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Mitochondrial Genetic Structure and Matrilineal Origin of White Sharks, *Carcharodon carcharias*, in the Northeastern Pacific: Implications for Their Conservation

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Abstract

White sharks (*Carcharodon carcharias*, WS henceforth) are globally and regionally threatened. Understanding their patterns of abundance and connectivity, as they relate to habitat use, is central for delineating conservation units and identifying priority areas for conservation. We analyzed mitochondrial data to test the congruence between patterns of genetic connectivity and of individual movements in the Northeastern Pacific (NEP) and to trace the matrilineal origin of immature WS from coastal California and Baja California to adult aggregation areas. We analyzed 186 mitochondrial control region sequences from sharks sampled in Central California (CC; $n = 61$), Southern California Bight (SCB; $n = 25$), Baja California Pacific coast (BCPC; $n = 9$), Bahía Vizcaíno (BV; $n = 39$), Guadalupe Island (GI; $n = 45$), and the Gulf of California (GC; $n = 7$). Significant mitochondrial differentiation between adult aggregation areas (CC, GI) revealed two reproductive populations in the NEP. We found general concordance between movement patterns of young and adult WS with genetic results. Young sharks from coastal California and Baja California were more likely born from females from GI. Mitochondrial differentiation of young-of-the-year from SCB and BV suggests philopatry to nursery areas in females from GI. These results provide a genetic basis of female reproductive behavior at a regional scale and point to a preponderance of sharks from GI in the use of the sampled coastal region as pupping habitat. These findings should be considered in Mexican and US management and conservation strategies of the WS NEP population.

Subject areas: Population structure and phylogeography; Conservation genetics and biodiversity
Key words: matrilineal origin, nursery areas, philopatry, population structure, white shark

The white shark [(WS), *Carcharodon carcharias*] occurs in temperate and subtropical regions of all major ocean basins Compagno et al. (2005). It has been recognized as a species vulnerable to overexploitation due to its low productivity, expressed as the intrinsic rate of increase or population rebound potential (Cailliet et al. 1985; Francis 1996; Pratt 1996; Curtis et al. 2014). Since the mid-1990s, it has become a widely protected species (Shivji et al. 2002), being listed as vulnerable in the Red List of the IUCN since 1996, and included in the Appendix II of CITES. In Mexico, it has been recognized as "threatened" since 2002 (DOF 2002), and fishing is prohibited by law (DOF 2007, 2014).

Despite this attention, basic facts about some biological aspects of its life cycle relevant for the development of conservation measures remain largely unknown. For instance, levels of genetic diversity, population connectivity, and trends in abundance remain largely unmeasured (Blower et al. 2012).

Very few population genetic studies have been conducted on WS, and what is known is generally consistent with its movement patterns. For instance, juvenile sharks tagged in eastern Australia remained in eastern nursery areas, and there is no record of them moving toward western shores (Bruce and Bradford 2012). This is consistent with the genetic differentiation found between eastern and western juvenile WS; a genetic structure guided by both parents as suggested by mitochondrial (mtDNA) and nuclear markers (Blower et al. 2012).

At a larger scale, there are few records of transoceanic movements of WS (Bonfil et al. 2005), and in the Pacific Ocean, there are no records documenting movements between the northwestern and northeastern Pacific. This is consistent with the genetic differentiation found between WS populations from Japan and California (Tanaka et al. 2011).

In the Northeastern Pacific (NEP), adult WS aggregate in two areas: Guadalupe Island (GI), in Mexico (Domeier and Nasby-Lucas 2008), and in the shelf waters off central California (CC), in the United States. (Boustany et al. 2002; Anderson and Pyle 2003; Weng et al. 2007a; Jorgensen et al. 2010). Adult sharks from both areas make long-distance seasonal migrations to a region in the subtropical eastern Pacific called SOFA—Shared Offshore Oceanic Area—as well as to the Hawaiian Islands (Boustany et al. 2002; Weng et al. 2007a; Domeier and Nasby-Lucas 2008).

Immature WS are found from the Southern California Bight (SCB), in the United States, to Baja California and the Gulf of California (GC), in Mexico (Weng et al. 2012), in productive nearshore waters, where most artisanal fisheries operate, making them vulnerable to incidental fishing mortality (Dewar et al. 2004; Weng et al. 2007b; Lowe et al. 2012; Santana-Morales et al. 2012). Some mature females appear to show philopatry to this area (Domeier and Nasby-Lucas 2013). Based on the presence of young-of-the year (YOY) WS, two hypothetical nursery grounds have been suggested: the SCB, in California (Weng et al. 2007b), and Bahía Vizcaíno (BV), in Baja California (Santana-Morales et al. 2012).

The spatial and temporal distributions of males and females at SOFA suggest a sex-biased migration and local spatial segregation. Adult male WS arrive first to GI around May–August and females after August, and both migrate to oceanic waters in December–March; the reason of these large-scale migrations is unknown (Domeier and Nasby-Lucas 2008). At SOFA, males are initially distributed in a concentrated core-area, whereas females move mainly in the periphery. Subsequently, when males start their return to GI, females move into the core-area (Domeier and Nasby-Lucas 2012). This lack of spatial and temporal overlap in the distribution of tagged adult male and females makes mating improbable (Domeier and Nasby-Lucas 2012; Jorgensen et al. 2012). Both sexes show philopatry to GI, where they return yearly (males) or every other year (females). It is assumed that the different return periodicity of females is related to breeding, since they move toward inshore waters of the SCB, Baja California, and the GC during the hypothetical breeding season (Domeier 2012).

Adult WS from CC show a comparable behavior. They have the same sex-biased distribution, migrate to SOFA and Hawaii, and are philopatric to CC (Jorgensen et al. 2012). The timing, distribution, and migration of CC sharks to oceanic waters are similar to those from Guadalupe (Weng et al. 2007a). There is no evidence of adult females from CC moving into nursery grounds in the Mexican Pacific coast, and there is only one record in the SCB of a female presenting recent mating-scars when tagged (Jorgensen et al. 2012).

This complex life cycle raises the need to genetically compare the two adult aggregation sites, to identify the genetic signature of YOY and juvenile sharks along the coast and in nursery areas, and to infer their matrilineal origin in order to understand the female reproductive strategies. If female adult aggregations were reproductively isolated and genetically differentiated, it would be possible to trace the matrilineal origin of coastal young WS to them. These genetic relationships are relevant because they would unveil the possible existence of more than one breeding population related to the philopatric behavior to the aggregation areas, GI and CC, and would provide the means to trace their offspring to these areas, which will increase our understanding of how adult WS make use of coastal regions in California and Baja California as nursery areas.

Consequently, we analyze the connectivity of WS in the NEP using mtDNA, a matrilineally inherited molecular marker, to test the hypothesis of congruence between known WS movements in the Northeastern Pacific (NEP) with those inferred from genetic analyses and to test if the matrilineal origin of young WS inhabiting coastal areas of California and Baja California can be traced to one or both aggregation areas in the region. This will shed light on the genetic patterns and reproductive dynamics of WS in the Northeastern Pacific and on their relative use of coastal nursery areas in California and Baja California. A better understanding of these biological aspects will have major implications for its management and conservation.

Material and Methods

Tissue Samples and DNA Extraction

White sharks were sampled in coastal areas between California and Baja California (SCB, BCPC, and BV), in the Gulf of California (GC), and GI (Figure 1). Muscle or skin tissue samples consisted of biopsies of free-ranging sharks or necropsies from incidental catches. All samples were kept frozen or preserved in 96% ethanol prior to genetic analyses. DNA was extracted using standard proteinase K digestion, purified using standard salting-out extraction protocols with LiCl, followed by organic extraction and subsequent ethanol precipitation (Aljanabi and Martínez 1997).

Amplification and Data Analyses

The mtDNA control region of WS was amplified with primers Pro-L2 and PheCacaH2 (Pardini et al. 2001) in 25 µL reactions containing 0.18 mM dNTPs, 1× PCR buffer (Tris HCl, 10 mM; KCl, 50 mM; and $MgCl_2$, 1.5 mM), 0.4 µM of each primer, 1 U of *Taq* DNAPol, and 20 ng of genomic DNA. Thermal cycling included an initial

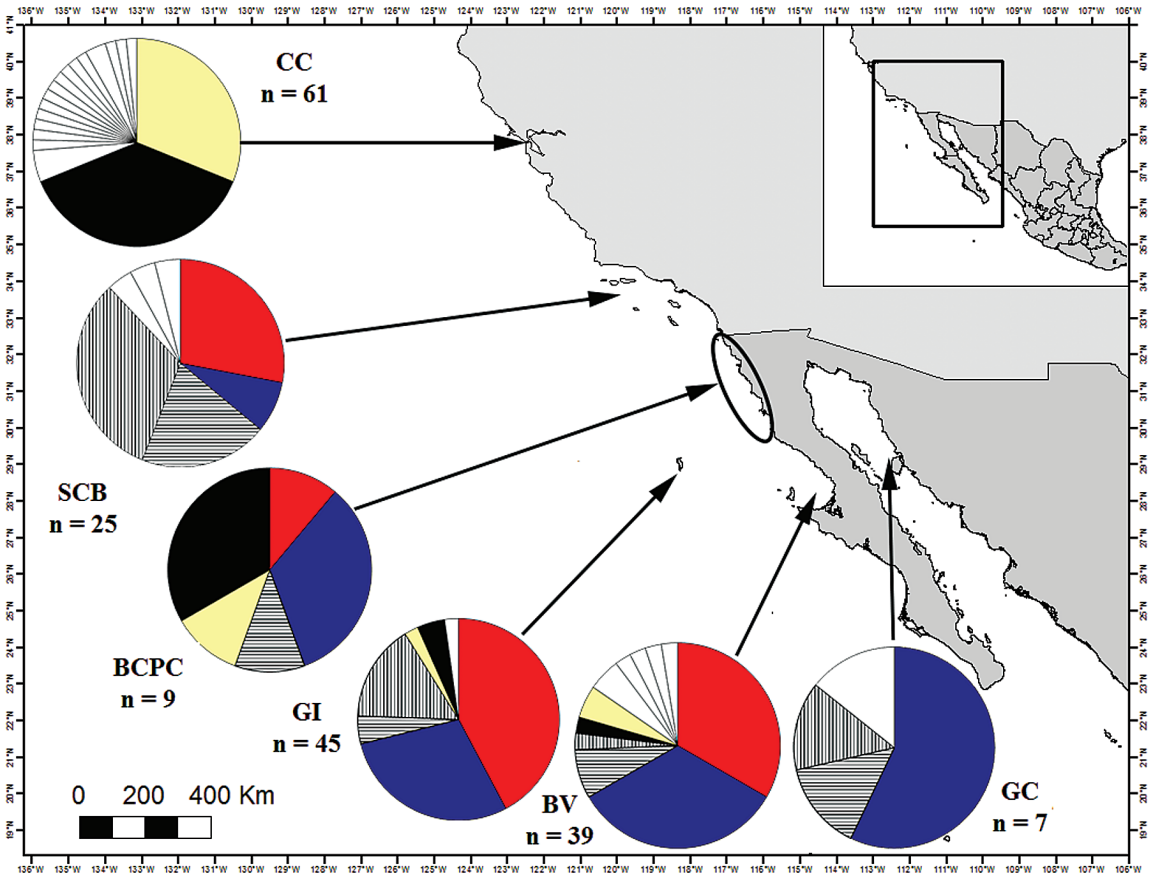

Figure 1. Mitochondrial variation in haplotype frequencies from Northeastern Pacific white shark samples. Each filled slice represents a shared haplotype, whereas white haplotypes are private. Studied areas are: central California (CC), Southern California Bight (SCB), Baja California Pacific coast (BCPC), Guadalupe Island (GI), Bahía Vizcaíno (BV), and the Gulf of California (GC).

denaturation of 5 min at 94 °C, 35 cycles of 15 s at 94 °C, 120 s at 63 °C and 45 s to 72 °C, and a final step of 5 min at 72 °C (Sandoval-Castillo et al. 2004). PCR products were enzymatically cleaned (exoSAP-IT, Affymetrix, Santa Clara, CA) before directly sequencing both DNA strands with an ABI 3130xl Genetic Analyzer. Base calling was verified with the program Codon Code Aligner v.1.6.3, and DNA sequences were aligned using MEGA v.5.10 (Kumar et al. 2004). The identification of distinct haplotypes and their frequencies was carried out using DNASP v4.0 (Rozas et al. 2003). Sequences produced in this study were from SCB, BCPC, BV, GI, GC, and CC (n = 2) (Genbank accession: KM014766-KM014781) and were analyzed with additional (n = 59) published sequences from CC (Genbank accession: GU002302–GU002321; (Jorgensen et al. 2010)).

We constructed a neighbor-joining (NJ) tree using MEGA v.5 (Tamura et al. 2011) to analyze mitochondrial lineages of WS from geographic areas worldwide including all mtDNA sequences available to us from the NEP, Northwestern Atlantic, South Africa, Australia, and New Zealand (GenBank accession: AY026196–AY026224; (Pardini et al. 2001)), and Mediterranean Sea (GenBank accession: HQ540294–HQ540298; (Gubili et al. 2011)).

Genetic diversity was estimated as haplotype (h) and nucleotide (π) diversities for mtDNA sequences as implemented in Arlequin v.3.5.1.2 (Excoffier et al. 2005). We tested the patterns of genetic differentiation using analyses of molecular variance implemented in Arlequin v.3.5.1.2 with a matrix of inter-haplotype distances based on a model on nucleotide evolution obtained with MODELTEST v3.7 (Posada and Crandall 1998) and PAUP* v4.0b10 (Swofford 2002).

Given the contrasting sex ratios of the GI and CC adult samples, we estimated the genetic differentiation (Φ_{st}) between sexes at each location, as implemented in Arlequin v3.5.1.2. In addition, we assessed the contrasting sex-ratios influence on the patterns of mitochondrial genetic structure using a resampling approach in which female and male control region haplotypes from GI and CC were randomly resampled with replacement to simulate 50 random samples possessing a close to even (f:m = 1:1.6 for CC similar to GI (Sosa-Nishizaki et al. 2012) or biased f:m = 1:3.8 for GI similar to CC (Dewar et al. 2013)) sex ratio. We computed pairwise Φ_{st} between samples of equivalent sex ratios (simulated biased GI or even CC with observed biased CC or even GI) as implemented in Arlequin v3.5.1.2.

In order to assess the parental origin of immature WS, we used their haplotype frequencies to calculate the most probable geographic origin of matrilineal haplotypes. We define this as the probability of randomly sampling a nonprivate (i.e., shared) mtDNA haplotype from an adult aggregation site (GI or CC) times the probability of sampling a shared haplotype with the adult aggregation area from the immature coastal sample (SCB, BCPC, BV, or GC). Given that haplotypes may also be shared between adult aggregation areas, we computed and added the probabilities independently for nonshared and shared haplotypes between adult aggregation areas (Equation 1).

$$P_O = (I_U * O_{unshared}) + (I_S * O_{shared}) \quad (1)$$

where P_o is defined as the probability of matrilineal origin to adult aggregation area O (GI or CC), I_u is the frequency among the immature WS sample of haplotypes shared with area O but unshared between adult aggregation areas, and $O_{unshared}$ is the frequency of haplotypes in area O unshared with the other aggregation area. I_s and O_{shared} refer to equivalent frequencies but of shared haplotypes between aggregation areas.

Data Archiving

In fulfillment of data archiving guidelines (Baker 2013), haplotype sequence data have been deposited with Dryad.

Results

We obtained 127 tissue samples of WS, of which those from SCB, BCPC, and BV were from immature specimens (22 females, 12 males, and 39 unknown); those from the GC included all developmental stages (2 females, 1 male, and 4 unknown), whereas those from GI were mostly from adults (13 females, 21 males and 11 unknown) and both tissue samples from CC were from adults (2 males). In total, we analyzed 186 mtDNA sequences [alignment length 1099 base pairs (bp)] of WS from the NEP.

Genetic Diversity

We found a total of 32 mtDNA haplotypes (see Supplementary Material online). BV and CC presented the highest number of different haplotypes, and the latter the highest number of private haplotypes (Table 1). In general, all localities had high values of haplotype diversity, BCPC and SCB showed the highest values (h = 0.83 and 0.80, respectively).

Phylogeography and Genetic Structure

Mitochondrial haplotypes from the NEP form a monophyletic group with those from New Zealand/Australia separated from Mediterranean, South Africa, and Atlantic in the NJ of all available WS control region sequences (Figure 2). We found a significant mtDNA genetic structure among all NEP geographic samples (Φ_{st} = 0.351; P < 0.001). This differentiation was driven mainly by the CC sample, which was very different from all the rest (Table 2).

In the comparison between sexes of adult WS from GI and CC, we found that 50% of the haplotypes were shared between males and females in GI, but in CC only 13% were shared and most of unshared haplotypes were present in males. There was no significant genetic differentiation between sexes in GI or in CC; however, the F_{ST} and P values were different in each location (Table 3). Whereas in CC, F_{ST} was essentially zero and largely nonsignificant (F_{ST} = −0.012; P = 0.51), in GI, it was larger and closer to being significant (F_{ST} = 0.044; P = 0.08). On the other hand, all comparisons of males and females between GI and CC produced large and highly significant F_{ST} values (Table 3). All comparisons of simulated and observed mitochondrial data sets with equivalent and biased sex ratios produced highly significant F_{ST} values between CC and GI (biased sex ratio 0.35 < F_{ST} < 0.46; unbiased sex ratio 0.34 < F_{ST} < 0.39).

Immature WS from near shore areas were significantly different, particularly SCB with respect to BCPC and BV (Φ_{st} = 0.10 and 0.11, respectively; both P < 0.05; Table 2), resulting from a frequent SCB haplotype being rare in BV and absent in BCPC (Figure 1). This distinction may be the result of distinct female assemblages using coastal nursery areas in the region (see below).

Matrilineal Origin of Immature Sharks

The matrilineal origin analysis showed that immature WS from BV are more likely related to females from GI (P_{GI} = 0.71) than from CC (P_{CC} = 0.05); as for the SCB's immature sharks, they have a 0.80 probability of being born from a GI mother, and they bear no matrilineal relationship with females from CC (Table 4). A high probability of matrilineal origin from GI was also observed in immature sharks from the GC (0.78). Juveniles from BCPC were also more likely born from a GI mother, but to a lesser extent than the others (P_{GI} = 0.54), than from a female from CC (P_{CC} = 0.31).

Discussion

This represents the most comprehensive study of white shark genetics in the NEP using a matrilineally inherited molecular marker (mtDNA), which allowed comparisons at a broad geographic scale to address habitat use of main nursery areas as they relate to adult aggregation sites.

We found two genetically distinct WS assemblages in the NEP, as evidenced by significant differences in mtDNA between adults from CC and GI. This distinction propagated to significant differences between CC and immature sharks from coastal sampled areas. Although CC WS have been previously reported as genetically distinct from other populations in the world (Jorgensen et al. 2010), our results highlight the complexity of the genetic patterns of WS in the NEP.

NEP WS Represent an Independent Population

Our phylogenetic results, which significantly extend the geographic sampling of previous studies in the NEP, confirm that WS from this region form a monophyletic mtDNA clade distinct from others in major distribution areas such as South Africa and Australia. Jorgensen et al. (2010) first established that WS from CC share an independent

Table 1. Genetic diversity of mtDNA sequences (1099 bp) from Northeastern Pacific samples of white sharks

Region	n	A	Ontogenetic stage	pA	h	π (%)
Central California[a]	61	18	Adult	16	0.77 ± 0.040	0.13 ± 0.09
Southern California Bight	25	7	Immature	3	0.80 ± 0.046	0.36 ± 0.21
BC Pacific Coast	9	5	Immature	0	0.83 ± 0.098	0.11 ± 0.08
Guadalupe Island	45	7	Both	1	0.73 ± 0.043	0.16 ± 0.11
Bahia Vizcaino	39	11	Immature	5	0.78 ± 0.046	0.11 ± 0.08
Gulf of California	7	4	Both	1	0.71 ± 0.181	0.10 ± 0.08
Total and average	186	36	—	—	0.77	0.0018

Sample size (n), number of haplotypes (A), private haplotypes (pA), haplotype diversity (h ± SD), and nucleotide diversity (π (%) ± SD) are given.
[a]Data from Genbank: accession numbers GU002302–GU002321 (Jorgensen et al. 2010).

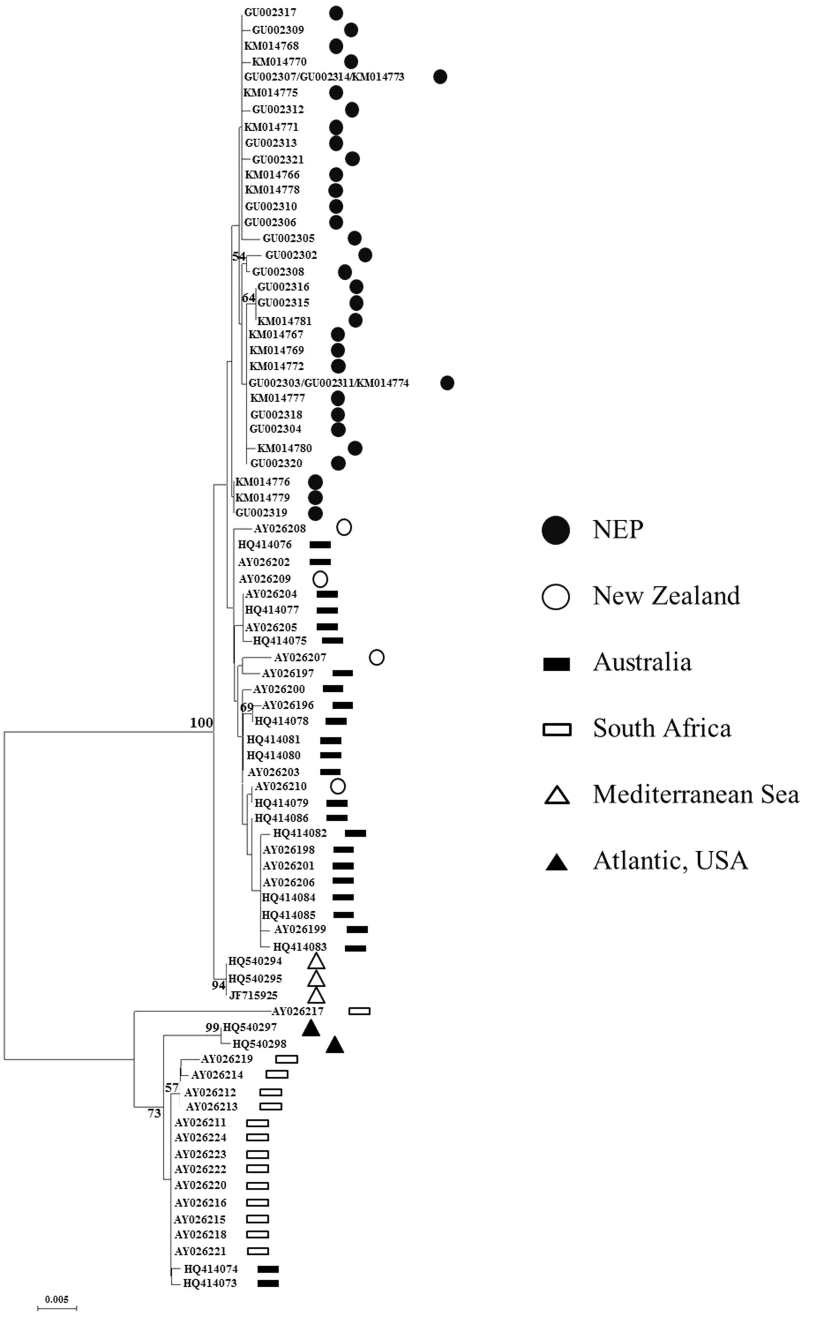

Figure 2. Neighbor-joining tree of available mitochondrial control region sequences from white sharks from Northeastern Pacific (present study and Jorgensen et al. (2010), black circles), New-Zealand (Pardini et al. 2001), white circles), Australia (Pardini et al. 2001; Blower et al. 2012), black squares), Mediterranean Sea (Gubili et al. 2011), white triangles), Atlantic, United States (Gubili et al. 2011), black triangles), and South Africa (Pardini et al. 2001), white squares). Numbers represent nonparametric bootstrap values.

mtDNA lineage more closely related to those in Australia/New Zealand. Another monophyletic lineage sister to the clade (NE Pacific, New Zealand/Australia) was found in sharks from Japan (Tanaka et al. 2011). Our extended results support that NEP WS represent a separate distinct population from other populations in the world.

Patterns of Genetic Variation in Mitochondrial DNA Suggest Female Philopatry to Nursery Areas

We found significant differences among sampled localities in the NEP. The mtDNA sequences revealed matrilineal genetic differentiation between WS from CC and those from SCB and Mexican waters, and among immature sharks from coastal localities. Given that mtDNA is maternally inherited, this differentiation could be affected by the different sex ratios observed in samples of the adult aggregation areas, where GI has a female:male ratio of 1.6 (Sosa-Nishizaki et al. 2012) and CC of 3.6 (Dewar et al. 2013; Jorgensen et al. 2010). Even though there was no statistically significant differentiation between sexes in both adult aggregation sites, as estimated by Φ_{ST}, the degree to which sexes were genetically undistinguishable and the distribution patterns of shared and unshared haplotypes between sexes differed between CC and GI, which could have potentially affected genetic structure in the face of significant sex-ratio bias in one of the localities. However, our simulation results equating sex-ratios between these samples (either as biased or unbiased)

Table 2. White shark's Φ*st* pairwise comparison between sampled regions

	CC	SCB	BCPC	GI	BV	GC
CC (n = 61)	—	***	***	***	***	***
SCB (n = 25)	0.39	—	*	ns	***	ns
BCPC (n = 9)	0.19	0.11	—	ns	ns	ns
GI (n = 45)	0.38	0.04	0.11	—	ns	ns
BV (n = 39)	0.40	0.10	0.10	0.01	—	ns
GC (n = 7)	0.45	0.04	0.09	0.07	0.07	—

Pairwise Φ_{st} are given below diagonal; significance is given above diagonal. *ns*, nonsignificant.
*$P < 0.05$; ***$P < 0.001$.

Table 3. Pairwise comparison between male and female white sharks from GI and CC

	GI females	GI males	CC females	CC males
GI females (n = 14)	—	0.075	0.000	0.000
GI males (n = 19)	0.04	—	0.000	0.000
CC females (n = 9)	0.42	0.32	—	0.511
CC males (n = 34)	0.41	0.39	–0.01	—

Pairwise Φ_{st} are given below diagonal; significance values are given above diagonal.

Table 4. Matrilineal origin probabilities (*PO*) of immature WS based on mtDNA haplotype frequencies from adult aggregation areas (CC and GI)

Immature WS	CC	GI
SCB	0.00	0.80
BCPC	0.31	0.54
BV	0.05	0.71
GC	0.00	0.78

show that the significant differentiation between CC and GI is unaffected by the presence or absence of sex ratio bias. In addition, the absence of records of WS movements from CC to the other sampled areas in this study supports these patterns of genetic structure. This differentiation at local scales was also found in Australia, where genetic differentiation of immature WS between eastern and western coasts agrees with a lack of movements between these areas (Blower et al. 2012).

Overall, the mitochondrial results suggest that adult female WS from GI may display philopatric behavior to distinct coastal parturition areas (SCB and BV) driving the matrilineal differentiation. However, more observations using bi-parentally inherited markers (nuclear DNA) are needed to understand the reproductive patterns between sexes. Efforts are currently being directed toward that goal. Sex-biased genetic differentiation and gene flow were found in WS from Australia/New Zealand and South Africa, based on mitochondrial and nuclear data (Pardini et al. 2001), raising the possibility to find similar patterns among NEP adult WS.

Congruence Between Movements and Genetic Heterogeneity

Mitochondrial sequences revealed significant differences between WS from both adult aggregation areas (GI and CC). Sharks from both regions display philopatric behavior, returning to their respective aggregation island after migrating to oceanic areas (Weng et al. 2007a; Domeier and Nasby-Lucas 2008). The lack of evidence that WS from each area interbreed (Domeier 2012) supports the genetic differences found between adults from GI and CC.

Tagging studies have assessed the relationship between NEP adults and immature WS, and our results support these relationships. Adult females from GI move to SCB, BV, GC and back to GI (Domeier and Nasby-Lucas 2008, 2013), and this is consistent with their being genetically indistinguishable from immature sharks sampled in these coastal localities (Table 2). On the other hand, adults from CC move to the oceanic area and back to CC, but there is no record of their moving to SCB or any coastal area to the south (Weng et al. 2007a; Jorgensen et al. 2010), which is consistent with the significant differences found between CC and the coastal sampled localities.

Despite their proximity, none of the shared haplotypes from CC is present in SCB. Additionally, even though SCB is a nursery area for WS and is close to CC, there are only few juveniles from SCB that have been recorded moving northward along the coast (Weng et al. 2007b), and there is only one recorded movement of an adult female from CC to the SCB. However, this lack of data may result from insufficient observations; hence, there is a need to increase tagging efforts of adult WS from CC and juveniles from SCB for a better understanding of the WS ontogenetic movements and to analyze those samples using nuclear molecular markers.

Most of the tagged immature WS from SCB move alongshore to the south and back (Weng et al. 2012). However, some sampled immature WS show matrilineal (mtDNA) genetic differentiation among areas; SCB was different from BCPC and BV, but neither was distinct from GI adults. If this pattern reflects a temporally consistent differentiation between mothers giving birth in these areas, it would suggest the existence of two female groups from GI each showing reproductive philopatry to their coastal parturition areas in SCB and BV, respectively, as previously discussed.

Mitochondrial findings also suggest that, at least during our sampling time, BCPC was more visited by immature sharks from BV. The suggested female philopatric behavior would be consistent with the movement of some presumably pregnant females to either SCB or BV (Domeier and Nasby-Lucas 2013). However, the number of tracked pregnant females from GI remains insufficient to fully corroborate this pattern. Most YOY from SCB remain in that nursery during early growth and start increasing their range of movements (mostly southward) after close to 1 year of life (Weng et al. 2007b, 2012). As juveniles, WS from SCB move southward to BCPC and even to BV. Although there are no records of movements of WS from BV, our results suggest that they can move to north to BCPC; however, studies on the movements of WS from BV are required to test this inference. The genetic differences between SCB and BCPC suggest variability in the magnitude of southward movements recorded from SCB, or that juvenile movements are not restricted to coastal regions, being capable of venturing into more oceanic waters (Weng et al. 2012).

Matrilineal Origin of Immature Sharks

The majority of immature WS sampled in coastal localities possessed a higher probability of having a mitochondrial lineage traceable to adults from GI. Although SCB is geographically closer to CC, sharks from SCB are more likely born from GI mothers. This is consistent with recorded movements of some adult females from GI to SCB during the hypothesized parturition season and of others from GI to near BV (Domeier and Nasby-Lucas 2013). The question remains about the temporal and spatial location of the progeny of adult WS from CC in the coastal areas of California and Baja California. This raises the need of conducting parentage analyses based on biparentally inherited markers to better

establish in both sexes the relationship between immature sharks from coastal nursery areas and the adults from aggregation sites.

Conservation Implications

Our results highlight the importance of the separated management strategies in the United States and Mexico, as implemented at present, for the conservation of WS reproductive populations. In Mexico, GI has been protected since 2005 (DOF 2005), which has led to conservation measures and increased interest in their biology and ecology, particularly in GI. We support the continuation of these conservation efforts, as they protect the future maintenance of the WS population.

Genetic differences between SCB and BV led us to suggest the existence of two independent nursery areas for WS in the NEP. These differences require the implementation of a national conservation strategy aimed to protect the Mexican nursery grounds for WS, namely BV. We suggest that a WS-oriented conservation measure should first map the high-risk areas in the bay that have shown the highest incidental catches, presumably related to the location of WS transit tracks, which should then be the focus of no take zones, or at least become out of reach of the bottom gillnet fisheries. Given the inferences stemming from our genetic results, there is also a need to track the movements of immature and YOY WS from BV.

Another conservation measure that needs to be promoted is the release of immature WS that are still alive during artisanal fishing operations. As reported by Santana-Morales et al. (2012), incidental catch of WS occurs along the west coast of Baja California. The Mexican government established a threatened status for the species (DOF 2002), and much more recently has established a total fishing ban for WS in Mexican waters (DOF 2014). However, this protected status could be improved by including a provision to release of incidentally caught WS as an extra protective measure. The release of live sharks will contribute to increased immature survivorship and, based on our results showing that most of these sharks are born from GI females, should prove beneficial to the maintenance of the adult population from the most important aggregation area in Mexico.

Finally, future genetic studies addressing the relationships of WS from different areas of the NEP should strive to extend the number of analyzed sharks from CC and to include nuclear analysis and parentage analysis to assess the bi-parental relationship between immature and adult WS.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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