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Local population structure and context-dependent isolation by distance in a large coastal shark

Jimiane L. Ashe[1], Kevin A. Feldheim[2], Andrew T. Fields[1], Eric A. Reyier[3], Edward J. Brooks[4], Martin T. O'Connell[5], Gregory Skomal[6], Samuel H. Gruber[7,8], Demian D. Chapman[1,*]

[1]Institute for Ocean Conservation Science/School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794-5000, USA

[2]Pritzker Laboratory for Molecular Systematics and Evolution, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605, USA

[3]Kennedy Space Center Ecological Program and InoMedic Health Applications, Kennedy Space Center, Cape Canaveral, FL 32920, USA

[4]Shark Research and Conservation Program, Cape Eleuthera Institute, Eleuthera, The Bahamas

[5]Nekton Research Laboratory, Pontchartrain Institute for Environmental Sciences, University of New Orleans, New Orleans, LA 70148, USA

[6]Massachusetts Shark Research Project, Division of Marine Fisheries, 1213 Purchase St., New Bedford, MA 02740, USA

[7]Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Miami, FL 33149, USA

[8]Bimini Biological Field Station Foundation, Miami, FL 33176, USA

ABSTRACT: Genetic diversity, population genetic structure and isolation by distance (IBD) were assessed in a viviparous coastal shark (the lemon shark *Negaprion brevirostris*) across 8 western Atlantic samples spaced between ~150 and 7000 km apart. Juveniles (N = 325) were sequenced at 2 mitochondrial loci (1729 bp) and typed at 9 nuclear encoded microsatellite loci. Analysis of mitochondrial sequences revealed higher diversity at low-latitude island samples compared to high-latitude continental samples, consistent with an equatorial center-of-origin for this species. There were 5 distinct groups across our sampling areas (Brazil, Louisiana, Cape Canaveral, Gullivan Bay and the Florida Keys/Bahamas/Virgin Islands; pairwise Φ_{ST} = 0.07–0.87) and all but one pair of the 8 samples also exhibited significantly different haplotype frequencies (pairwise F_{ST} = 0.10–0.51). Bayesian analysis indicated that the Brazil and Louisiana samples were generally isolated from the others, but most of the rest were diverged although still connected or recently connected by migration. In contrast, structure was only detected between the most distant sample (Brazil) and all of the others using the microsatellite markers (pairwise F_{ST} = 0.03–0.06). There was a significant pattern of IBD for all markers and measures of genetic differentiation (r^2 = 0.65–0.81, $p < 0.05$–0.01), but not after removing the Brazil sample. There was evidence that glacial and post-glacial historical processes and sex-specific differences in philopatry affected IBD. Because of the relatively fine-scale population structure of this and other large coastal shark species more attention should be paid to local processes in the conservation and fisheries management of these species.

KEY WORDS: Mitochondrial DNA · Microsatellites · Phylogeography · Population structure

INTRODUCTION

The genetic diversity of marine fish populations is determined by a combination of historical demography, drift, selection and the introduction of genetic material from outside populations mediated by dispersal and reproductive mixing ('gene flow'). In broadcast spawning fish, gene flow can occur through larval advection or active movements of older stages (Cowen & Sponaugle 2009). Active

*Corresponding author: demian.chapman@stonybrook.edu

movements alone determine gene flow in fish that have direct development, including sharks and their relatives (Frisk et al. 2014). Any geophysical feature that inhibits individual movements or causes a break in their distribution will restrict gene flow, which promotes population genetic differentiation. This type of population genetic structure may also develop within a continuously distributed species when gene flow between distant populations is limited because reproduction is more likely to occur between proximate individuals. In such cases, it is expected that geographical and genetic distances between populations will be positively correlated, a pattern known as isolation by distance (IBD) (Wright 1943).

Large coastal sharks are often widely distributed and have the ability to move long distances (Kohler et al. 1998). Population genetic structure has often been found on regional geographic scales when large oceanic expanses or thermal barriers (e.g. the Benguela upwelling off southwest Africa) inhibit gene flow (e.g. Benavides et al. 2011a,b). Correspondingly, stock assessments are usually performed assuming that population structure develops at large, regional scales or when populations are obviously subdivided by a geophysical barrier (Cortés 2004). Population structure between sites separated by <1000 km and in the absence of barriers has been examined in relatively few species of large coastal shark (Keeney et al. 2005). Given that some individual sharks exhibit fidelity to mating or parturition sites (Feldheim et al. 2002a, 2004, 2014, DiBattista et al. 2009, Mourier & Planes 2013) it is conceivable that structure at this smaller spatial scale is also common. Determining whether or not this is the case is an important step to properly scale stock assessments in situations where fishing does not affect all populations proportionally. Assessments are urgently needed to inform management, given high levels of exploitation and evidence of exploitation-driven declines in many coastal shark populations (Worm et al. 2013, Dulvy et al. 2014).

Many commercially important coastal sharks belong to the family Carcharhinidae, and one of the most intensively studied species in this lineage is the lemon shark *Negaprion brevirostris* (e.g. Feldheim et al. 2002a, 2004, 2014, Chapman et al. 2009a, DiBattista et al. 2009). A cosmopolitan ancestral lemon shark species diverged into separate Atlantic and Indo-Pacific species with the cessation of gene flow after the closure of the Tethyan corridor 12 to 20 million years ago (Schultz et al. 2008). The Atlantic species, *N. brevirostris*, is continuously distributed in the western Atlantic from the southern USA to southern Brazil, with physically and genetically disjunct populations also occurring in the eastern Atlantic and eastern Pacific (Schultz et al. 2008). Lemon sharks generally conform to the model life history proposed for coastal sharks by Springer (1967), where adult females give birth in shallow, protected nursery sites and juveniles disperse from these areas to join the adult population. Juveniles remain in their natal nursery site until reaching sizes of ~90 cm in subtropical and tropical nursery sites but migrate to overwintering habitat after their first summer at higher latitude nursery sites at the margin of their distribution (Chapman et al. 2009a, Reyier et al. 2014). Older juveniles may remain close to or return to their natal site for many years (Chapman et al. 2009a). Mature lemon sharks of both sexes make long-distance coastal movements (>750 to 1000 km) and cross relatively narrow oceanic stretches, such as the Florida Straits (Kohler et al. 1998, Feldheim et al. 2001, 2014). Despite this, many females giving birth at nursery sites at Bimini, Bahamas and Marquesas Key, Florida, practice parturition site-fidelity on a biennial cycle (Feldheim et al. 2002a, 2004, 2014, DiBattista et al. 2009). Some females home to the exact nursery site where they were born to give birth ('natal philopatry'; Feldheim et al. 2014). Males rarely sire offspring in the same nursery site more than once (Feldheim et al. 2002a, 2004, DiBattista et al. 2009), indicating either male-biased dispersal or that mating takes place when adults born in different places are admixed.

There are no obvious geophysical barriers that would restrict movements and reproductive mixing between lemon sharks in the western Atlantic. Nonetheless, population structure exists between sampled nursery sites at the northern and southern range extremes (i.e. Florida/Bahamas and Brazil; Feldheim et al. 2001, Schultz et al. 2008). Population structure is observed at this same scale for other large coastal sharks in the western Atlantic, including scalloped hammerhead (*Sphryna lewini*; Chapman et al. 2009b), bull (*Carcharhinus leucas*; Karl et al. 2011) and nurse sharks (*Ginglymostom cirratum*; Castro 2011). This could be a simple function of the distance between these sites (~5000 to 7000 km) given that the longest known distance travelled by many of these species is ~1000 km (Kohler et al. 1998, Feldheim et al. 2001). Given the level of parturition site-fidelity and natal philopatry documented in gravid lemon sharks, it is also plausible that there is even finer scale population genetic structure among nursery sites within this continuous range, at least at maternally inherited markers, given that these behaviors have only been documented in females. If there were finer scale population

structure, we would also expect a pattern of IBD, assuming that gene flow mediated by straying or homing error is most likely to occur between proximate populations. Here we use both mitochondrial and nuclear encoded genetic markers to assess the genetic diversity, structure and gene flow among lemon shark populations in the western Atlantic at spatial scales ranging from 10^2 to 10^3 km. We tested the following hypotheses: (1) all samples have similar levels of genetic diversity, (2) there is population structure at finer geographic scales than just between the northern and southern range extremes and (3) population genetic differentiation is positively correlated with geographical distance between sampling sites.

MATERIALS AND METHODS

Sampling

Juvenile lemon sharks (N = 325) were sampled at 8 sites (Fig. 1). Seven of these sites were in the Northern Hemisphere and 3 occurred along ~1500 km of the continental margin of North America (Gullivan Bay [GB, N = 30 ind.] and Cape Canaveral [CC, N = 42] in Florida and the Chandeleur Islands [CI, N = 40] in Louisiana, northern Gulf of Mexico). We hereafter refer to these as the 'continental' samples. The remaining Northern Hemisphere samples were collected around islands from ~130 to 1730 km off the North American coast, over a total distance of ~1900 km. These were Marquesas Key (MK, N = 40) in Florida, Bimini (BI, N = 48) and Eleuthera (EL, N = 45) in the Bahamas and the US Virgin Islands (VI, N = 36) in the eastern Caribbean. We hereafter refer to these as the 'island' samples. The most distant site from all of the others was in the Southern Hemisphere, at Atol das Rocas in Brazil (RO, N = 44). All individuals included in a sample were measured to the nearest 0.1 cm for pre-caudal length (PCL), fork length (FL) and total length (TL), sexed and had a small piece of fin removed and stored in 20% DMSO (Seutin et al. 1991) for genetic analysis. All samples were composed of individuals from 67 to 90 cm TL, with the exception of Gullivan Bay, likely meaning that that all but this latter sample were composed of individuals in close proximity to their natal site (Chapman et al. 2009a). Besides Gullivan Bay, which included individuals up to 170 cm, the only exception was the Cape Canaveral sample. All Cape Canaveral

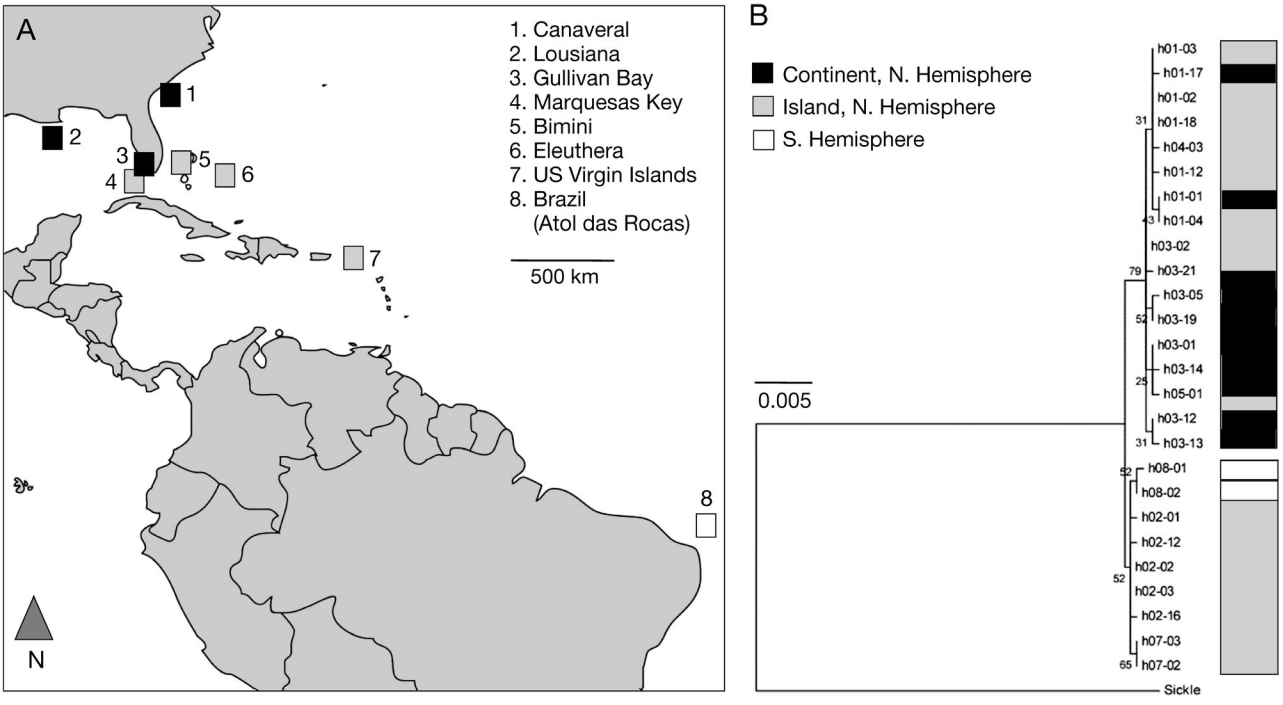

Fig. 1. (A) North and South America showing locations where juvenile lemon sharks *Negaprion brevirostris* were sampled. Northern Hemisphere continental sampling locations are shown in black, Northern Hemisphere island sampling locations, in grey and the Southern Hemisphere site (Atol das Rocas, Brazil), in white. (B) Maximum-parsimony tree depicting the relationships between concatenated mitochondrial haplotypes. The bar to the right of the tree shows whether haplotypes are found primarily in Northern Hemisphere continental (black), Northern Hemisphere island (grey), or Southern Hemisphere (white) samples. The out-group is a sicklefin lemon shark *Negaprion acutidens*. The scale bar in (B) shows genetic distance

individuals were <90 cm TL; however, the sharks only overwinter at this site and are most likely born in northern Florida, Georgia, or the Carolinas (Reyier et al. 2014). All individuals in all locations were tagged with passive integrated transponders and/or dorsal fin roto-tags prior to release, preventing the accidental inclusion of the same individual more than once in the dataset.

Laboratory analysis

Genomic DNA was extracted from tissues using the Qiagen blood and tissue extraction kit (Qiagen). Sequences from the entire mitochondrial control region (CR, 1080 bp) were PCR-amplified using CR proline transfer RNA light-strand forward primer Pro-L (5′-AGG GRA AGG AGG GTC AAA CT-3′) and ribosomal RNA heavy-strand reverse primer 282 12S (5′-AAG GCT AGG ACC AAA CCT-3′) (Keeney et al. 2003). This locus was previously found to have relatively low nucleotide and haplotype diversity in our sampling region, and there were shared haplotypes between the Bahamas/Florida and Brazil, which are over 7000 km apart (Schultz et al. 2008). We therefore had concerns that CR would have limited resolution on its own and may be insufficient to test our hypotheses. Partial sequences from the mitochondrial ND2 gene (650 bp) were obtained to supplement our analyses, using forward (5′-TGT ATT AAC CAT CCT AAT TTC AAG-3′) and reverse (5′-GGT GTT AGG GCA GAA GGA TGG ATA-3′) primers designed from GenBank Accession No. U91418. For both loci, PCR was carried out in 50 µl volumes containing 1 µl DNA template (~20 ng), 1× CoralLoad PCR buffer, 200 µM of each deoxyribonucleotide (dNTPs), 1 U HotStar *Taq* DNA Polymerase (Qiagen) and 0.25 µM forward and reverse primers. Cycling parameters included an enzyme activation step of 95°C for 15 min, 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, and a final extension at 72°C for 10 min with a MultiGene thermal cycler (Labnet International). PCR products were purified with ExoSAP-IT (Affymetrix) and sequenced using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems) with a Bio-Rad DYAD thermal cycler (Bio-Rad Laboratories). The resulting products were precipitated with 125 mM EDTA and 100% ethanol and run on an ABI 3730 DNA Analyzer (Applied Biosystems). Resulting sequences were validated by eye and aligned in Geneious Pro 5.1.7 (Drummond et al. 2010), where haplotypes of CR and ND2 were identified manually. Polymorphic sites that defined haplotypes were sequenced in both forward and reverse directions in 1 to 10 ind. to ensure they were valid. Once separate haplotypes of ND2 and CR were identified for each individual, a new concatenated sequence file was created with the contiguous sequence of the 650 nucleotides from ND2 followed by the 1080 nucleotides from CR, for a total of 1729 bp for each individual (hereafter referred to as mtND2-CR). ND2 sequences were translated in Geneious Pro 5.1.7 to determine whether any mutations changed the amino acid sequence of the protein and could therefore be influenced by selection. All population genetic analyses were run using this concatenated mtND2-CR sequence.

All sampled sharks were genotyped at 9 polymorphic microsatellite markers that are described elsewhere (Feldheim et al. 2002a,b, 2004, DiBattista et al. 2008, 2009). An independent analyst rescored a subset of genotypes scored by the primary analyst (J.L.A.) to determine the error rate and make corrections. Individuals that were homozygotes or had weak bands were re-amplified up to 3 times (see DiBattista et al. 2008 for more information on the quality control protocols used for this dataset).

Statistical analysis

Haplotype diversity (h) and nucleotide diversity (π) of the mtND2-CR were calculated in DnaSP 4.0 (Rozas et al. 2003) for the entire dataset and for each sample individually. The relationship between all of the concatenated mitochondrial haplotypes was visualized using a maximum-parsimony tree in Paup 4.0 (Swofford 2003), and a minimum-spanning network was implemented in TCS 1.21 (Clement et al. 2000). Global population differentiation was first assessed using an analysis of molecular variance (AMOVA) implemented in Arlequin 3.5.1.2 (Excoffier & Lischer 2010), using the Tamura-Nei model of sequence evolution, which in jModeltest2 (Darriba et al. 2012) was the highest ranked model that is also available in Arlequin 3.5. The use of alternative models available in Arlequin 3.5.1.2 had no substantial effect on the results. Pairwise Φ_{ST} and haplotype frequency-based F_{ST} were calculated between samples, with the significance of all positive values assessed with 10 000 permutations of the data. Bonferroni adjustments of α were used to correct for multiple tests.

Multilocus microsatellite genotypes were compared between all individuals within a sample to identify potential littermates or identical genotypes using the program ML-Relate (Kalinowski et al. 2006).

Microsatellite diversity for each sample was expressed as the number of alleles (k) and the observed and expected heterozygosity (H_o and H_e, respectively). Each sample/locus combination was tested for Hardy-Weinberg equilibrium in Arlequin 3.5.1.2 using the Markov chain exact probability test (Guo & Thompson 1992) with 100 000 iterations and 1000 dememorization steps. Bonferroni adjustments of α were used to correct for multiple tests. These loci have previously been shown to sort independently so no further linkage testing was performed (Feldheim et al. 2002a,b, 2004, DiBattista et al. 2008, 2009). Differentiation between samples at microsatellite loci was assessed using pairwise frequency-based F_{ST} as implemented in GENEPOP 4.0 (Raymond & Rousset 1995). Bonferroni adjustments of α were used to correct for multiple tests. STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to infer the number of populations (clusters) within the dataset. It was run for a burn-in period of 15 000 Markov chain Monte Carlo (MCMC) steps followed by 350 000 MCMC steps using the admixture model (with/without *a priori* location) for $K = 1$ to 5 for 10 independent runs each to determine convergence. To infer the correct number of clusters K, Pritchard et al. (2000) suggest determining the convergence of the mean estimate of the ln probability of ΔK. Further, we used the ΔK metric suggested by Evanno et al. (2005) to determine the statistically most supported number of clusters as implemented in STRUCTURE HARVESTER 0.6.8 (Earl & vonHoldt 2012).

Genetically differentiated populations can still be connected by gene flow. We used the Bayesian MCMC program IMa2 (Hey & Nielsen 2007) to estimate the time since divergence (t) and rate of gene flow (m), both scaled by the mutation rate, for each of the 28 pairwise comparisons of our samples in order to resolve differentiated, isolated populations from those that were differentiated but still connected by migration. These analyses were only conducted for mitochondrial sequence data because of a general lack of structure observed in microsatellites (see 'Results'). The IMa2 model assumes that the populations (i.e. samples) compared have no genetic input from unsampled populations, but, when these assumptions have been violated, IMa2 has still been shown to distinguish between populations that are completely isolated and those that have diverged but maintained some gene flow (Machado et al. 2002, Won & Hey 2005, Niemiller et al. 2008). Preliminary runs were used to establish priors for final runs. Final runs consisted of a burn-in period of at least 200 000 genealogies and a post-burn-in period of 1 000 000 genealogies. Each run used 40 to 150 chains with geometric heating. For each pairwise comparison the posterior probability distribution of t was examined to determine the probability that the divergence time was zero, which would indicate a lack of divergence between the 2 populations (Portnoy et al. 2010). For comparisons with a probable non-zero divergence, the posterior probability distribution of m was evaluated to determine the probability of zero gene flow, which indicates the 2 populations are fully isolated from each other (Won & Hey 2005, Niemiller et al. 2008). A log-likelihood ratio test was used to create log-likelihood ratio statistics (2LLR) to compare the model fit without migrations to the null model, which included migration (Hey & Nielsen 2007), for comparisons that had an m value close to zero or a peak probability close to the probability of zero. Parameter estimations were left in mutation-scaled format to avoid the uncertainty caused by violating the assumption of the model (Wakeley 2000, Strasburg & Rieseberg 2010).

We tested for IBD by using a Mantel test to correlate each pairwise measure of genetic differentiation (i.e. pairwise Φ_{ST}, F_{ST} for mtDNA and F_{ST} for microsatellite data) with the corresponding minimum geographical distances between sampling sites. We measured the distance between each site assuming that lemon sharks would minimize travel across deep open water, which corresponds to the 'coastal distance' used by Schultz et al. (2008). The only exception was all of the distances between the Brazil site and each of the other sites, which were measured assuming that dispersing sharks would island-hop along the windward Caribbean islands as opposed to travelling the much longer distance around the North and Central American continental margin. The tests were conducted using the web interface IBDWS 3.23 (Jensen et al. 2005; http://ibdws.sdsu.edu/~ibdws/). Significance was assessed by 30 000 randomizations of the data. We also examined finer scale IBD by conducting Mantel tests with the 7 Northern Hemisphere samples (i.e. all samples excluding Brazil) and also on the 4 island samples alone (i.e. Marquesas Keys, Bimini, Eleuthera and US Virgin Islands).

RESULTS

Twenty-six mtND2-CR haplotypes were found among 325 juvenile lemon sharks sampled from Cape Canaveral, Florida, USA, to Atol Das Rocas, Brazil (Figs. 1–3, GenBank Accession Numbers FJ008700–FJ008710 and KP303694–KP30371). All samples were screened for identical genotypes and littermates, the

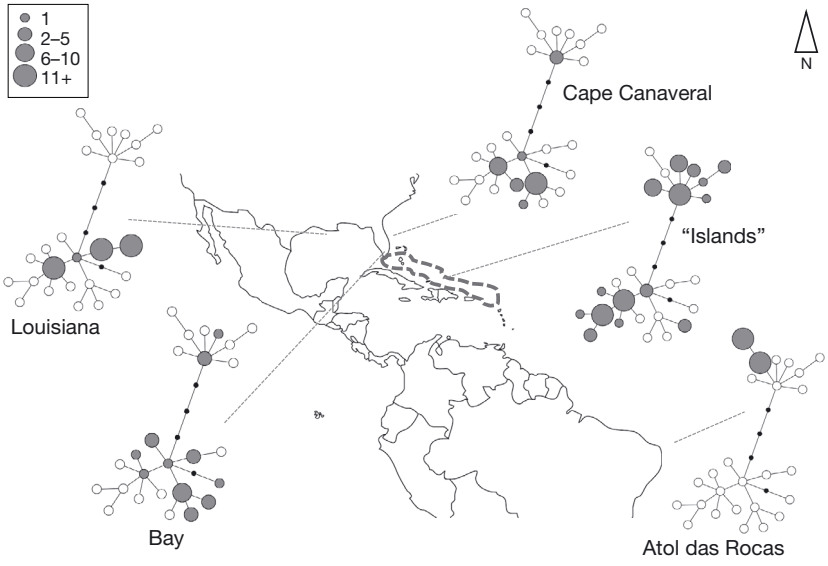

Fig. 2. Maximum-parsimony networks for each sampling location for lemon sharks *Negaprion brevirostris* (the Northern Hemisphere island sites are grouped together). Grey circles in the network represent haplotypes that were sampled in that particular location; white circles are haplotypes that were not found in that location but sampled elsewhere. The size of the circle is proportional to the number of individuals in the sample that possessed that haplotype (see key for details)

inclusion of which could skew mitochondrial haplotype frequencies and cause deviations from Hardy-Weinberg expectations in the microsatellites. None were found. Translations of ND2 haplotypes showed no variation in amino acid sequences of the sampled individuals. There were 8 singleton haplotypes, all of which were verified by sequencing them in both directions. All of these singletons were only sampled once in this dataset but have been observed in other individuals that we have sequenced but that were not included in this study because they were not sampled in the study sites or age group we are reporting here. Overall h was 0.825, and overall π was 0.0091 (Table 1). The low-latitude Northern Hemisphere island samples contained a wide range of haplotypes that were widely distributed within the network and phylogenetic tree, while most of

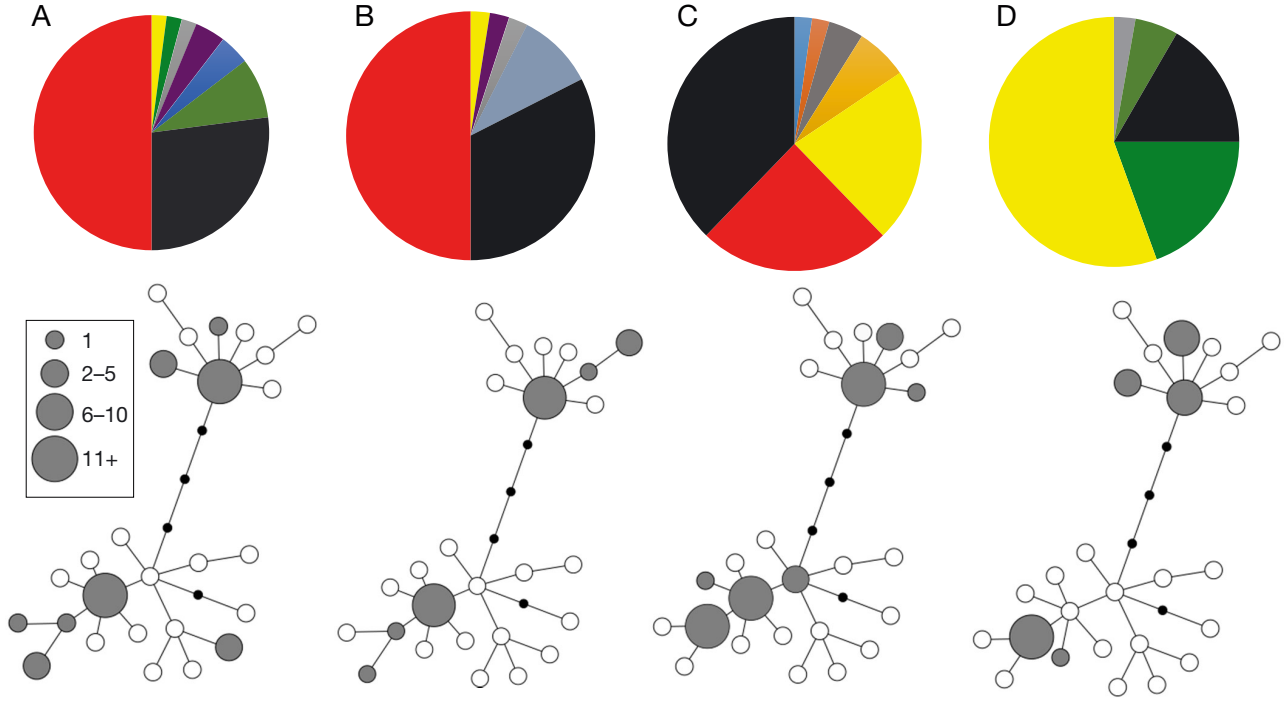

Fig. 3. Haplotype frequencies (upper panel) and maximum-parsimony networks (lower panel) for the island samples of lemon sharks *Negaprion brevirostris*, arranged from west to east. (A) Marquesas Key, (B) Bimini, (C) Eleuthera, (D) US Virgin Islands. Grey circles in the network represent haplotypes that were sampled in that particular location; white circles are haplotypes that were not found in that location but sampled elsewhere. The size of the circle is proportional to the number of individuals in the sample that possessed that haplotype

Table 1. Sample size (N), mitochondrial ND2-control region (mtND2-CR) haplotype (h) and nucleotide diversity (π), average microsatellite allelic diversity (k), observed heterozygosity (H_o) and expected heterozygosity (H_e) for 8 samples of juvenile lemon sharks *Negaprion brevirostris*

Site	N	h	π	k	H_o	H_e
Canaveral	42	0.58188	0.00086	7.625	0.76613	0.79562
Bimini	48	0.67908	0.00158	8.750	0.77250	0.77250
Marquesas	40	0.64872	0.00163	8.500	0.78049	0.76916
Eleuthera	45	0.75758	0.00157	8.125	0.83503	0.78350
US Virgin Is.	36	0.74545	0.00158	5.500	0.76799	0.75369
Gullivan Bay	30	0.87126	0.00156	7.500	0.79810	0.78977
Louisiana	40	0.69615	0.00076	7.750	0.80202	0.78407
Rocas	44	0.40592	0.00023	7.125	0.72086	0.73442
Total	325	0.82500	0.00195	7.609	0.78039	0.78940

the high-latitude Northern Hemisphere continental samples and the 1 Southern Hemisphere sample contained a more restricted range of haplotypes (Figs. 1–3). The exception to this pattern was the Gullivan Bay continental sample, which contained a diverse range of haplotypes. Microsatellite locus allelic diversity (k), H_o and H_e are shown for each sample in Table 1. All samples were in Hardy-Weinberg equilibrium at all loci after correction for multiple tests. The continental Northern Hemisphere and Brazil samples typically exhibited lower genetic diversity than the Northern Hemisphere island samples (Table 1, Figs. 1 & 2).

All measures of genetic differentiation at both types of markers indicated structure between Brazil and all of the other sites (Tables 2 & 3, Figs. 1 & 2). Nonetheless, the most probable number of populations contributing to the entire collection of 325 ind. detected by STRUCTURE 2.3.4 and based on microsatellite loci was 1. Nearly all of the Northern Hemisphere samples, including the island samples, exhibited significantly different haplotype frequencies, as detected by significant pairwise F_{ST} values based on concatenated mtND2-CR haplotype frequency differences and Fisher's exact tests (Table 2, Fig. 3). Corresponding pairwise Φ_{ST} values, which also incorporate the genetic distance between haplotypes as well as frequency, were significantly different from zero in all pairwise comparisons involving Cape Canaveral, and the Louisiana and Gullivan Bay continental samples (Table 2). Pairwise Φ_{ST} was not significantly different from zero between any combination of the Marquesas Keys, Bimini, Eleuthera and

Table 2. Pairwise F_{ST} (above diagonal) and Φ_{ST} (below diagonal) values based on mtND2-CR sequence for lemon sharks *Negaprion brevirostris*. **Bold** values are significantly different from zero ($p < 0.05$). Values marked by asterisks are significant after correction for multiple tests

	Canaveral	Bimini	Marquesas	Eleuthera	Virgin Is.	Gullivan	Louisiana	Brazil
Canaveral		**0.292***	**0.384***	**0.282***	**0.385***	**0.095***	**0.322***	**0.508***
Bimini	**0.269***		−0.005	**0.068***	**0.293***	**0.175***	**0.191***	**0.454***
Marquesas	**0.334***	−0.007		**0.061***	**0.309***	**0.186***	**0.208***	**0.475***
Eleuthera	**0.288***	−0.004	−0.013		**0.139***	**0.121***	**0.214***	**0.417***
US Virgin Is.	**0.331***	−0.004	−0.001	0.001		**0.226***	**0.332***	**0.483***
Gullivan	**0.069***	**0.216***	**0.216***	**0.175***	**0.211***		**0.179***	**0.381***
Louisiana	**0.284***	**0.399***	**0.399***	**0.353***	**0.332***	**0.241***		**0.452***
Brazil	**0.835***	**0.671***	**0.658***	**0.581***	**0.683***	**0.731***	**0.872***	

Table 3. Pairwise F_{ST} values (above diagonal) based on microsatellites for lemon sharks *Negaprion brevirostris*. **Bold** values are significantly different from zero ($p < 0.05$). Values marked by asterisks are significant after correction for multiple tests

	Canaveral	Bimini	Marquesas	Eleuthera	Virgin Is.	Gullivan	Louisiana	Brazil
Canaveral		0.006	−0.001	0.001	−0.004	−0.001	−0.001	**0.057***
Bimini			0.002	0.004	0.009	0.011	0.004	**0.059***
Marquesas				0.002	0.002	0.001	0.004	**0.049***
Eleuthera					0.003	0.007	0.004	**0.052***
Virgin Is.						−0.004	0.004	**0.034***
Gullivan							−0.004	**0.042***
Louisiana								**0.050***
Brazil								

the US Virgin Islands (i.e. the island samples; Table 2). Pairwise F_{ST} values based on microsatellite allele frequencies were not significantly different from zero for any of the Northern Hemisphere samples and were from 1 to 3 orders of magnitude lower than the corresponding mitochondrial-based F_{ST} for these samples (Tables 2 & 3).

IMa2 (Hey & Nielsen 2007) was used to estimate *t* and *m* between all sample pairs (N = 28). We only used the mitochondrial sequence data for these analyses because of the lack of significant differentiation (*F*-statistics) or clusters detected using microsatellite markers. Changing the mixing terms and the prior values was found to shift distributional peaks in the preliminary runs of IMa2, but no peak shifted between a zero and non-zero value. Pairwise comparisons of samples resulted in the pair being categorized into one of the following groups: non-divergent, diverged with migration and diverged without migration (Table 4). Four pairwise comparisons had a posterior probability of *t* that peaked at or near zero, indicating that these samples were non-divergent (Bimini with each of Eleuthera, Marquesas Keys and the Virgin Islands, and Virgin Islands and Marquesas; Table 4). The remaining pairwise comparisons had a non-zero divergence value, and the migration (*m*) parameters were then evaluated. The posterior probability distribution of *m* for 10 of these pairwise comparisons indicated that the most likely value of *m* was zero, indicating that the samples were from divergent populations that were not connected by migration since diverging (Table 4). The posterior probability distribution of *m* in the remaining 14 pairwise comparisons indicated gene flow in at least 1 direction (Table 4). The peak of the posterior probability distribution of *m* for 4 pairwise comparisons indicated that *m* was zero in 1 direction (Canaveral to Virgin Islands, Canaveral to Marquesas, Canaveral to Eleuthera, Gullivan to Louisiana) and that the peak probability was greater than zero in the other direction. Because the peak probability was at least 2.5 times greater than the zero probability, the log-likelihood ratio test was not used to test these cases. Ten pairwise comparisons had at least 1 peak prob-

Table 4. IMa2 (Bayesian MCMC program) estimates of the time since divergence (*t*) and migration rate (*m*) between all sample pairs (N = 28) for lemon sharks *Negaprion brevirostris*. We report zero (Y) or non-zero (N) values only. Dashes indicate a value that was not evaluated because other model predictions had determined the value (e.g. a comparison which has a divergence time of zero must have bidirectional gene flow and a migration rate >0)

Pairwise comparison		IMa2 result	*t* = 0	*m* = 0	Gene flow direction
Bimini	Eleuthera	Non-divergent	Y	–	–
Bimini	Marquesas	Non-divergent	Y	–	–
Bimini	Virgin Is.	Non-divergent	Y	–	–
Marquesas	Virgin Is.	Non-divergent	Y	–	–
Bimini	Canaveral	Diverged with migration	N	N	Bidirectional
Eleuthera	Marquesas	Diverged with migration	N	N	Bidirectional
Eleuthera	Virgin Is.	Diverged with migration	N	N	Bidirectional
Gullivan	Virgin Is.	Diverged with migration	N	N	Bidirectional
Canaveral	Virgin Is.	Diverged with migration	N	N	Virgin Is. to Canaveral
Eleuthera	Canaveral	Diverged with migration	N	N	Eleuthera to Canaveral
Gullivan	Louisiana	Diverged with migration	N	N	Louisiana to Gullivan
Canaveral	Marquesas	Diverged with migration	N	N	Marquesas to Canaveral
Canaveral	Rocas	Diverged with migration	N	N	Rocas to Canaveral
Bimini	Gullivan	Diverged with migration	N	N	Bimini to Gullivan
Bimini	Louisiana	Diverged with migration	N	N	Bimini to Louisiana
Gullivan	Canaveral	Diverged with migration	N	N	Gullivan to Canaveral
Bimini	Rocas	Diverged without migration	N	Y	–
Eleuthera	Rocas	Diverged without migration	N	Y	–
Gullivan	Rocas	Diverged without migration	N	Y	–
Louisiana	Rocas	Diverged without migration	N	Y	–
Marquesas	Rocas	Diverged without migration	N	Y	–
Virgin Is	Rocas	Diverged without migration	N	Y	–
Eleuthera	Louisiana	Diverged without migration	N	Y	–
Canaveral	Louisiana	Diverged without migration	N	Y	–
Marquesas	Louisiana	Diverged without migration	N	Y	–
Virgin Is	Louisiana	Diverged without migration	N	Y	–
Marquesa*s*	Gulliva*n*	Diverged without migration	N	Y	–
Eleuthera	Gullivan	Diverged without migration	N	Y	–

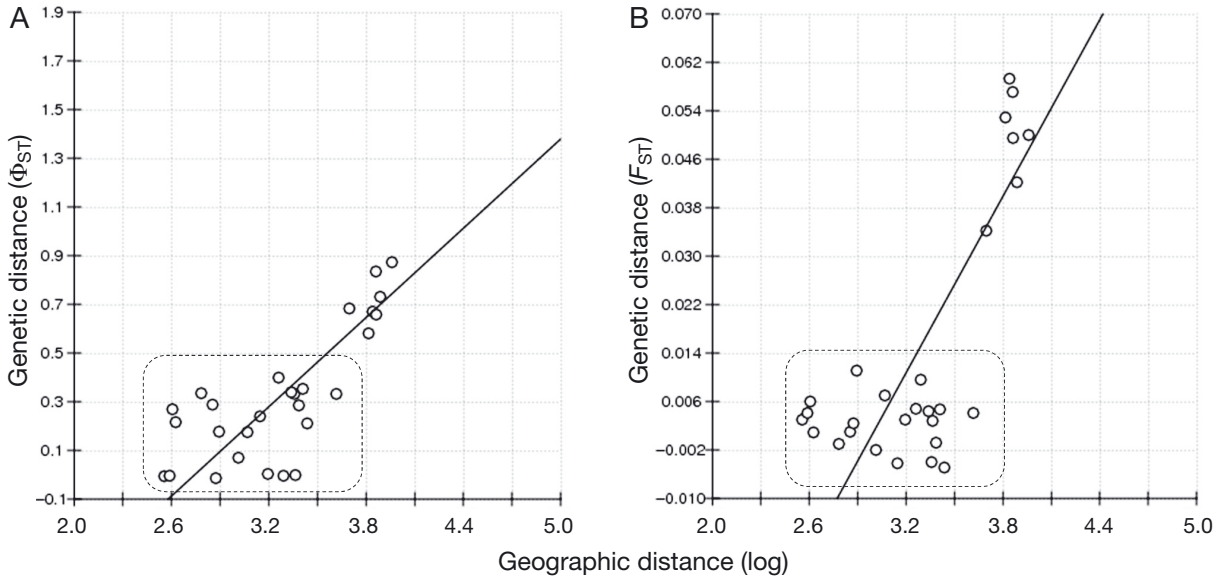

Fig. 4. Correlation between pairwise genetic distance: (A) F_{ST} based on microsatellite markers and (B) Φ_{ST} based on concatenated mitochondrial sequences and geographic distance separating samples for lemon sharks *Negaprion brevirostris*. Dashed box encloses Northern Hemisphere samples, in which there was no significant correlation

ability of *m* close to zero probability. The log-likelihood ratio test indicated no significant difference between the null model and the model where *m* was equal to zero for 2 of these comparisons (Eleuthera/Gullivan [2LLR = 4.991, p = 0.08, df = 2] and Eleuthera/Louisiana [2LLR = 3.674, p = 0.16, df = 2]), indicating that these samples can be categorized as diverged without migration. The log-likelihood ratio test was significantly different ($p < 0.05$) between the null model and a model with *m* equal to zero for the remaining 8 pairwise comparisons, indicating divergence with migration between these populations. In 4 of these pairwise comparisons (Bimini to Louisiana, Bimini to Gullivan, Gullivan to Canaveral, Rocas to Canaveral), a model with the *m* in one direction was equal to zero, while the *m* in the other direction was greater than zero.

There was a significant pattern of IBD with all measures of genetic differentiation and all marker types used when all of the samples were included in the analysis (mtDNA F_{ST}: $r^2 = 0.65$, $p < 0.01$; mtDNA Φ_{ST}: $r^2 = 0.76$, $p < 0.01$; microsatellite F_{ST}: $r^2 = 0.81$, $p < 0.01$; Fig. 4). When the Brazil sample was removed, the correlations were no longer significantly different from zero (mtDNA F_{ST}: $r^2 = 0.20$, $p = 0.06$; mtDNA Φ_{ST}: $r^2 = 0.12$, $p = 0.12$; microsatellite F_{ST}: $r^2 = 0.01$, $p = 0.6$; Fig. 4). There was a significant pattern of IBD within the island populations in the mitochondrial DNA and measured by F_{ST} ($r^2 = 0.91$, $p < 0.01$; Fig. 5), but not the Φ_{ST} ($r^2 = 0.04$, $p = 0.49$) or microsatellite-based F_{ST} ($r^2 = 0.12$, $p = 0.37$).

DISCUSSION

Lemon sharks have a wide, continuous distribution in the western Atlantic and are seemingly unimpeded by geophysical barriers to dispersal, yet we found evidence of a complex and relatively fine-scale

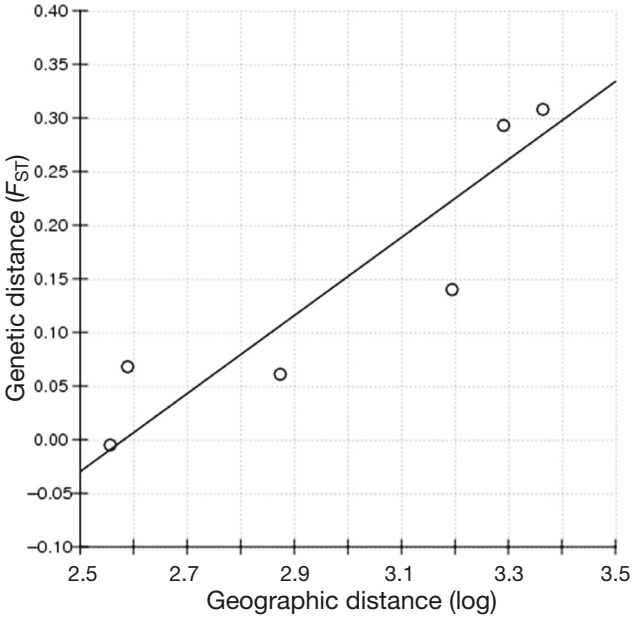

Fig. 5. Correlation between pairwise genetic distance (F_{ST} based on concatenated mitochondrial haplotype frequencies) and geographic distance in each pair of Northern Hemisphere island samples for lemon sharks *Negaprion brevirostris*

population structure across this range. We rejected the hypothesis that levels of genetic diversity in each sample were the same, because there are several lines of evidence that the high-latitude continental populations that we sampled (Louisiana, Gullivan Bay and Canaveral) are less diverse and derived from the lower latitude populations in the Northern Hemisphere. First, haplotypes predominantly found in the island samples were distributed throughout the phylogenetic tree and network, while the haplotypes dominating the continental samples are restricted to one of the clusters. Additionally, 2 of the 3 continental samples exhibit very low nucleotide diversity compared to the island samples. The Gullivan Bay continental sample is composed of a more diverse range of haplotypes than Louisiana or Canaveral, suggesting that this area of southern Florida could be a mixing zone between Atlantic, Gulf and some island populations. Alternatively, our sample from this site is composed of larger juveniles that may be less tied to their natal site than the juveniles <90 cm TL that were sampled everywhere else (Chapman et al. 2009a). Additional evidence that the high-latitude continental populations are derived stems from the fact that every Bayesian pairwise comparison indicating unidirectional gene flow between a pair of populations was in the south to north direction, except 1 case that was between a pair of the continental populations. Since lemon sharks are a primarily tropical and subtropical species, exhibiting physiological adaptations for living in warm, shallow water (Compagno 1984, Bushnell et al. 1989), it is intuitive that the high-latitude populations in the Northern Hemisphere would have been colonized from ancestral low-latitude populations. This pattern has also been seen in other primarily tropical and subtropical marine animals in this region, such as sea turtles and manatees (Garcia-Rodriguez et al. 1998, Shamblin et al. 2012).

The Brazil sample has the lowest nucleotide and haplotype diversity and, as previous studies have indicated (Feldheim et al. 2001, Schultz et al. 2008), is genetically isolated from all of the Northern Hemisphere samples regardless of the marker (mitochondrial and nuclear), measure of differentiation (F_{ST} and Φ_{ST}) and type of analysis (standard and all but one of the Bayesian pairwise comparisons). Unlike Schultz et al. (2008) we found no shared haplotypes between Brazil and the Northern Hemisphere samples. This occurred because we used both CR and ND2, and they only used CR. The Brazil sampling location, Atol Das Rocas, is a small platform located ~230 km from mainland South America and it is possible that it is not representative of continental genetic diversity in this region due to founder effects or small effective population size. This sample also drives the IBD pattern observed in the complete dataset. Tagging and telemetry data have revealed that movements of ~200 to 800 km are common in subadult and adult lemon sharks, with the longest known dispersal event involving a male shark born at Bimini being recaptured >1000 km away in the Gulf of Mexico (Feldheim et al. 2001, 2014). The combined genetic and movement data indicate that male or female lemon sharks are unlikely to disperse between Brazil and the other sites given the large distances separating them. Previous population genetics of large coastal sharks have typically found structure over distances of >1000 km (Dudgeon et al. 2013). Regional philopatry (i.e. individuals moving between regions but preferentially returning to their natal region to breed) has frequently been discussed as a possible explanation for this pattern in certain western Atlantic shark populations (Schultz et al. 2008, Chapman et al. 2009b, Karl et al. 2011). We suggest that a more parsimonious explanation is that movement coupled with reproductive mixing beyond ~1000 km is exceptional in many of these species.

The genetic structure observed among lemon sharks at smaller spatial scales in the Northern Hemisphere (i.e. 150 to 2000 km) is not entirely a function of geographic distance. We conclude this because IBD was evident in both mitochondrial and nuclear genetic markers only when the Brazil sample was included in the analysis. This occurred for 2 reasons. First, we did not detect differentiation between any of the Northern Hemisphere samples using microsatellite markers, regardless of distance. Male lemon sharks rarely sire offspring in the same nursery site more than once (Feldheim et al. 2002a, 2004, DiBattista et al. 2008), which suggests that reproducing males are not as site-faithful as females and may therefore be vectors of gene flow at larger geographic scales. It is also possible that mating takes place at a time and place where adults from multiple nursery sites are admixed, with females only later segregating to specific nursery sites to give birth. While we did observe population structure within the Northern Hemisphere in the mitochondrial marker, the effect of distance between sites was inconsistent. The 3 continental samples (Louisiana, Gullivan Bay and Cape Canaveral) are separated by ~1500 km and are strongly differentiated (i.e. exhibited moderate to high pairwise Φ_{ST}) from each other and all other samples. Two of these sites contain a small number of unique haplotypes,

while the third contains a wide variety of haplotypes. In contrast, the island samples that were collected across ~1900 km (Marquesas Keys, Bimini, Eleuthera and the US Virgin Islands) are not differentiated from one another (i.e. pairwise Φ_{ST} between these samples were not significantly different from zero) because they were all composed of several of the most differentiated haplotypes. The differences in the degree of structure along similar distances in continental and island samples observed in the mitochondrial marker coupled with an absence of structure in the nuclear markers undermine the effect of simple distance on genetic differentiation in the Northern Hemisphere.

There are several potential hypotheses that could explain why the stretch of uninterrupted coastal shelf habitat from Louisiana to Cape Canaveral harbors more strongly differentiated lemon shark populations than the discontinuous series of banks and islands occurring across a similar geographic distance from the lower Florida Keys to the Virgin Islands. The phylogeographic history of these populations is very different given the effects of the most recent Wisconsin glaciation on these regions. During this period the land area of the Florida peninsula was much larger due to the lower sea level and physically divided the Atlantic and Gulf of Mexico basins (Grimm et al. 1993). Correspondingly, there is a phylogenetic break occurring between Atlantic and Gulf of Mexico populations of many marine species, even those that are continuously distributed across these regions today (Bowen & Avise 1990, Avise 1992). Under this model the differentiation of the Louisiana (northern Gulf) and Canaveral (Atlantic) lemon shark lineages could have been caused by the long-term isolation of these populations during the Wisconsin glaciation, coupled with contemporary philopatry or limited migration of females maintaining separation even after sea levels rose. Supporting this interpretation, the Louisiana sample is, with 1 exception, diverged and isolated (i.e. without migration) from all of the others in the Bayesian pairwise analyses. Southwest Florida (Gullivan Bay) may represent a mixing zone between Atlantic and Gulf lineages. Indeed, the pairwise Bayesian analyses commonly inferred migration between this and other Northern Hemisphere populations. The Canaveral sample is differentiated and diverged from all of the island populations, but the pairwise Bayesian analysis indicates that it has recently been connected to this region by migration.

The islands of the Florida Keys, Bahamas and Caribbean were all components of much larger landmasses during the Wisconsin glaciations. The island sites we sampled were only formed as sea levels rose and these landmasses were inundated after the glaciers retreated ~10 000 yr ago. All of these island samples are each composed of several of the most divergent haplotypes, which drives down pairwise Φ_{ST} because nucleotide diversity within populations is comparable to nucleotide diversity between them. There is still fine-scale population structure in lemon sharks because the haplotype frequencies within each sample are distinct (i.e. pairwise F_{ST} values, which are based on haplotype frequencies and do not include genetic distance information, are all significantly different from zero, with the exception of the Bimini and Marquesas Key comparison). Similar discordance between pairwise Φ_{ST} and F_{ST} in mitochondrial DNA data has been found in other marine species, such as sea turtles and marine mammals sampled at fine geographic scales (O'Corry-Crowe et al. 1997, Rosel et al. 1999, Shamblin et al. 2012). We concur with these authors that researchers should consider whether distance-based Φ_{ST} or frequency-based F_{ST} is the most appropriate way to assess fine-scale population structure in the mitochondrial DNA of their study species (O'Corry-Crowe et al. 1997, Rosel et al. 1999, Shamblin et al. 2012).

There is a strong pattern of IBD in pairwise mitochondrial F_{ST} between these island samples. IBD usually arises because mating is more likely to occur between proximate individuals, yet this is irrelevant for a maternally inherited marker. We therefore interpret our findings to indicate that females primarily give birth at or near their birthplace (Feldheim et al. 2014). This interpretation is consistent with the high levels of site-fidelity and natal philopatry observed in female lemon sharks in at least some of these islands (Feldheim et al. 2002a, 2014). We therefore conclude that female-mediated gene flow is restricted between nearly all Northern Hemisphere sites (i.e. continental and island alike), but the samples from the continental sites are more divergent from one another because they have been separated since the Wisconsin glaciation and, according to the Bayesian analysis, 2 of the 3 (Canaveral and Louisiana) are divergent and do not exchange migrants. In contrast, the island sites were more recently colonized from the same or several very similar ancestral source populations during the postglacial period. According to the Bayesian analysis, most of the island sites have diverged but have recently exchanged migrants among themselves and in many cases with close continental sites (Gullivan Bay and Cape Canaveral). We speculate that this source population or populations lived along the margins of the low-

latitude landmasses during the glacial period. While many females giving birth at Bimini are site-faithful and/or practice natal philopatry, a significant number of females only bred there once during a nearly 2 decade monitoring program (Feldheim et al. 2014). These individuals may be occasional strays that are vectors of ongoing or historic connectivity between some of our samples. The pattern of IBD observed in the island samples suggests that stray females may primarily originate at proximate rather than more distant nursery sites.

The genetic population structure of lemon sharks from Florida's east coast to Brazil is complex and influenced by a combination of distance, female site-fidelity and/or natal philopatry and historical processes. There are at least 2 fully differentiated populations in this region, which are broadly defined as Northern and Southern Hemisphere stocks (although more sampling is needed to define their boundaries). There is restricted female-mediated gene flow within the Northern Hemisphere, supporting direct observations that females practice site-fidelity to specific nursery sites and exhibit natal philopatry (Feldheim et al. 2002a, 2004, 2014). Although connected by male-mediated and, in certain cases, some female-mediated gene flow, it is possible that lemon shark populations have some level of internal dynamics because they are largely composed of discrete groups of adult females adhering to site-fidelity and/or natal philopatry. No amount of male immigration can compensate for a local decline in females under these conditions, although the Bayesian analyses and direct observations suggest there is, or has been, straying of some term females between some proximate nursery sites that could contribute to some form of metapopulation dynamics. Overall, the combined analyses and direct observations indicate that lemon sharks may be vulnerable to localized overfishing, a pattern that has been documented but never fully explained in some other coastal sharks (Hueter et al. 2005). For this reason, lemon sharks and perhaps other similar coastal carcharhinids should be assessed and managed on subregional geographic scales and should be fished only after comprehensive assessments of population structure have been completed, in order to avoid causing local collapse of populations.

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