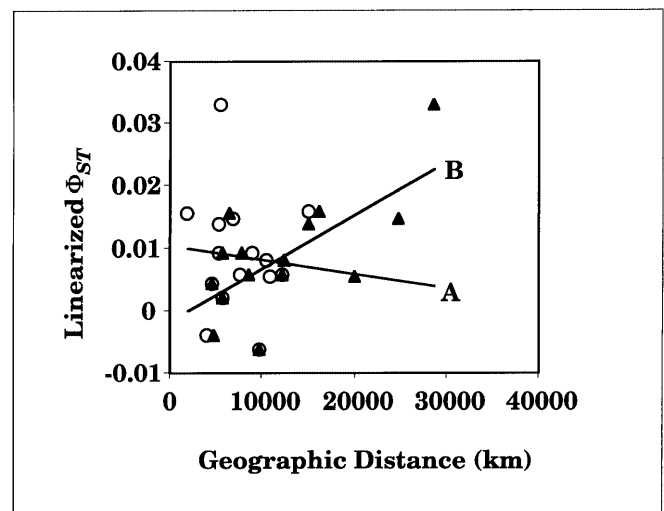
Population	Aust	Japan	Hawaii	Cal/Mex	Eq Pac	Chile
Australia (Aust)		0.032	0.014	0.005	0.002	0.006
Japan	0.001 ^b		0.004	0.006	0.014	0.015
Hawaii	0.030 ^a	0.221		-0.004	0.015	0.008
California–Mexico (Cal/Mex)	0.176	0.172	0.696		0.009	0.009
Central Equatorial Pacific (Eq Pac)	0.318	0.035 ^a	0.027 ^a	0.084		-0.006
Chile	0.175	0.028 ^a	0.124	0.098	0.837	

^a Significant p values

^b Significant p values after Bonferroni correction

**Fig. 2** *Xiphias gladius*. Map of Pacific basin showing U-shape pattern of genetic connectivity [lines connect pairs of demes not significantly different from each other as indicated in Table 3; demes are a Japan; b Hawaii; c California–Mexico; d Chile; e Central Equatorial Pacific; f Australia; dashed lines show latitudinal distribution of swordfish (Palko et al. 1981)]

against geographic distance in Fig. 3, assuming two different scenarios of migration in the Pacific. For the first scenario (circles in Fig. 3), geographic distances were measured between collection sites assuming that gene flow occurs along direct, linear paths between any two Pacific demes. Such an assumption would be appropriate for a highly migratory swordfish travelling randomly among a group of demes under an island model of migration. For this scenario, the slope of the

**Fig. 3** *Xiphias gladius*. Linear regression analysis for two scenarios of migration in Pacific swordfish. Linearized Φ_{ST} s are plotted against geographic distance between populations assuming either: migration follows direct straight path between two demes as in an island model (A, \circ); or migration follows U-shaped pattern of Fig. 2 (B, \blacktriangle). Regression equations and coefficients are as follows: A: $y = -2 \times 10^{-7}x + 0.0105$; $r^2 = 0.007$; $p = 0.762$; B: $y = 8 \times 10^{-7}x - 0.0016$; $r^2 = 0.464$; $p = 0.005$

regression line (A) in Fig. 3 is nearly flat, and is not different from zero ($p = 0.762$), indicating little correlation between genetic divergence and geographic distance. For the second scenario (triangles in Fig. 3), distances were measured between demes as straight-line paths arranged along the U-shaped pattern in Fig. 2. The positive slope of the regression line (B) observed for this scenario is significantly different from zero ($p = 0.005$), supporting a correlation between Φ_{ST} and geographic distance. If Pacific demes truly belonged to a single panmictic population, no significant correlation

should exist. This result indicates a trend in the data that can be examined further.

We used the Mantel test to further test the correlation between Φ_{ST} and geographic distance. Again, if geographic distance between demes is measured along the U-shaped corridor in Fig. 2, then a significant correlation exists between Φ_{ST} and geography ($p = 0.04$). For this test, Australia was not included as it differs significantly from Japan and only increases the statistical confidence ($p = 0.001$). Conversely, if we assume that all swordfish demes are connected by the shortest and most direct linear path between them, then the Mantel test produces a non-significant result ($p = 0.49$).

Discussion

Analysis of control region sequences in *Xiphias gladius* supports genetic population subdivision within the Pacific basin. This conclusion is based largely on the AMOVA, which provides unambiguous and highly significant evidence for rejecting the null hypothesis that genetic variation is randomly distributed among the six Pacific demes (Table 2). However, the AMOVA says little about the geographic corridors of gene flow and the pattern of population subdivision. Below, we discuss ways to infer geographical population structure and corridors of gene flow/migration in Pacific swordfish. We also discuss the implications these results have for management of the Pacific swordfish fishery.

Phylogenetic reconstruction

Phylogenetic analysis can elucidate patterns of geographic partitioning and gene flow among subpopulations, provided gene lineages have accumulated adequate mutations over time and that populations have been isolated long enough to allow for genetic drift. A phylogenetic approach is commonly used to discern evolutionarily significant management units, or ESUs (Ryder 1986; Waples 1991; Dizon et al. 1992; Vogler and DeSalle 1994). The rapid accumulation of mutation in the control region, as well as the simplicity of mtDNA inheritance through a single maternal line, provides a genetic marker highly susceptible to genetic drift and sensitive enough to test for structure among most populations (Avice 1994).

In swordfish, the level of polymorphism in the control region is sufficient to discern numerous unique mitochondrial lineages. Yet the phylogeny fails to provide evidence of discrete clades, despite attempts to enhance the phylogenetic signal using the Tamura–Nei distance estimate as well as a close relative, Clade II, to root the tree of Clade I haplotypes. The phylogeny is best described as star-like, possessing many unique lineages coalescing to a single node in the tree. The large homoplasy index (70%) calculated for this unresolved

tree is consistent with an occurrence of numerous mutational reversions along its branches, causing the actual genetic distance between relatives to be underestimated (Wakeley 1996; Yang 1996). Yet there is another caveat that can be used to explain this tree.

If a population experiences a bottleneck and subsequent rapid expansion, numerous mtDNA lineages could be generated, all tracing ancestry to a single node in the phylogeny (Slatkin and Hudson 1991). The high haplotypic diversity (99.9%) observed in Pacific swordfish mitochondrial lineages, as well as the coalescence of haplotypes to a single node, is compatible with a small population expanding and colonizing the Pacific. A similar scenario has been proposed to explain the high haplotypic diversity and star-like phylogeny of mtDNA lineages observed in our own species, *Homo sapiens sapiens* (e.g. Vigilant et al. 1991; Takahata et al. 1995). Yet human populations show significant levels of subdivision (Excoffier et al. 1992). Therefore, the lack of phylogeographic partitioning in the tree does not necessarily imply contemporary population panmixia.

Population structure and gene-flow corridors

Although our results show that genetic variation is not randomly distributed among Pacific demes, a pattern of population structure and gene flow has been difficult to verify statistically. Analysis of pairwise population Φ_{ST} s provides strong support for divergence between Australian and Japanese samples. Beyond this, the biologically accepted level of statistical significance ($p \leq 0.05$) is too stringent to reveal the subtle genetic differences that distinguish pairs of northern and southern swordfish populations in the Pacific, especially when the Bonferroni correction for multiple p values is applied. Nonetheless, we have observed a pattern in the data that was tested further.

The observed U-shaped pattern indicates that northern and southern populations in the eastern Pacific are genetically continuous, while those in the west have diverged. This pattern outlines a system of equatorial currents in the Pacific around which swordfish appear to migrate. Interestingly, phylogeographic relationships of mtDNA haplotypes among subspecies of the Pacific sardine follow a similar pattern (Bowen and Grant 1997). The importance of concordance between evolutionarily unrelated species is often used to lend support to observations in biogeography and molecular ecology (e.g. Avice and Ball 1990; Reeb and Avice 1990). We invoke this philosophy here to hypothesize that the U-shaped pattern of genetic connectivity observed in Pacific swordfish populations could be real, despite being weakly supported statistically.

Regression analyses similar to that of Slatkin (1993) and Rousset (1997) were used to test the null hypothesis of panmixia against two patterns of dispersal, one assuming an island model of migration and the other assuming a stepping stone model along the hypothesized

U-shaped corridor. If our sampled demes are significantly subdivided, a positive correlation between genetic divergence and geographic distance should exist. However, genetic isolation by geographic distance was observed only in the stepping-stone model. This was somewhat surprising for an endothermic, highly migratory species that presumably has no barriers to gene flow. Thus, we believe the U-shaped pattern illustrated in Fig. 2 reveals an important biogeographic pattern of population structure and gene flow in swordfish that may be applicable to other pelagic species in the Pacific.

By rejecting the island model of migration, we are left with two hypotheses. First, the Pacific may be composed of two distinct swordfish populations that overlap in the eastern Pacific. Alternatively, there may be several smaller populations exchanging genes along a U-shaped stepping-stone network (Kimura and Weiss 1964; Maruyama 1971) to produce a gradient of genetic differentiation around the Pacific. If F_{ST} (or Φ_{ST}) is very low, as in the case of swordfish, then migration rates estimated from them will be high. In theory, excessive migration among demes over time could cause the whole array of demes to behave as a single panmictic unit (Kimura and Maruyama 1971). However, our genetic data cannot distinguish between contemporary gene flow and that caused by recent common ancestry.

Fishery management

This study is the first to support swordfish population structure in the Pacific. However, the results leave two hypotheses that involve very different strategies of management. The first hypothesis is that there are at least two distinct populations of swordfish in the Pacific with range overlap in the east. The second hypothesis suggests that the six sampled demes represent several subpopulations interconnected by gene flow among neighbors. While the first hypothesis requires a plan to manage two discrete fishery stocks, the second hypothesis leaves open the possibility that stock depletion through overfishing would be overcome eventually through immigration from neighboring demes along the U-shaped corridor. By combining more recent fishery data with our genetic results, one hypothesis appears to be more applicable to swordfish than the other.

If the Pacific contains several self-sustaining subpopulations connected by a stepping-stone network of gene flow, then each should have its own breeding ground and geographic abundance would not necessarily fluctuate dramatically. Although larvae and reproductive adults are widely distributed, Nishikawa et al. (1985) describe high densities of larval swordfish occurring in two main areas, one encompassing the northwest and central Pacific (April through June), and the other in the southwest Pacific (October through December). Average annual catch data show three main regions of swordfish abundance, one in the northwestern Pacific, another in the southwest, and a third area in the

southeastern Pacific (Carocci and Majkowski 1996). Sosa-Nishizaki and Shimizu (1991) compiled 15 yr of temporal and spatial trends in monthly catch per unit effort data (CPUE) from the Japanese long-line fishery, and showed swordfish abundance extending continuously across the northern Pacific into the east during the autumn and winter months. In the summer, the northern fishery is restricted to the western Pacific. Catch in the eastern Pacific is fairly continuous across the equator throughout the year. This information helps to explain our results. It seems more likely that swordfish are comprised of two or three large populations with overlapping ranges in the eastern Pacific rather than a stepping-stone network of stationary subpopulations that exchange genes. Certainly, the lack of genetic divergence in the Mexico samples collected over a 6 mo time frame may hint of more than one stock moving into the northeastern Pacific at different times of the year.

A combination of tagging, catch analysis, and genetic techniques focused along the U-shaped corridor in the Pacific may ultimately provide fishery biologists with more precise information for managing swordfish, including location of stock boundaries and their temporal stability. Future work should include tagging studies to determine if the high degree of genetic similarity among some Pacific demes is due to contemporary gene flow (a concern for management) or is caused by recently shared common ancestry (of less concern). Sophisticated tags are now available that record geographic position and transmit the data to satellites for downloading without the recapture of individuals (Block et al. 1998). Areas of potential overlap, especially in the eastern Pacific, should be temporally monitored, with additional genetic sampling using both nuclear and mitochondrial markers to verify whether genetic composition changes seasonally. Also, more studies should be undertaken to locate spawning areas and gather information on the dispersal patterns of larvae.

We have elucidated a subtle pattern of population differentiation and gene flow among Pacific populations of swordfish showing regions of genetic similarity and those of genetic divergence more compatible with the management unit (MU) described by Moritz (1994) than with the phylogenetic ESU described above. The MU recognizes subtle, yet differentiable population segments connected by limited contemporary gene flow that are useful for conservation. Given the information presented in this paper, we encourage managers to now consider the existence of at least a northern and southern swordfish stock, both of which migrate through the coastal zones of several nations and into international waters.

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