

Global population structure of the spiny dogfish *Squalus acanthias*, a temperate shark with an antitropical distribution

A. VERÍSSIMO, J. R. MCDOWELL and J. E. GRAVES

Virginia Institute of Marine Science, College of William & Mary, P.O. Box 1346, Gloucester Point, VA 23062, USA

Abstract

The spiny dogfish (*Squalus acanthias*) is a temperate, coastal squaloid shark with an antitropical distribution in the Atlantic and Pacific oceans. The global population structure of this species is poorly understood, although individuals are known to undergo extensive migrations within coastal waters and across ocean basins. In this study, an analysis of the global population structure of the spiny dogfish was conducted using eight polymorphic nuclear microsatellite markers and a 566-bp fragment of the mitochondrial ND2 gene region. A low level of genetic divergence was found among collections from the Atlantic and South Pacific basins, whereas a high level of genetic divergence was found among Pacific Ocean collections. Two genetically distinct groups were recovered by both marker classes: one exclusive to North Pacific collections, and one including collections from the South Pacific and Atlantic locations. The strong genetic break across the equatorial Pacific coincides with major regional differences in the life-history characters of spiny dogfish, suggesting that spiny dogfish in areas on either side of the Pacific equator have been evolving independently for a considerable time. Phylogeographic analyses indicate that spiny dogfish populations had a Pacific origin, and that the North Atlantic was colonized as a result of a recent range expansion from the South American coast. Finally, the available data strongly argue for the taxonomic separation of the North Pacific spiny dogfish from *S. acanthias* and a re-evaluation of the specific status of *S. acanthias* is warranted.

Keywords: antitropical distribution, population structure, spiny dogfish, temperate shark

Received 2 December 2009; revision received 3 February 2010; accepted 12 February 2010

Introduction

During the last two decades, significant progress has been made in our understanding of the patterns of genetic population structure of elasmobranchs. Recent studies have shown that the level of intraspecific genetic differentiation in elasmobranch species appears to be highly correlated with dispersal ability. In addition, the level of population structure recovered in these studies generally agrees with that expected based on the species' maximum size and habitat preference (e.g. coastal, pelagic or benthic), variables intimately

associated with elasmobranch vagility (Musick *et al.* 2004). For instance, low levels of genetic differentiation among populations were reported for highly migratory, large, oceanic species with global distributions, with population structure being detected only between ocean basins (e.g. shortfin mako *Isurus oxyrinchus* Schrey & Heist 2003; basking shark *Cetorhinus maximus* Hoelzel *et al.* 2000; whale shark *Rhincodon typus* Castro *et al.* 2007). In contrast, higher levels of intraspecific genetic differentiation across similar spatial scales have been reported for less vagile, demersal or benthic species with more restricted geographic ranges (e.g. shovelnose guitarfish *Rhinobatus productus* Sandoval-Castillo *et al.* 2004; thornback ray *Raja clavata* Chevolut *et al.* 2006; zebra shark *Stegostoma fasciatum* Dudgeon *et al.* 2008).

Correspondence: A. Verissimo, Fax: +1 804 684 7157; E-mail: averissimo@vims.edu

Exceptions to the low levels of genetic differentiation predicted for highly vagile elasmobranchs have nonetheless been reported. For instance, in species where females exhibit site fidelity to nursery areas, discrete populations can be found along areas of continuous habitat, as in the blacktip shark *Carcharhinus limbatus* or the sandbar shark *Carcharhinus plumbeus* (Schrey & Heist 2003; Keeney & Heist 2006; Portnoy 2008). Unsuitable environmental conditions and/or habitat discontinuities can also severely limit dispersal, even for highly vagile elasmobranch species. For example, open oceanic waters appear to be a barrier to gene flow in species such as the scalloped hammerhead *Sphyrna lewini* and the lemon shark *Negaprion brevirostris* (Feldheim *et al.* 2001; Duncan *et al.* 2006; Schultz *et al.* 2008), whereas warm equatorial waters also seem to function as barriers to gene flow in temperate species such as the tope *Galeorhinus galeus* (Chabot & Allen 2009).

Habitat use in elasmobranch taxa is heavily influenced by water temperature conditions (Simpfendorfer & Heupel 2004). As such, changes in water temperature can alter the dispersal patterns and the distribution of populations and species. Although the Earth's climate has changed dramatically throughout geological time, high latitude regions have experienced considerably more environmental variation than regions at mid and lower latitudes (Zachos *et al.* 2001; Ravelo *et al.* 2004; Brierley *et al.* 2009; Liu *et al.* 2009). The relative stability of tropical and warm-temperate regions and their vast spatial distribution in the world's oceans have facilitated uninterrupted gene flow in highly vagile, tropical and warm-temperate shark species, effectively reducing the potential for genetic divergence. In contrast, the variability in the temperature regime at mid and high latitudes (Zachos *et al.* 2001; Ravelo *et al.* 2004; Brierley *et al.* 2009; Chiang 2009; Hollis *et al.* 2009) has probably affected the distributions of temperate and boreal elasmobranchs, and produced patterns of population structure distinct from those of their warm water relatives. However, the patterns of population structure of temperate and boreal elasmobranchs are not well known.

In this study, we investigated the global population structure of a highly vagile, temperate elasmobranch—the spiny dogfish *Squalus acanthias*. The spiny dogfish is a common coastal shark with an antitropical distribution in the Pacific and Atlantic oceans, where it is generally found in continental shelf waters ranging from 6 to 10 °C (Burgess 2002; Shepherd *et al.* 2002; Compagno *et al.* 2005). The species is known to undergo seasonal north–south migrations along coastal areas or inshore–offshore movements triggered by changes in bottom water temperatures (Hisaw & Albert 1947; Holden 1965; Burgess 2002; Stenberg 2005; Campana *et al.* 2007). Long-distance migrations, including

both eastward and westward trans-Atlantic movements and westward trans-Pacific movements, have also been reported for the spiny dogfish, confirming its high dispersal ability (Holden 1967; Templeman 1976; McFarlane & King 2003). Considering the wide geographic distribution and potential for long-distance dispersal of the spiny dogfish, we hypothesize that restricted gene flow/genetic divergence along the species' range is associated with regions of unsuitable environmental conditions, such as warm waters at low latitudes. To test this hypothesis, we used highly polymorphic molecular markers, nuclear microsatellite loci and nucleotide sequences from a mitochondrial DNA gene region (ND2), to estimate the levels of genetic diversity and genetic differentiation among sample collections from throughout the geographic range of the species. We also inferred the contemporary and historical patterns and processes responsible for the current distribution of genetic variation.

Materials and methods

Sample collection, DNA extraction and analysis

Spiny dogfish collections were obtained from throughout the species' range and include locations in the North Atlantic: Irish Sea (UK), Ireland (IRE), Massachusetts (MA) and Virginia (VA); the western South Atlantic: Argentina (ARG); the North Pacific: California (CA), Washington-Oregon border (WAOR) and Japan (JA); and the South Pacific: Chile (CH) and New Zealand (NZ) (Table 2). Despite our best efforts, no collections were obtained from southern Africa, or the Mediterranean or Black seas. Individual samples consisted of muscle tissue or fin clips and were preserved in 95% ethanol and stored at 4 °C, or in a 20% solution (v/v) dimethyl sulphoxide (DMSO) buffer saturated with NaCl (Seutin *et al.* 1991) and stored at room temperature. Total genomic DNA (gDNA) was extracted from each individual sample using one of two methods: Chelex® resin according to the protocol of Estoup *et al.* (1996), or the Qiagen DNeasy Tissue kit according to the manufacturer's instructions.

All individuals were genotyped for a total of eight nuclear microsatellite loci. Four loci (*DF U285*, *DF T289*, *DF J451* and *DF J445*) were obtained from McCauley *et al.* (2004) and were chosen after testing for consistent amplification and conformation of genotypic distributions to the expectations of Hardy–Weinberg Equilibrium (HWE). The remaining four microsatellite loci were developed *de novo* as described below. One microsatellite locus, *Saca GA11* (GenBank Accession number GU553360), was obtained through the development and screening of a GA-repeat enriched genomic library

according to the protocol of Glenn & Schable (2005). Clones with DNA fragments exhibiting GA motifs over six repeat units in length were selected and the corresponding flanking regions used for primer design. Three additional microsatellite loci were obtained from the *Squalus acanthias* expressed-sequenced tag library available on GenBank (dbEST ID 47209646). The library was screened for dinucleotide repeats over 7 units in length using the Sequence Repeat Identification Tool (Temnykh *et al.* 2001), and primer pairs were designed for 19 potential loci. All loci were screened for high levels of polymorphism and conformation of genotypic distributions to HWE expectations, and three loci were chosen: *Saca3853*, *Saca4234* and *Saca6396* (Accession nos ES883853, ES324234, DV496396, respectively). Annealing temperatures and polymerase chain reaction (PCR) conditions were optimized for each microsatellite locus (see Table 1 and below for details). Microsatellite genotyping was conducted by amplifying each individual sample via PCR in 5 μ L reactions containing 5–15 ng of gDNA, 0.0375 μ M of forward primer labelled with a T3 tail, 0.15 μ M of reverse primer, 0.1 μ M of T3 primer fluorescently label (e.g. NED, PET, VIC or 6FAM), 0.2 mM each dNTP, 1.5 mM MgCl₂, 0.025 units *Taq* DNA polymerase, and 1 \times *Taq* buffer (Qiagen) and autoclaved milli-Q water. PCR conditions consisted of an initial denaturation of 3 min at 94 °C, followed by 35–45 cycles of 1 min at 94 °C, 35 s–1 min at the corresponding annealing temperature and 35 s–1 min at 72 °C, and a final extension step for 7 min at 72 °C. The products were run on an ABI Prism 3130xl (Applied Biosystems). Genotypes were scored manually with the software GeneMarker version 1.3 (Softgenetics; LLC). The presence of null alleles or of scoring errors in genotyping was tested for each locus using Micro-Checker (van Oosterhout *et al.* 2004). Conformation of genotypic distributions to HWE expectations for each locus within each population, tests of linkage disequilibrium between each pair of loci within and among all popula-

tions, number of alleles per locus and population, and observed and expected heterozygotes were calculated in Genepop version 4.0 (Raymond & Rousset 1995; Rousset 2008).

Mitochondrial DNA sequences of the NADH dehydrogenase 2 (ND2) gene region were obtained for each individual via PCR amplification using species-specific primers designed based on the complete mitochondrial genome sequence of *S. acanthias* available in GenBank (Accession no. NC_002012): ND2_F 5'-TTCCTCACACAAGCAACCGC-3' and ND2_R 5'-GATGGTGGCTGGGATGGC-3'. PCR master mixes of 25 μ L reactions included 10–20 ng gDNA, 1 μ M of each primer, 200 μ M each dNTP, 0.025 units *Taq* polymerase and 1 \times *Taq* buffer with 1.5 mM MgCl₂ (Qiagen), and autoclaved milli-Q water. PCR conditions consisted of an initial denaturation of 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C, and a final extension step for 7 min at 72 °C. The amplicons were cleaned with the QIAquick PCR Purification kit (Qiagen) according to the manufacturer's protocol, and the forward strands were sequenced using the ABI Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems). Reactions were run on an ABI Prism 3130xl genetic analyser (Applied Biosystems). The resulting DNA sequences were imported into Sequencher version 4.8 (Gene Codes Corp.) and checked for quality and accuracy in nucleotide base assignment. All individual sequences were aligned in MacVector version 8.0 (MacVector, Inc.) using the ClustalW multiple alignment algorithm (Thompson *et al.* 1994). Haplotypes were confirmed by sequencing the reverse strand of one individual of each haplotype.

Statistical analysis

The total number of alleles and observed (H_O) and expected heterozygosities (H_E) were calculated for all microsatellite loci in Genepop version 4.0 (Raymond and Rousset 1995; Rousset 2008). Allelic richness (R_s , an estimate of the number of alleles standardized for unequal sample sizes) was estimated in ESTAT version 2.9.3.2 (Goudet 2002). Mitochondrial DNA ND2 sequence diversity indices were calculated in DnaSP version 5 (Librado & Rozas 2009) including number of polymorphic sites, number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π), average number of nucleotide differences within (k), as well as number of fixed differences between haplotypes. Levels of among-population genetic differentiation were estimated by pairwise F_{ST} (microsatellites), or Φ_{ST} (mtDNA), in Arlequin version 3.11 (Excoffier *et al.* 2005). In the case of the F_{ST} -tests, the statistical power (i.e. rejection of the H_O of genetic homogeneity among

Table 1 Nuclear microsatellite loci of the spiny dogfish *Squalus acanthias*, with respective motif and repeat units, annealing temperature (T_a) and total number of alleles scored at each locus (A)

Marker name	Repeat motif	T_a (°C)	A
DF U285	[CT] ₁₁	54	11
DF T289	[TCC] ₇	57	11
DF J451	[AC] ₁₀	54	9
DF J445	[AC] ₁₀	59	23
<i>Saca</i> GAll	[TG] ₉ C[TG]	62	9
<i>Saca</i> 6396	[CA] ₁₉	56	22
<i>Saca</i> 3853	[TC] ₉ GC[TC] ₂	56	14
<i>Saca</i> 4234	[TG] ₁₄	58	11

two subpopulations when it is false) and the alpha level (i.e. rejection of H_0 when it is true) were estimated with the POWSIM software (Ryman & Palm 2006) using a sampling scheme of nine subpopulations with 45 individuals each, and one subpopulation with 20 individuals. The analyses were conducted using 1000 dememorizations, 100 batches and 1000 iterations per batch. A visual representation of among-population differentiation based on microsatellite allele frequencies was constructed using a principal component analysis (PCA) as implemented in PCA-GEN version 1.2 (J. Goudet, <http://www2.unil.ch/popgen/softwares/pcagen.htm>). The significance of total inertia and each axis' inertia was tested by 10 000 randomizations of the data.

The Structure software version 2.2 (Pritchard *et al.* 2000; Falush *et al.* 2003) was used to infer the population structure of spiny dogfish using nuclear microsatellite data. An initial analysis was conducted for K -values between 1 and 10, using a 'no-admixture model' with independent frequencies and default parameters since individuals are expected to belong to only one population with each population having different allelic frequencies. A second round of analysis was conducted to look for structure within the major groups recovered by the first analysis. For this purpose, the total dataset was divided into groups comprising the collections assigned to each of the population clusters retrieved in the first analysis. The K -values tested ranged from 1 to the maximum number of different collections in each subset, and the admixture ancestry model with correlated allelic frequencies was used, as some mixing between subpopulations (i.e. admixed individuals) is expected. Ten replicates were obtained for each K -value tested, with each replicate including 15 000 steps of burn-in followed by 35 000 steps. Criteria for choosing the best K -values followed those indicated in the software manual.

Hierarchical population structure was evaluated for each type of marker through an analysis of molecular variance (AMOVA) as implemented in Arlequin. For microsatellite data, the AMOVA was performed on a locus-by-locus basis and integrated over all loci, based on allelic frequency data; for mtDNA ND2 data, the AMOVA was performed based on a distance matrix of pairwise differences. In either case, significance was estimated using 10 000 iterations (Excoffier *et al.* 1992). Maximum-parsimony ND2 haplotype networks (Polzin & Daneschmand 2003) were constructed using the median joining algorithm (Bandelt *et al.* 1999) with default parameters and a transition to transversion ratio of 11:1 (as estimated by DnaSP) using the Network 4.5.1.0 software (<http://www.fluxus-engineering.com>). Only haplotypes occurring in more than two individuals were included in the haplotype network to highlight the geographic distribu-

tion of shared haplotypes. Divergence from an ancestral population of size N_0 at T -generations in the past was estimated in Arlequin for each of the recovered populations groups. The value of T , scaled by the mutation rate μ , i.e. $\tau_D = 2\mu T$, was estimated assuming isolation after divergence and constant but unequal daughter population sizes (Excoffier *et al.* 2005).

Past reduction of effective population size (bottleneck) was investigated with nuclear microsatellites using the ratio (M) test of Garza & Williamson (2001) for each locus and each population independently and compared to the critical values (M_c). The M_c was calculated based on seven loci, $\theta = 10$ and the conservative parameters of 90% one-step mutations and a mean size of non-one-step mutations of 3.5 (Garza & Williamson 2001). The excess heterozygosity test implemented in Bottleneck version 1.2.02 (Piry *et al.* 1999) was also used to infer past bottleneck events. The discrepancy between heterozygosity values was tested using a Wilcoxon's test under the null hypothesis of no significant heterozygosity excess (Piry *et al.* 1999). The two methods were used to infer the relative timing (older vs. recent) and severity (long or short duration) of detected bottleneck events (Williamson-Natesan 2005). Demographic analyses conducted using mtDNA mitochondrial sequences were performed in Arlequin. Mismatch distributions were obtained for each population unit (10 000 permutations) to infer changes in population size based on the frequency of pairwise differences among haplotypes (Schneider & Excoffier 1999). Relative time since lineage expansion (t) was obtained from the mismatch distribution analysis and estimated from $\tau_G = 2\mu t$ where τ_G is obtained from the mismatch distribution, and μ is the mutation rate assumed to be equal for all populations. Deviations from selective neutrality were also tested for the mitochondrial ND2 sequences with Tajima's D (Tajima 1989) and Fu's F (Fu 1997) (10 000 permutations, $\alpha = 0.05$) based on an infinite-site model without recombination.

Results

Genetic diversity

A total of 467 individuals was screened for variation at eight nuclear microsatellite loci. Complete genotypes were generated for 454 individuals while the remaining 13 individuals had missing data at one of the eight loci. Genotypic distributions conformed to HWE expectations for all locus/population combinations. Only one test of linkage disequilibrium in one collection remained significant after Bonferroni correction (JA, *DFT289* vs. *DFJ445*) but no loci were in linkage disequilibrium globally (data not shown). The number of

Table 2 Genetic diversity indices for the spiny dogfish, *Squalus acanthias*, integrated over all microsatellite loci and all mtDNA ND2 haplotypes from each sampling location. *N*, number of samples; H_O , observed mean heterozygosity; H_E , expected mean heterozygosity; Mean *A*, mean number of alleles; Mean R_s , mean allelic richness; *H*, number of haplotypes (unique haplotypes); *h*, haplotype diversity; π , nucleotide diversity; *k*, mean number of nucleotide differences between haplotypes

		Nuclear microsatellites					Mitochondrial ND2 sequences				
		<i>N</i>	H_O	H_E	Mean <i>A</i>	Mean R_s	<i>N</i>	<i>H</i>	<i>h</i>	π	<i>k</i>
North Pacific											
Japan	JA	49	0.56	0.56	6.25	4.87	46	21 (8)	0.93	0.0047	2.64
Washington/Oregon	WAOR	50	0.55	0.55	6.88	5.10	49	24 (9)	0.93	0.0054	3.02
California	CA	49	0.54	0.55	6.50	4.85	45	21 (9)	0.89	0.0048	2.73
South Pacific											
New Zealand	NZ	46	0.69	0.65	7.00	5.51	40	18 (9)	0.73	0.0022	1.23
Chile	CH	18	0.64	0.64	6.25	6.25	18	8 (3)	0.64	0.0016	0.88
North Atlantic											
Virginia	VA	55	0.65	0.65	8.25	6.19	43	11 (4)	0.57	0.0015	0.84
Massachusetts	MA	48	0.62	0.62	7.00	5.66	41	13 (6)	0.68	0.0017	0.95
Ireland	IRE	56	0.62	0.62	8.00	5.89	39	15 (9)	0.71	0.0024	1.33
Irish Sea	UK	48	0.63	0.64	7.38	5.88	48	14 (7)	0.56	0.0016	0.89
South Atlantic											
Argentina	ARG	48	0.64	0.64	7.63	5.93	43	16 (6)	0.71	0.0027	1.54

alleles per locus varied between 9 (*DF J451* and *Saca GA11*) and 23 (*DF J445*) (mean: 13.5) and the mean allelic richness (R_s) varied between 4.85 (CA) and 6.25 (CH) (Tables 1 and 2). The North Pacific collections had lower mean heterozygosities (H_O : 0.54–0.56; H_E : 0.55–0.56) and mean R_s (4.85–5.10) compared to those of other regions (H_O : 0.62–0.69; H_E : 0.62–0.65; R_s : 5.51–6.25).

A 566-bp fragment of the mtDNA ND2 locus was sequenced for 412 spiny dogfish, resulting in 103 haplotypes. There were 83 polymorphic positions of which 40 were parsimony informative and 43 were singleton mutations. The absolute number of pairwise differences between distinct haplotypes ranged from 1 to 13 (mean: 4.84). Overall nucleotide diversity was 0.0086 and haplotype diversity was 0.839. Singleton haplotypes occurred in 63% of the individuals, while 36–60% of haplotypes in each collection occurred in <3 individuals. Only 33 haplotypes were shared by two or more collections, with none being shared between North Pacific and non-North Pacific collections. North Pacific collections showed higher haplotype diversities (0.89–0.93) and divergences (2.64–3.02) compared to other regions (0.56–0.73 and 0.84–1.54, respectively).

Genetic differentiation and population structure analysis

Power analysis of microsatellite data indicated that a pairwise F_{ST} -level of 0.01 could be detected 100% of the time and for an alpha value of 0.057. Pairwise F_{ST} and Φ_{ST} -tests indicated low, nonsignificant genetic differen-

tiation among collections within the Atlantic, South Pacific and North Pacific as well as among those from the Atlantic and South Pacific (Table 3). Conversely, strong and significant genetic differentiation was found between collections from the Atlantic and the North Pacific as well as between those from the North and South Pacific. These results are consistent with the results of the PCA analysis, in which North Pacific collections were clearly separate from all others (PCA 1: $F_{ST} = 0.03624$, 79% of total inertia, $P = 0.001$; Fig. 1). Differentiation of North Atlantic collections from the southern NZ, CH and ARG collections is also suggested by the PCA but the axis separating these two groups was not significant (PCA 2: $F_{ST} = 0.00317$, 7% of total inertia, $P = 1.00$). This result holds true when considering only those collections outside the North Pacific, and the former without the ARG collection which showed an intermediate position between North Atlantic and South Pacific collections (data not shown).

The Structure analysis showed that the log probability $\ln \Pr(D)$ of the full microsatellite dataset had the largest rate of change at $K = 2$, while $\ln \Pr(D)$ increased only slightly for $K > 2$ (see Supporting Information). Moreover, the assignment percentages of each collection to one of the clusters when $K = 2$ exceeded 95% in all replicates (see Supporting Information) while assignment percentages were approximately equal among clusters for $K > 2$ (data not shown). The above results indicated $K = 2$ as the best estimate of the true K -value, corresponding to one cluster including only North Pacific collections (JA, WAOR and CA), and another cluster including Atlantic and South Pacific collections.

Table 3 Levels of genetic divergence among populations of *Squalus acanthias*. Pairwise F_{ST} below diagonal; pairwise Φ_{ST} above diagonal. Numbers in bold have P -values <0.001

		Φ_{ST}									
		JA	WAOR	CA	NZ	CH	VA	MA	IRE	UK	ARG
JA					0.170	0.198	0.249	0.193	0.178	0.258	0.178
WAOR	-0.005				0.170	0.198	0.248	0.193	0.178	0.257	0.179
CA	0.014	0.007			0.190	0.220	0.270	0.213	0.199	0.279	0.199
NZ	0.091	0.092	0.074			-0.018	0.004	-0.002	-0.010	0.007	-0.014
CH	0.084	0.080	0.069	0.001			-0.007	-0.009	-0.012	-0.010	-0.018
VA	0.094	0.092	0.065	0.005	0.007			0.000	-0.002	-0.012	0.003
MA	0.084	0.083	0.064	0.007	0.007	0.001			-0.001	0.002	0.002
IRE	0.094	0.091	0.066	0.005	0.004	-0.002	0.001			0.003	0.000
UK	0.094	0.093	0.068	0.002	0.002	-0.005	0.002	-0.003			0.002
ARG	0.075	0.073	0.055	-0.002	-0.003	0.000	-0.001	-0.012	0.005		

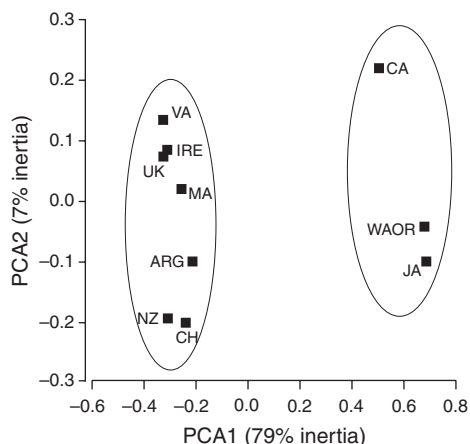


Fig. 1 Principal component analysis (PCA) of microsatellite allelic frequencies (eight loci) for the spiny dogfish *Squalus acanthias*. Abbreviations of locations are as indicated in the text. Circles around locations highlight separation according to PCA1 ($P = 0.01$).

Separate analyses of these two subsets of data provided no evidence of further population substructuring (see Supporting Information).

Results of the AMOVA analysis were generally consistent between nuclear microsatellite loci and the mtDNA ND2 gene region (Table 4). The null hypothesis of global panmixia was rejected due to significant genetic heterogeneity among collections (F_{ST} : 0.040, Φ_{ST} : 0.676, $P < 0.001$). Strong genetic divergence was detected between Pacific and Atlantic collections (F_{CT} : 0.034, Φ_{CT} : 0.477, $P < 0.01$) as well as among collections within the Pacific Ocean: the North Pacific group including JA, WAOR and CA, was significantly different from the South Pacific group including NZ and CH (F_{CT} : 0.081, Φ_{CT} : 0.753, $P < 0.001$). Given the continuous distribution of the spiny dogfish around the tip of South America

Table 4 Analysis of molecular variance (AMOVA) for *Squalus acanthias*. Numbers in bold have P -values <0.001 (***), 0.01 (**) or 0.05 (*)

	Microsatellites	mtDNA
Global panmixia		
F_{ST}	0.040***	0.676***
Between oceans		
F_{ST}	0.055***	0.744***
F_{SC}	0.022***	0.511***
F_{CT}	0.034***	0.477**
North vs. South Pacific		
F_{ST}	0.085***	0.752***
F_{SC}	0.004*	-0.005
F_{CT}	0.081***	0.753***
Southern Group vs. North Atlantic		
F_{ST}	0.004	0.007
F_{SC}	-0.001	-0.002
F_{CT}	0.005***	0.009***
North Pacific vs. North Atlantic		
F_{ST}	0.081***	0.797***
F_{SC}	0.001	-0.004
F_{CT}	0.080***	0.798***

and the absence of significant differentiation between any of the southern collections (ARG, CH and NZ), a 'North Atlantic-only' group (MA, VA, IRE and UK) and a 'Southern' group (ARG, CH and NZ) were compared. Low but significant genetic heterogeneity was detected between these groups with both types of markers (F_{CT} : 0.005, Φ_{CT} : 0.009, $P < 0.001$), whereas no differentiation was detected within groups (F_{ST} and Φ_{ST} were both non-significant). When the ARG collection was removed, genetic differentiation among North Atlantic and South Pacific collections was still recovered (F_{CT} : 0.05, Φ_{CT} : 0.008, $P < 0.01$), although there was a slight decrease in the Φ_{CT} and P -values. Small but significant

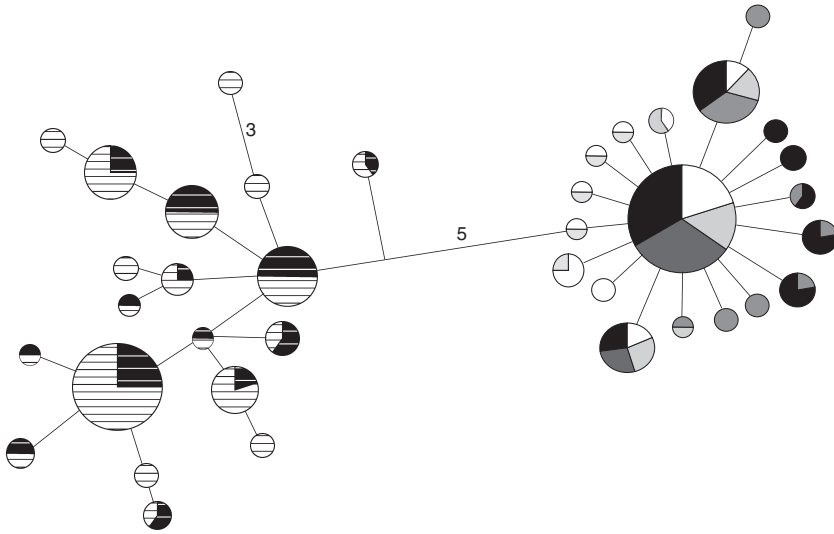


Fig. 2 Maximum-parsimony haplotype network of the mitochondrial DNA ND2 for the spiny dogfish *Squalus acanthias*. Haplotypes are represented by circles with sizes proportional to absolute frequency in the total sample. Colour codes are as follows: black with white stripes—JA; white with black stripes—WAOR and CA; white—NZ and CH; light grey—ARG; dark grey—IRE and UK; black—MA and VA. All branches correspond to one nucleotide substitution between haplotypes except where indicated with a number.

differentiation was found between CA and WAOR (F_{SC} : 0.007, $P < 0.05$) but this result was driven by only one locus ($DF\ T289$).

Phylogeographic analysis

Examination of the haplotype network based on the mtDNA ND2 region recovered two lineages separated by a minimum of five nucleotide substitutions: one lineage was exclusively represented by Atlantic and South Pacific sequences while the other lineage included all North Pacific haplotypes. Haplotypes in the North Pacific lineage were most closely related to haplotypes found in the NZ and ARG collections (Fig. 2). In addition, several common and geographically widespread haplotypes were found in the North Pacific clade, separated by one to five substitutions ($K_{NP} = 2.79$), from which other less frequent haplotypes were derived (Fig. 2). In contrast, the South Pacific and Atlantic lineage had a star-shaped network with one very common, central haplotype (39% of all individuals in the group) and with only the three most common haplotypes being shared across collections. Other shared haplotypes had lower frequencies and were geographically constrained: six additional haplotypes were found only in New Zealand and South America collections while three additional ones were present only in the North Atlantic. Estimates of time since population divergence from a common ancestor were in general agreement with the results described above: relative divergence times were largest for the North Pacific group, with slightly larger estimates from the North Atlantic group than from the Southern group ($\tau_D = 5.35$ vs. $\tau_D = 5.05$, respectively). The estimated divergence time between the later two groups produced a negative value ($\tau_D = -0.88$).

Demographic analysis

The geographic groups recovered in the AMOVA (i.e. North Pacific, North Atlantic and Southern Group) were used for all demographic analyses described below. Past events of population size reduction, or bottlenecks, were not detected by either ratio tests (mean observed $M > 0.7$, $M_c \approx 0.675$) or excess heterozygosity tests (overall P -values > 0.37), except for the Southern group in which the ratio M ($M = 0.673$, variance = 0.054) was lower than the critical value. Tajima's D (NP: -1.66 , $P = 0.018$; SG: -2.49 and NA: -2.50 , $P < 0.001$) and Fu's F -statistics were significantly negative in all cases (NP: -26.6 ; SG: -28.7 ; NA: -32.1 , $P < 0.001$) in conformance with a model of population growth, while mismatch distributions indicated that the sudden expansion model of population growth could not be rejected for any of the three geographic groups. Assuming an equal mutation rate for all populations, the relative time since population expansion was three times larger in the North Pacific ($\tau_G = 3.0$, 95% CI: 1.55–3.88) than in the other two populations, with the Southern group ($\tau_G = 1.1$, 95% CI: 0.66–1.59) having very similar values to the North Atlantic ($\tau_G = 1.0$, 95% CI: 0.24–1.88).

Discussion

Global population structure

The null hypothesis of global panmixia in the spiny dogfish *Squalus acanthias* was rejected by analyses of both mtDNA ND2 nucleotide sequence ($\Phi_{ST} = 0.676$, $P < 0.001$) and nuclear microsatellite data ($F_{ST} = 0.04$, $P < 0.001$). Significant genetic differentiation was

consistently detected by both types of molecular markers between geographic regions, and corresponded to two distinct genetic groups: a North Pacific group, and a non-North Pacific group including Atlantic and South Pacific collections. The strong genetic divergence between the groups was supported by the existence of fixed nucleotide differences at the mtDNA level, and very distinct allelic frequencies and the presence of private alleles (North Pacific: 10, Atlantic and South Pacific: 31) at the nuclear microsatellite loci. A small but statistically significant level of differentiation was also detected by both mtDNA ND2 sequences and nuclear microsatellites when comparing North Atlantic (MA, VA, IRE and UK) and southern collections (NZ, CH and ARG).

The high level of genetic divergence detected across the equatorial Pacific is in agreement with results from previous studies on the molecular genetics and general biology of the spiny dogfish. Analyses of the mtDNA control region (CR) and cytochrome oxidase I (COI) gene region also showed marked genetic differences between North Pacific and non-North Pacific collections (Franks 2006; Ward *et al.* 2007; Hauser 2009). Furthermore, strong genetic differentiation was detected at nuclear microsatellite loci between eastern North Pacific collections and those from Chile and the northeastern USA (Maine) (Franks 2006). The pronounced genetic divergence found across the equatorial Pacific is coincident with regional differences in the life history of the spiny dogfish. For instance, the age and length at sexual maturity of North Pacific spiny dogfish is attained between 29–35 years and 92–100 cm in females, and between 16–19 years and 70–80 cm in males, while maximum sizes and ages are up to 122–130 cm and 81 years, respectively (Ketchen 1972, 1975; Jones & Geen 1977; Saunders & McFarlane 1993). Outside North Pacific waters, the age and length at sexual maturity is attained earlier and at smaller sizes, between 10–16 years and 72–82 cm in females, and between 6–10 years and 60–64 cm in males, with maximum sizes and ages reaching only 110–117 cm and 40 years, respectively (Templeman 1944; Holden & Meadows 1964; Holden 1965; Nammack *et al.* 1985; Hanchet 1988; Campana *et al.* 2007; Ellis & Keable 2008). Overall, these results are consistent with long-term isolation of spiny dogfish populations across the Pacific equator.

In contrast, Atlantic and South Pacific collections of the spiny dogfish were not significantly different (Table 3; Figs 1 and 2). The lack of marked genetic differences across such a large area is remarkable and suggests that some level of gene flow is occurring, or has occurred until recently, among locations separated by hundreds to several thousands of miles. Several independent tagging studies have reported multiple cases of

long-distance movements in *S. acanthias*, including trans-oceanic migrations in the North Pacific and North Atlantic (Holden 1967; Templeman 1976; McFarlane & King 2003). A few long-distance migrants per generation are enough to prevent the genetic differentiation between geographically distant locations. This fact, combined with the long generation times found in Atlantic and South Pacific spiny dogfish (10–16 years), means that the hypothesis of ongoing gene flow throughout the Atlantic and South Pacific cannot be dismissed. However, a small but highly significant level of genetic differentiation between collections on either side of the Atlantic equator were found in our study ($F_{ST} = 0.005$ and $\Phi_{ST} = 0.009$, $P < 0.001$). Moreover, we also found that low frequency haplotypes were geographically limited to either the southern collections or the North Atlantic collections. These particular haplotypes are relatively derived (Fig. 2) and have probably originated recently; therefore, their spatial distribution should reflect recent dispersal events which, to the exception of one haplotype shared between ARG and IRE, appear not to include trans-equatorial crossings. Franks (2006) also found small but significant differentiation between Chilean and northeastern US collections at nuclear microsatellite loci ($F_{ST} = 0.013$, $P < 0.001$). These results support the hypothesis that gene flow has historically occurred between locations on either side of the Atlantic equator but it has recently become restricted.

Previous studies of genetic population structure of widely distributed and highly vagile marine elasmobranchs with warm temperate and/or tropical distributions have generally found limited gene flow and/or genetic differentiation across areas of habitat discontinuity (see Introduction for more details and Hoelzel *et al.* 2000; Pardini *et al.* 2001; Schrey & Heist 2003; Castro *et al.* 2007). In the case of cold temperate and boreal species, regions of constricted gene flow/genetic differentiation may coincide with warm-temperate and tropical waters at low latitudes. Indeed, the pronounced differences in life history and genetic diversity between North Pacific and non-North Pacific spiny dogfish is consistent with the existence of a barrier to gene flow at low latitudes. Similarly, and despite the comparatively smaller levels of genetic divergence between North Atlantic and southern hemisphere collections of spiny dogfish (NZ, CH and ARG), our results also suggest a recent restriction of gene flow across the equatorial Atlantic.

Phylogeographic reconstruction

The current distribution of genetic diversity observed for the spiny dogfish appears to have resulted from a

series of discrete range expansion events followed by population divergence across areas of unsuitable environmental conditions (e.g. warm, low latitude waters). The clear break in both genetic diversity and life history strategies found for fish on either side of the Pacific equator, and the large estimate of time since population divergence calculated for the North Pacific lineage suggest a relatively old divergence between the northern and southern Pacific populations. Moreover, evidence of an old and severe bottleneck (significant *M*-test, Williamson-Natesan 2005) in combination with sudden population expansion in the Southern group are consistent with a scenario of population size reduction and long-term maintenance of small effective sizes (Rogers & Harpending 1992), such as would result from a dispersal (founder) event of fish from the North to the South Pacific. Our data therefore point to a North Pacific origin of the current spiny dogfish populations with subsequent dispersal into South Pacific waters. Dispersal into Atlantic waters appears to have occurred along the South American coast as indicated by the presence of both genetic lineages in the Pacific basin and the closer interclade relationship exhibited by North Pacific and NZ and ARG collections. Dispersal into the North Atlantic basin appears to be the last step in the population history of *S. acanthias* apparently occurring relatively recently and in association with a rapid increase in population size in the South Pacific and Atlantic.

A North Pacific origin and subsequent dispersal into the Atlantic and South Pacific was also proposed by Franks (2006) based on the observation of non-North Pacific mtDNA control region haplotypes nesting within North Pacific ones. However, and contrary to our findings, Franks (2006) proposed a northern link between the Pacific and Atlantic oceans with subsequent range expansion into the South Atlantic and South Pacific, although no data was provided to support this hypothesis. Our alternative hypothesis of a southern link between the Pacific and Atlantic oceans is in agreement with the results of Jones & Geen (1976), in which a 'South American stock', mostly represented by Chilean samples in their study, presented values of mean total number of vertebrae that were intermediate between North Pacific and North Atlantic spiny dogfish. A very different perspective on the population history of the spiny dogfish is provided by the fossil record, which argues for a North Atlantic origin and later dispersal into the North Pacific. The oldest unambiguous fossil of *S. acanthias* in the North Atlantic dates back to the Early Pliocene of Belgium (Herman 1974 in Capetta 2006), while the oldest record in the North Pacific is of a younger age (Pleistocene of California; Fitch 1967 & 1968 in Capetta 2006). Nevertheless, the possibility of

an incomplete fossil record in the North Pacific cannot be discounted.

In the absence of a well-calibrated molecular clock for *S. acanthias* or any of its squaloid relatives, there are considerable limitations to inferring an approximate timing of events. The only estimates of mutation rate for elasmobranch taxa refer solely to the noncoding mtDNA CR (in the order of 10^{-5} mutations per generation for the whole mtDNA CR) of two warm-temperate sharks, the bonnethead *Sphyrna lewini* and the blacktip shark *Carcharhinus limbatus* (Duncan *et al.* 2006; Keeney & Heist 2006). The equivalent mutation rates are likely considerably lower in the temperate spiny dogfish due to its potentially lower metabolic rate and longer generation time (Avise *et al.* 1992; Martin & Palumbi 1993; Martin 1999). Nevertheless, the application of the above estimate should serve as an indication of the most recent time of events in the history of *S. acanthias*. As such, population divergence across the equatorial Pacific must have occurred earlier than 7.8 Myr (i.e. before the late Miocene). On another account, the fossil record places *S. acanthias* in the North Atlantic by the Early Pliocene (5.3–3.6 Myr; Herman 1974 in Capetta 2006). If the presence of spiny dogfish in the North Atlantic was indeed preceded by range expansion from the south, dispersal into the North Atlantic must have occurred before the Pliocene.

Taxonomic status of the North Pacific spiny dogfish

The North Pacific spiny dogfish was originally given species status and designated as *Squalus suckleyi* Girard 1854. Later, it was considered a subspecies of the cosmopolitan *S. acanthias* and designated *S. a. suckleyi* (see Jones & Geen 1976 and references therein). Its taxonomic validity was subsequently addressed by Bigelow & Schroeder (1948) and Jones & Geen (1976), who did not find evidence supporting the separation of the North Pacific spiny dogfish into a distinct taxonomic unit based on morphological and meristic characters. Since then, only one species has been considered as valid, namely *S. acanthias*.

Recently, molecular studies have consistently found strong genetic divergence between North Pacific and non-North Pacific spiny dogfish at three different mtDNA gene regions (control region, COI and ND2) and at nuclear microsatellite loci (Franks 2006; Ward *et al.* 2007; this study). In addition, two reciprocally monophyletic lineages have been found with two of the three mitochondrial (COI and ND2, but not CR) and with nuclear DNA markers, corresponding to a North Pacific-only clade and a 'rest of the world' clade (Ward *et al.* 2007; this study). All these data are consistent with long-term cessation of gene flow between the

North Pacific spiny dogfish and those from other regions. In addition, the genetic divergence among groups is coincident with distinct life history strategies: North Pacific fish reach maturity at an older age, have larger maximum sizes and live longer than fish occurring outside North Pacific waters (see above for references). Based on the above data, the North Pacific spiny dogfish should be considered as an independent management and/or conservation unit, as proposed by Hauser (2009). From a conservation genetics' perspective, the removal of genetic diversity from the North Pacific will result in the loss of unique genetic variation. Ultimately, the available data strongly argue for the taxonomic separation of the North Pacific spiny dogfish from *S. acanthias* and, as such, a re-evaluation of the specific status of *S. acanthias* incorporating molecular and morphological analyses is warranted.

Acknowledgements

The authors are indebted to all institutions and individuals who provided tissue samples, namely P. Chase and S. Rowe (NOAA/NEFSC), G. Johnston (Marine Institute); J. Ellis (CE-FAS), D. Stevens and R. O'Driscoll (NIWA); C. Healey (Royal Ontario Museum); D. Ebert, M. Boyle and E. Loury (MLML/PSRC); J. Yamamoto (Hokkaido University); A. Massa (INIDEP); N. Straube (Stuttgart Museum of Natural History); F. Concha; K. Neill. A. Verissimo was funded by the Fulbright Commission PhD scholarship 2005/2006, and by Fundação para a Ciência e Tecnologia (SFRH/BD/40326/2007). This is contribution number 3073 of the Virginia Institute of Marine Science.

References

- Awise JC, Bowen BW, Lam T, Meylan AB, Bermingham E (1992) Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in Testudines. *Molecular Biology and Evolution*, **9**, 457–473.
- Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.
- Bigelow HB, Schroeder WC (1948) Fishes of the western North Atlantic, part 1. *Memoirs of the Sears Foundation for Marine Research*, **1**, 59–576.
- Brierley CM, Fedorov AV, Liu Z, Herbert TD, Lawrence KT, LaRiviere JP (2009) Greatly expanded tropical warm pool and weakened Hadley circulation in the Early Pliocene. *Science*, **323**, 1714–1718.
- Burgess GH (2002) Spiny dogfishes—Family Squalidae. In: *Bigelow and Schroeder's Fishes of the Gulf of Maine* (eds Colette BB, Klein-MacPhee G), pp. 54–57. Smithsonian Institution Press, Washington, DC.
- Campana SE, Gibson JF, Marks L, Joyce W, Rulifson R, Dadswell M (2007) *Stock Structure, Life History, Fishery and Abundance Indices for Spiny Dogfish (Squalus acanthias) in Atlantic Canada*. Canadian Science Advisory Secretariat, Research Document 2007–2089.
- Capetta H (2006) *Elasmobranchii post-Triadici (index generum et specierum)—Fossilium Catalogus I: Animalia 142 (series ed. Rieggraf W)*. Backhuys Publish, Leiden.
- Castro ALF, Stewart BS, Wilson SG *et al.* (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). *Molecular Ecology*, **16**, 5183–5192.
- Chabot CL, Allen LG (2009) Global population structure of the tope (*Galeorhinus galeus*) inferred by mitochondrial control region sequence data. *Molecular Ecology*, **18**, 545–552.
- Chevolot MH, Hoarau G, Rijnsdorp AD, Stam WT, Olsen JL (2006) Phylogeography and population structure of thornback rays (*Raja clavata* L., Rajidae). *Molecular Ecology*, **15**, 3693–3705.
- Chiang JCH (2009) The tropics in Paleoclimate. *Annual Review in Earth and Planetary Sciences*, **37**, 263–297.
- Compagno LJV, Dando M, Fowler S (2005) *Sharks of the World*. Princeton University Press, Princeton, NJ.
- Dudgeon CL, Broderick D, Ovenden R (2008) IUCN classification zones concord with, but underestimate, the population genetic structure of the zebra shark *Stegostoma fasciatum* in the Indo-West Pacific. *Molecular Ecology*, **18**, 248–261.
- Duncan KM, Martin AP, Bowen BW, de Couet G (2006) Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). *Molecular Ecology*, **15**, 2238–2251.
- Ellis JR, Keable J (2008) Fecundity of Northeast Atlantic spurdog (*Squalus acanthias*). *ICES Journal of Marine Science*, **65**, 979–981.
- Estoup A, Larigiader CR, Perrot E, Chourrout D (1996) Rapid one tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology*, **5**, 295–298.
- Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
- Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, **7**, 574–578.
- Feldheim KA, Gruber SH, Aschley MV (2001) Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Molecular Ecology*, **10**, 295–303.
- Franks J (2006) *Phylogeography and population genetics of spiny dogfish (Squalus acanthias)*. Master's Thesis, University of Washington, Seattle, WA.
- Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.
- Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.
- Glen TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.
- Goudet J (2002) *FSTAT: a program to estimate and test gene diversities and fixation indices (version 2.9.3.2)*. Available from <http://www2.unil.ch/popgen/softwares/fstat.htm>. Updated from Goudet (1995).

- Hanchet S (1988) Reproductive biology of *Squalus acanthias* from the east coast, South Island, New Zealand. *New Journal of Marine and Freshwater Research*, **22**, 537–549.
- Hauser L (2009) The molecular ecology of dogfish sharks. In: *Biology and Management of Dogfish Sharks* (eds Gallucci VF, McFarlane GA, Bargmann GG), pp. 229–252. American Fisheries Society, Bethesda, MD.
- Hisaw FL, Albert A (1947) Observations on the reproduction of the spiny dogfish, *Squalus acanthias*. *Biological Bulletin*, **92**, 187–199.
- Hoelzel AR, Shivji MS, Magnussen J, Francis MP (2000) Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). *Biology Letters*, **2**, 639–642.
- Holden MJ (1965) The stocks of spurdogs (*Squalus acanthias* L.) in British waters, and their migrations. *Fishery Investigations*, **24**, 1–19.
- Holden MJ (1967) Transatlantic movement of a tagged spurdogfish. *Nature*, **214**, 1140–1141.
- Holden MJ, Meadows PS (1964) The fecundity of the spurdog (*Squalus acanthias* L.). *Journal Du Conseil Permanent International Pour L'Exploration De La Mer*, **28**, 418–424.
- Hollis CJ, Handley L, Crouch EM *et al.* (2009) Tropical sea temperatures in the high-latitude South Pacific during the Eocene. *Geology*, **37**, 99–102.
- Jones BC, Geen GH (1976) Taxonomic reevaluation of the spiny dogfish (*Squalus acanthias* L.) in the Northeastern Pacific Ocean. *Journal of the Fisheries Research Board of Canada*, **33**, 2500–2506.
- Jones BC, Geen GH (1977) Reproduction and embryonic development of spiny dogfish (*Squalus acanthias*) in the Strait of Georgia, British Columbia. *Journal of the Fisheries Resource Board of Canada*, **34**, 1286–1292.
- Keeney DB, Heist EJ (2006) Worldwide phylogeography of the blacktip shark (*Carcharhinus limbatus*) inferred from mitochondrial DNA reveals isolation of western Atlantic populations coupled with recent Pacific dispersal. *Molecular Ecology*, **15**, 3669–3679.
- Ketchen KS (1972) Size at maturity, fecundity, and embryonic growth of the spiny dogfish (*Squalus acanthias*) in British Columbia waters. *Journal of the Fisheries Research Board of Canada*, **29**, 1717–1723.
- Ketchen KS (1975) Age and growth of dogfish *Squalus acanthias* in British Columbia waters. *Journal of the Fisheries Research Board of Canada*, **32**, 43–59.
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.
- Liu Z, Pagani M, Zinniker D *et al.* (2009) Global cooling during the Eocene-Oligocene climate transition. *Science*, **323**, 1187–1190.
- Martin AP (1999) Substitution rates of organelle and nuclear genes in sharks: implicating metabolic rate (again). *Molecular Biology and Evolution*, **16**, 996–1002.
- Martin AP, Palumbi SR (1993) Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 4087–4091.
- McCauley L, Goecker C, Parker P, Rudolph T, Goetz F, Gerlach G (2004) Characterization and isolation of DNA microsatellite primers in the spiny dogfish (*Squalus acanthias*). *Molecular Ecology Notes*, **4**, 494–496.
- McFarlane GA, King JR (2003) Migration patterns of Spiny Dogfish (*Squalus acanthias*) in the North Pacific Ocean. *Fisheries Bulletin*, **101**, 358–367.
- Musick JA, Harbin MM, Compagno LJV (2004) Historical Zoogeography of the Selachii. In: *Biology of Sharks and Their Relatives* (eds Carrier JC, Musick JA, Heithaus MR), pp 33–78. CRC Press, Boca Raton, FL, USA.
- Nammack MF, Musick JA, Colvocoresses JA (1985) Life history of spiny dogfish off the Northeastern United States. *Transactions of the American Fisheries Society*, **114**, 367–376.
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
- Pardini AT, Jones CS, Noble LR *et al.* (2001) Sex-biased dispersal of great white sharks. *Nature*, **412**, 139–140.
- Piry S, Lukart G, Cornuet J-M (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.
- Polzin T, Daneschmand SV (2003) On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters*, **31**, 12–20.
- Portnoy DS (2008) *Understanding the reproductive behavior and population condition of the sandbar shark (Carcharhinus plumbeus) in the Western North Atlantic: a molecular approach to conservation and management*. PhD Thesis, College of William and Mary, Williamsburg.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure from multilocus genotype data. *Genetics*, **155**, 945–959.
- Ravelo AC, Andreansen DH, Lyle M, Lyle AO, Wara MW (2004) Regional climate shifts caused by gradual cooling in the Pliocene epoch. *Nature*, **429**, 263–267.
- Raymond M, Rousset F (1995) Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *Heredity*, **86**, 248–249.
- Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.
- Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.
- Ryman N, Palm S (2006) POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology*, **6**, 600–602.
- Sandoval-Castillo J, Rocha-Olivares A, Villavicencio-Garayzar C, Balart E (2004) Cryptic isolation of Gulf of California Shovelnose Guitarfish evidenced by mitochondrial DNA. *Marine Biology*, **145**, 938–988.
- Saunders MW, McFarlane GA (1993) Age and length at maturity of the female spiny dogfish, *Squalus acanthias*, in the Strait of Georgia, British Columbia, Canada. *Environmental Biology of Fishes*, **38**, 49–57.
- Schneider S, Excoffier L (1999) Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics*, **152**, 1079–1089.
- Schrey AW, Heist EJ (2003) Microsatellite analysis of population structure in the shortfin mako (*Isurus oxyrinchus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **60**, 670–675.

- Schultz JK, Feldheim KA, Gruber SH, Aschley MV, McGovern TM, Bowen BW (2008) Global phylogeography and seascape genetics of the lemon sharks (genus *Negaprion*). *Molecular Ecology*, **17**, 5336–5348.
- Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, **69**, 82–90.
- Shepherd T, Page F, MacDonald B (2002) Length and sex-specific associations between spiny dogfish (*Squalus acanthias*) and hydrographic variables in the Bay of Fundy and Scotian Shelf. *Fisheries Oceanography*, **11**, 78–89.
- Simpfendorfer CA, Heupel MR (2004) Assessing habitat use and movement. In: *Biology of Sharks and Their Relatives* (eds Carrier JC, Musick JA, Heithaus MR), pp 553–572. CRC Press, Boca Raton, FL, USA.
- Stenberg C (2005) Life history of the piked dogfish (*Squalus acanthias* L.) in Swedish waters. *Journal of the Northwest Atlantic Fisheries Sciences*, **35**, 155–164.
- Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.
- Temnykh S, DeClerk G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. *Genome Research*, **11**, 1441–1452.
- Templeman W (1944) The life-history of the spiny dogfish (*Squalus acanthias*) and the vitamin A values of dogfish liver oil. *Newfoundland Department of Natural Resources Research Bulletin*, **15**, 1–102.
- Templeman W (1976) Transatlantic migrations of spiny dogfish (*Squalus acanthias*). *Journal of the Fisheries Research Board Canada*, **33**, 2605–2609.
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.
- Ward RD, Holmes BH, Zemlak TS, Smith PJ (2007) DNA barcoding discriminates spurdogs of the genus *Squalus*. In: *Descriptions of new dogfishes of the genus Squalus (Squaloidea: Squalidae)* (eds Last PR, White WT, Pogonoski JJ), pp. 117–130. CSIRO, Hobart.
- Williamson-Natesan EG (2005) Comparison of methods for detecting bottlenecks from microsatellite loci. *Conservation Genetics*, **6**, 551–562.
- Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rythms, and aberrations in global climate 65 Ma to present. *Science*, **292**, 686–693.

A. Verrissimo is currently a graduate student at the Virginia Institute of Marine Science (VIMS), College of William and Mary and this study is part of her PhD research on the patterns of population structure of deep water squaloid sharks. J.R. McDowell is a population geneticist who is interested in the genetic structure and conservation of marine organisms. J.E. Graves, a professor at VIMS, studies the processes of molecular evolution in marine organisms, with a focus on the population structure and movements of large pelagic fishes.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Supporting Information tables referred to in the text.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.