

Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*)

K. M. DUNCAN,* A. P. MARTIN,† B. W. BOWEN‡ and H. G. DE COUET*

*Department of Zoology, University of Hawaii, 2538 The Mall, EDM 152, Honolulu, Hawaii, 96822, USA, †Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado, 80309, USA, ‡Hawaii Institute of Marine Biology, PO Box 1346, Kāne'ohe, Hawaii, 96744, USA

Abstract

Large marine fishes typically have little population genetic structure. The exceptions are associated with sedentary behaviour, disjunct distributions, or reproductive philopatry. Scalloped hammerhead sharks (*Sphyrna lewini*) incorporate the contrasting traits of oceanic habitat (usually associated with high dispersal) and possible fidelity to nursery grounds (for reproductive females). To evaluate the expectations of these contrasting behaviours, we examined the global genetic structure of *S. lewini* based on collections ($n = 271$ individuals) from 20 nursery areas. A 548-bp fragment of mitochondrial DNA control region revealed 22 polymorphic sites, 24 haplotypes, and three lineages distinguished by 2.56–3.77% sequence divergence. Coalescence analyses based on a provisional molecular clock indicate an origin in the Indo-West Pacific with late Pleistocene radiations into the central Pacific (Hawaii) and eastern Pacific (Central America), as well as recent interchange between oceans via southern Africa. Population subdivisions are strong (overall $\Phi_{ST} = 0.749$, $P < 0.0001$ and among oceans $\Phi_{ST} = 0.598$, $P < 0.0098$). Genetic discontinuity within oceans ($\Phi_{ST} = 0.519$, $P < 0.0001$) is primarily associated with oceanic barriers (migration across oceans $M \approx 0$), with much less structure along continental margins ($M > 10$). We conclude that nursery populations linked by continuous coastline have high connectivity, but that oceanic dispersal by females is rare. Although we cannot rule out philopatry to natal nurseries, oceanic barriers appear to have a much stronger influence on the genetic architecture of this species and may indicate a mechanism for recent evolutionary radiations in the genus *Sphyrna*.

Keywords: biogeography, cosmopolitan marine species, dispersal, mitochondrial DNA, nursery habitat, philopatry

Received 25 October 2005; revision accepted 6 February 2006

Introduction

Widely distributed marine fishes, including tunas, billfishes and sharks, typically exhibit high dispersal and little population structure across vast regions (Graves 1998; Heist 2004). Exceptions to this pattern of high genetic connectivity invariably involve species that are sedentary, have disjunct distributions, or display some degree of

philopatry, such as anadromous fishes (e.g. salmon, Grant & Waples 2000; Waples *et al.* 2001). Recent studies indicate, however, that some marine teleosts (bony fishes) and some elasmobranchs (sharks, skates, and rays) may have more population structure than expected given their cosmopolitan distributions (Graves 1998; Hueter *et al.* 2002; Heist 2004). Among sharks, significant genetic structure for maternally inherited mitochondrial DNA (mtDNA) is reported in most species surveyed to date (e.g. gummy shark *Mustelus antarcticus*, Gardner & Ward 1998; great white shark *Carcharodon carcharias*, Pardini *et al.* 2001; blacktip sharks *Carcharhinus limbatus*, Keeney *et al.* 2003, 2005; shortfin mako *Isurus oxyrinchus*, Schrey & Heist 2003)

Correspondence: Kanesa M. Duncan, University of Hawaii, CRDG, 1776 University Ave., UHS 3 #121, Honolulu, HI, 96822, USA. Fax: 01-808-956-7260; E-mail: kanesa@hawaii.edu

and has been attributed, in part, to reproductive behaviour. Unlike large oceanic teleosts, sharks lack pelagic larvae; young are laid in demersal egg cases or born live, and dispersal is accomplished exclusively by juvenile and adult stages. Sharks do not exhibit parental care, but many species use shallow, coastal nurseries that are geographically distinct from adult feeding areas (Springer 1967; Lund 1990). This habitat partitioning may indicate philopatry, which would explain the unexpected degree of population structure found in sharks, including some widely distributed, highly vagile species. Even if they are not loyal to specific nurseries, reproduction in many species is strongly affiliated with sheltered, coastal habitat.

The scalloped hammerhead shark (*Sphyrna lewini*) is circumglobally distributed in tropical waters (Compagno 1984). This species, and perhaps all hammerhead sharks (Sphyrnidae), have geomagnetic orientation and navigation abilities, possibly enhanced by their unique laterally expanded head (Klimley 1993; Montgomery & Walker 2001; Kajiura & Holland 2002; Meyer *et al.* 2005). Seasonal aggregations of scalloped hammerheads at seamounts (Klimley & Nelson 1981, 1984; Klimley & Butler 1988) and the predictable appearance of adults in nursery grounds (Clarke 1971; Snelson & Williams 1981; Compagno 1984; Branstetter 1990; Castro 1993; Simpfendorfer & Milward 1993) suggest a capacity for philopatry. Recent genetic evidence indicates that the nominal *S. lewini* may actually comprise two species (Abercrombie *et al.* 2005; Quattro *et al.* 2006), and there are currently seven described species within the genus *Sphyrna*. Four of the described species are small and occupy coastal habitat with restricted geographical range, including *S. corona*, *S. media*, *S. tudes* and *S. tiburo*. The last species includes two subspecies separated by the Isthmus of Panama (Gilbert 1967; Compagno 1984; Martin 1993). In contrast, *S. lewini*, *S. mokarran* and *S. zygaena* are larger, ocean-going, and more widely distributed.

The global distribution of the larger hammerhead sharks implies some level of *trans*-oceanic dispersal. *Sphyrna lewini* is abundant along continental margins and around mid-oceanic islands in tropical waters (Compagno 1988), but it may not be a truly oceanic species. Tagging data indicate that long distance forays are rare (Kohler & Turner 2001). Moreover, the barriers to dispersal between tropical oceans are substantial. The link between East Pacific and West Atlantic oceans closed with the Isthmus of Panama 3.1–3.5 Ma (Coates & Obando 1996; Coates *et al.* 2004), and the Tethys link between the Indian and Atlantic oceans closed 12–20 Ma (Ricou 1987). The distribution of *S. lewini* across formidable barriers raises questions about the cohesion of this species' gene pool. Are geographically disjunct populations linked by contemporary gene flow or by historical dispersal events during different climatic or biogeographical regimes?

Although recent dispersal events by fishes between the Indian and Atlantic Oceans are rare (Rocha *et al.* 2005), the Agulhas current system occasionally projects warm water masses westward around the horn of South Africa (Peeters *et al.* 2004), as indicated by tropical Indian Ocean biota that appear at the oceanic island of St. Helena in the South Atlantic (Lubbock 1980). The distribution of scalloped hammerheads in every tropical ocean basin indicates historical dispersal when corridors were available, but it is also possible that geographically disjunct populations are linked by continual gene flow. Insight into the genetic architecture of *Sphyrna lewini* will advance our understanding of the evolution and speciation of large, mobile, marine fishes and can inform conservation strategies that may help protect vulnerable species.

Here, we present the results of a study of mitochondrial DNA sequence variation in hammerhead sharks collected from across the species' range. The data indicate substantial genetic variation, uncommon for such a large marine predator, and marked population genetic structure indicating restricted dispersal across deep ocean habitat. Relatively minor genetic structure along continental margins does not support the hypothesized philopatric behaviour of breeding females.

Methods

Sample collection, DNA extraction and sequencing

Scalloped hammerhead specimens, consisting of fin, muscle, or liver, were acquired by fishing or purchased from fish markets during 1999–2004. Specimens were collected from multiple locations in each of three ocean basins, including (i) Pacific: Baja California, Pacific Panama, Hawaii, the Philippines, Taiwan, and eastern Australia; (ii) Indian: Thailand, Western Australia, Seychelles, and South Africa; and (iii) Atlantic: Western Africa, Brazil, Atlantic Panama, Gulf of Mexico, and East Coast USA (Fig. 1). Small tissue samples (< 1 ml) from each specimen were preserved in ethanol (> 85%) or a saturated salt buffer (Amos & Hoelzel 1991). When possible, specimens were collected from juvenile sharks (fork length < 60 cm) within a nursery area to avoid the confounding effect of sampling adults in feeding areas where distinct breeding populations may overlap (Bowen *et al.* 2005). Samples from six other hammerhead species (genus *Sphyrna*) were included as outgroups and to provide a relative comparison for interpreting mtDNA diversity within *S. lewini*.

Genomic DNA was isolated from 15 to 20 mg of tissue, using a QIAGEN DNeasy Tissue kit (QIAGEN) as described by the manufacturer. The mtDNA control region (CR) and the phenylalanine tRNA were amplified (~1200 bp total) by polymerase chain reaction (PCR) using primers complementary to sections of the proline tRNA (light strand



Fig. 1 Map with the range of *Sphyrna lewini* in yellow (adopted from Compagno 1988). Numbers indicate sample sizes from each location.

primer: Pro-L 5'-AGGGRAAGGAGGGTCAAACCT-3') and 12S rRNA (heavy strand primer: 282 H 5'-AAF-GCTAFFACCAAACCT-3', Keeney *et al.* 2003, J. Patton, unpublished). Each PCR consisted of 200 μ M of each dNTP, 10 \times *Taq* reaction buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25 $^{\circ}$ C) and 1% Triton X-100), 2 mM MgCl₂, 0.25 μ M of each primer, 0.5 U *Taq* DNA polymerase, and 50 ng DNA template in a volume of 50 (μ l). PCR conditions were modified slightly from Keeney *et al.* (2003): 2-min denaturation at 92 $^{\circ}$ C, followed by 30 cycles of 1 min at 95 $^{\circ}$, 1 min at 58 $^{\circ}$, 1 min at 72 $^{\circ}$, and finished with 6 min at 72 $^{\circ}$ for final extension. PCR products were either purified using a QIAGEN QIAquick PCR Purification kit, or were separated by electrophoresis on a 1% agarose gel at 70 V for 90 min and excised with a QIAGEN QIAquick Gel Extraction kit (QIAGEN).

To screen for variable positions within the control region (1086–1087 bp), 20 *S. lewini* individuals were sequenced using primers Pro-L and 282-H. The majority of the parsimony-informative sites occurred within domain 1 of the control region, and specimens with a common haplotype were sequenced only in the forward direction (using primer Pro-L). A 548-bp segment of this sequence was used for subsequent analyses. Haplotypes observed in one or a few individuals were sequenced in the reverse direction using primer SLcr-H (5'-ACATTCTCCATCCCCTTG TG-3') to confirm nucleotide assignments. Sequencing was conducted on an automated sequencer using the dye-termination method (Applied Biosystems).

Phylogenetic and phylogeographic analysis of sequences

Sequences were aligned with SEQUENCHER 4.2 (Gene Codes Inc.), and alignment results were confirmed by eye. Sequences were grouped into haplotypes and analysed in PAUP 4.0 (Swofford 2003) for number of polymorphic sites, transitions, transversions and nucleotide composition. Gaps were treated as missing data. Haplotype and nucleotide diversities were calculated with ARLEQUIN 3.0 (Excoffier *et al.* 2005), which implements equations 8.4 and 10.5 of Nei (1987). Genetic diversity within and among populations was estimated with an analysis of molecular variance (AMOVA, Excoffier *et al.* 1992; ARLEQUIN 3.0, Excoffier *et al.* 2005). To eliminate a potentially confounding signal from migratory adult sharks, within and among population variation was estimated separately for juveniles and adults.

Haplotypes of *Sphyrna lewini* were aligned with sequences from six outgroup hammerhead species ($n = 1$ for *S. corona*, *S. media*, *S. tiburo* and *S. tudes*, $n = 8$ for *S. mokarran* and $n = 9$ for *S. zygaena*) using SEQUENCHER 4.2 (Gene Codes Inc.). The likelihood criteria in MODELTEST 3.0 (Posada & Crandall 1998) identified the HKY85 + G ($\alpha = 0.6615$) + I model of sequence evolution as appropriate for *S. lewini*. This model of evolution was used in calculating a distance matrix of haplotypes and in reconstructing maximum likelihood trees in PAUP 4.0 with 1000 bootstrap replicates, heuristic search method and nearest neighbor interchange branch swapping. Gene genealogies for *S. lewini* were

estimated with statistical parsimony criteria in TCS 1.13 (Templeton *et al.* 1992; Clement *et al.* 2000).

Molecular clock calibration

A control region molecular clock for *S. lewini* was estimated from comparisons of West Atlantic and East Pacific *S. lewini*. These populations were probably isolated by formation of the Isthmus of Panama, 3.1–3.5 Ma (Coates & Obando 1996; Coates *et al.* 2004). Intrapopulation divergence of mtDNA lineages prior to separation by the isthmus was corrected by $d_{\text{corr}} = d - (\pi_1 + \pi_2)/2$ (see Cann *et al.* 1987), and the remaining divergence between the Atlantic and Pacific populations was used to estimate a molecular clock.

Population age and size

We tested for evidence of population expansion using Fu's *F* test in ARLEQUIN 3.0 (Fu 1996). The mismatch analysis in ARLEQUIN 3.0 included a raggedness index to determine goodness of fit to a unimodal distribution (Harpending 1994). Population size was calculated by $\theta = 2N_f\mu$, where N_f is the effective female population size and μ is the mutation rate per generation. Because of a dichotomy of opinions on *S. lewini* growth rate, population size was calculated for two-generation times: 5.7 and 16.7 years (see Branstetter 1987; Chen *et al.* 1990; Liu & Chen 1999; Anislado-Tolentio & Robinson-Mendoza 2001; Cortes 2004). Population age was calculated by $\tau = 2t\mu$, where *t* is age in generations.

Migration rate and population isolation

Adjacent pairs of populations with sample sizes > 7 were analysed using MDIV, a Bayesian Markov chain Monte Carlo method for the joint estimation of migration rate and isolation time between populations on a pairwise basis (Nielsen & Wakeley 2001). For each data set, we used the finite sites model. Migration and population divergence time were estimated for values from 0.02 to 10 in increments of 0.02. The parameter value with the highest probability was adopted as the best estimate, and upper and lower credibility limits were within $\pm 2 \log(L)$ U from the best estimate (where *L* is the likelihood function). Samples from the Gulf of Mexico and eastern North America were pooled when compared to the South African sample.

To assess whether the direction of dispersal events could be inferred based on the current distribution of haplotypes, parsimony mapping of geographical location was conducted using MACCLADE 3.3 (Maddison & Maddison 1992). Nodes that were not resolved were considered hard polytomies in the trees.

Results

Sequence characteristics and variation

Mitochondrial control region sequences were resolved for 271 individuals of *Sphyrna lewini*, 21 individuals of the other recognized hammerhead species, and seven individuals of an undescribed, cryptic Atlantic lineage most closely related to *S. lewini* (Abercrombie *et al.* 2006; Quattro *et al.* 2005). Sequence divergence between species ranged from 7.8% to 24.3%. The cryptic Atlantic lineage differed from individuals of *S. lewini* (*sensu stricto*) by 5.6–7.5%. Within *S. lewini* (*sensu stricto*), we uncovered 24 haplotypes defined by 22 polymorphic sites (Appendix 1). Haplotype diversity was high ($h = 0.80 \pm 0.020$ SD, Table 1) with maximum sequence divergence between individuals at 3.8% (average $d = 1.3\%$). We found a similar level of variation in *Sphyrna zygaena* ($h = 0.83 \pm 0.127$ SD, $n = 9$) and *Sphyrna mokarran* ($h = 0.64 \pm 0.184$ SD, $n = 8$), for which we surveyed individuals from widely separated localities.

Parsimony, maximum likelihood, and statistical parsimony reconstructions yielded similar topologies (Figs 2 and 3). Several features of the maternal genealogy are noteworthy. First, the inferred root of the tree was an ancestor most similar to haplotypes sampled from southern Africa (SL22) and the Indo-West Pacific (SL23 and SL24, Fig. 2). Second, derived from these basal haplotypes (SL22–24) were two divergent lineages with strong geographical orientations. The first lineage includes the most common haplotype (SL1) and is distributed from the eastern Pacific to the Indian Ocean. The second lineage, which includes the second most common haplotype (SL19), occurs in the North Atlantic, South Atlantic, and the Indo-West Pacific. Within the lineages, some of the relatively common haplotypes are restricted to either the Pacific Ocean (e.g. SL2), the Atlantic Ocean (e.g. SL15), or the Indo-West Pacific (e.g. SL24, Fig. 3).

The genetic structure evident in the gene trees was confirmed by an analysis of molecular variance (AMOVA), which revealed significant partitioning of genetic variation among oceans and among locations. Genetic variation was similarly structured in analyses of adults, juveniles, and all individuals (Table 2). Estimates of divergence time and migration rate between pairs of adjacent populations revealed that the structure within oceans was primarily due to genetic discontinuities across ocean basins (Table 3). For all adjacent pairs of populations separated by deep ocean, MDIV estimates of divergence time were always significantly different from zero and the estimated migration rate was low ($M \leq 1$, Table 3). For example, the number of migrants per generation between Hawaii and the eastern Pacific was $M = 0.34$ –0.42. Similarly $M = 0.02$ –0.06 between Western Australia and South

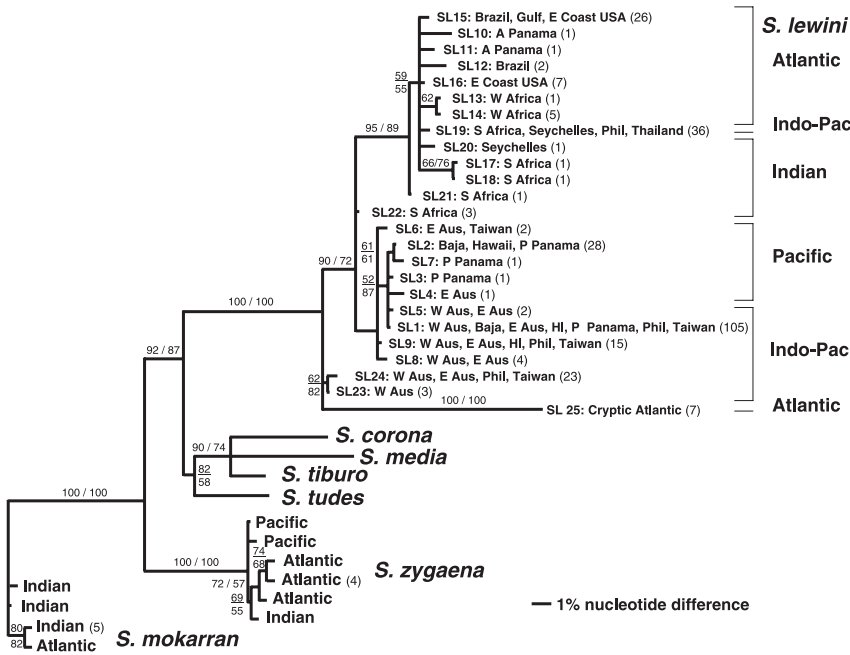


Fig. 2 Overview of genus *Sphyrna* based on mitochondrial control region haplotypes showing levels of divergence within and between species. The topology is based on maximum likelihood and numbers above and below branches indicate bootstrap values greater than 50% (1000 pseudo replicates) from maximum likelihood and maximum parsimony analyses, respectively. For *S. mokarran*, *S. zygaena* and *S. lewini* the number of individuals (for haplotypes with more than one individual) is indicated in parenthesis. For *S. lewini*, haplotype numbers are indicated by SL# before terminal taxa location (SL# corresponds to Appendix 1 and Fig. 3). Location abbreviations are as follows: A, Atlantic; Aus, Australia; E, East; HI, Hawai'i; P, Pacific; Phil, Philippines; and W, West.

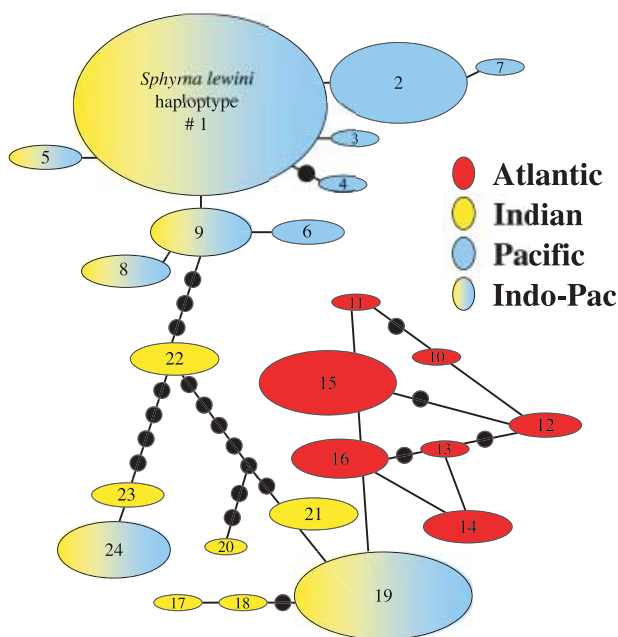


Fig. 3 Haplotype network for *Sphyrna lewini* constructed by statistical parsimony in rcs 1.13 (Templeton *et al.* 1992; Clement *et al.* 2000). Haplotypes are indicated by ovals, which are sized in proportion to the number of individuals with that haplotype. Haplotype number corresponds to Appendix 1 and Fig. 2. Ocean basins are indicated by colours: Atlantic (red), Indian (yellow), Pacific (blue), and shared Indian-Pacific (yellow fading to blue). Each connecting line represents a single mutation. Black dots represent hypothetical missing ancestors. Haplotypes are connected only through ≤ 9 mutations, as indicated by the 95% connection confidence interval determined for this data set in rcs 1.13.

Africa or the Seychelles. In contrast, for all pairs of adjacent populations along contiguous continental margins, divergence time was never significantly different from zero, and estimated migration rates were large ($M = 1.2-9.9$). Moreover, all the upper credibility limits were greater than 10 (Table 3). Overall, *MDIV* analysis revealed strong genetic discontinuities across deeper water coupled with low differentiation along continuous continental margins.

Because ancestral states were equivocal, parsimony mapping of geography onto the haplotype tree provided little insight into the direction of dispersal events over the evolutionary period encompassed by haplotype diversity. There were, however, two exceptions. First, a cluster of western Indian Ocean haplotypes was nested within an Atlantic lineage, suggesting a dispersal event from the Atlantic to the Indian Ocean. Second, most of the Pacific Ocean haplotypes coalesce in the Indo-West Pacific, suggesting a potential centre of origin.

Molecular clock calibration

We estimated $d = 0.028$ for transisthmanian comparisons of *S. lewini* (Atlantic $n = 2$, Pacific $n = 7$). When corrected for the variance in populations prior to the closure of the Panamanian Isthmus, $d_{corr} = 0.027$. This corresponds to 0.8% divergence between lineages per million years. This rate is similar to the value obtained from transisthmanian comparisons of lemon sharks (*Negaprion brevirostris*, J. Schultz, unpublished).

Table 2 *Sphyrna lewini*. AMOVA: the proportion of haplotype variation attributed to differences within and among populations for all *S. lewini* individuals, juveniles only and adults only. Values with a * are significant at $P < 0.0001$, values with ** are significant at $P = 0.00098$, and values with *** are significant at $P = 0.022$

Comparison of <i>S. lewini</i>	All <i>S. lewini</i> ($n = 271$)		<i>S. lewini</i> juveniles (207)		<i>S. lewini</i> adults ($n = 64$)	
	% variation	Φ_{ST}	% variation	Φ_{ST}	% variation	Φ_{ST}
Among populations	74.9%	0.749*	74.6%	0.746*	77.2%	0.772*
Within populations	25.2%		25.4%		22.8%	
Hierarchical						
Among oceans Φ_{CT}	59.8%	0.598**	61.8%	0.618**	52.6%	0.526***
Among populations within oceans						
Φ_{sc}	20.9%	0.519*	20.1%	0.525*	27.5%	0.579*
Within populations Φ_{ST}	19.3%	0.807*	18.2%	0.818*	20.0%	0.800*

Comparison	$T > 0$	M	Credibility limits	Pairwise Φ_{ST}
Along continental margins				
P. Panama-Baja	No	1.4	0.06→10	0.002
Taiwan-Philippines	No	2.5	0.26→10	0.100
Taiwan-E. Australia	No	9.9	2.40→10	0.016
Philippines-E. Australia	No	3.3	0.66→10	0.122*
E. Australia-W. Australia	No	2.2	0.58→10	0.397*
S. Africa-Seychelles	No	1.2	0.06→10	0.009
Across ocean basins				
Hawaii-Baja	Yes	0.34	0.00–2.5	0.448*
Hawaii-P. Panama	Yes	0.42	0.04–2.4	0.629*
Hawaii-Taiwan	Yes	0.60	0.16–1.5	0.330*
Hawaii-E. Australia	Yes	1.20	0.20–8.3	0.171*
W. Australia-S. Africa	Yes	0.06	0.00–0.38	0.991*
W. Australia-Seychelles	Yes	0.02	0.00–0.24	0.736*
S. Africa-NW Atlantic	Yes	0.08	0.00–0.56	0.573*

Table 3 Results from MDIV (Nielsen & Wakeley 2001) analysis. T is the estimated time of divergence between the two populations; yes or no indicates whether the two populations exhibited likelihood values of T that were significantly different from zero. M is the estimated number of migrants per generation. Credibility limits are lower and upper limits of M . Pairwise Φ_{ST} values were estimated in ARLEQUIN 3.0 (Excoffier *et al.* 1992, 2005). Values with a * are significant at $P < 0.05$

Population age and size

Three of the populations examined (Thailand, Brazil, Atlantic Panama) had small sample sizes ($n < 5$) and were not analysed for all population parameters. In the remaining populations, with the exception of Hawaii, Fu's F_S test was not significant. The mismatch assumption of a unimodal distribution ($P > 0.05$) was accepted for all samples except the Baja population. Therefore, population parameters should be evaluated with caution for the Baja and Hawaii populations (Table 1). Estimates of female effective population size ranged from 550 to 31 000 000. The oldest population expansion estimate was for the Indo-West Pacific (Taiwan; 740 000 years), and the youngest estimates were for the eastern Atlantic (43 000 years), eastern Australia (44 000 years), and eastern Pacific (73 000 years; Table 1).

Discussion

Most genetic analyses of cosmopolitan marine fishes reveal a trend toward low levels of genetic structure across ocean

basins, high dispersal between populations, and shallow population histories (see Grant & Bowen 1998; Waples 1998). Conversely, gene flow is lower among fishes with disjunct distributions (Graves 1998). Scalloped hammerhead sharks are widely distributed, but they are also dependent on discrete coastal nursery areas, a factor that may promote population structure (Keeney *et al.* 2003, 2005; Bowen *et al.* 2005). Therefore, we must consider the possibility that these sharks are faithful to natal sites, a condition that should result in peripatric populations. Our results indicate that *Sphyrna lewini* has strong population structure on a global scale and mtDNA lineages that appear to have been isolated within ocean basins for hundreds of thousands of years. However, nurseries connected by continuous shallow-water habitat were not significantly different, indicating low natal philopatry in this species.

Prior to interpreting these results, we address two caveats:

- 1 Our molecular clock is provisional. Knowlton & Weigt (1998) demonstrated that species with different ecologies experienced the severance of gene flow at different times with respect to the closure of the Isthmus of Panama.

While concordant divergence rate estimates across shark species (*S. lewini* and *Negaprion brevirostris*) are reassuring, our molecular clock estimate (0.8%/Myr between lineages) is a first approximation. Furthermore, the generation time of *S. lewini* is controversial (see Branstetter 1987; Liu & Chen 1999; Cortes 2004). Since generation time and mutation rate are primary parameters in coalescence analyses, corresponding values for population age and size should be regarded as qualitative indicators, rather than precise estimates. For example, differences in coalescence time estimates for Baja California (73 000 years) and Pacific Panama (110 000 years) may not be meaningful, but we can say with some confidence that both values are in the late Pleistocene, and probably within the most recent glacial cycle, beginning approximately 120 000 years ago.

- 2 Abercrombie *et al.* (2005) and Quattro *et al.* (2006) demonstrate a cryptic Atlantic species within *S. lewini*. We have excluded this lineage from our population level analysis under the assumption that it is a distinct taxon. Including it would increase the level of structure but would not affect our analysis of gene flow between populations of *S. lewini* (*sensu stricto*).

Distinct genetic structure across ocean basins

The scalloped hammerhead shark has population subdivisions between oceans as well as within ocean basins. Statistical parsimony and population age calculations reveal a pattern of genetic differentiation that includes some elements of the stepping stone model of dispersal (Kimura & Weiss 1964). Consistent with this analysis, MDIV shows little genetic structure among populations connected by coastline. For all pairwise population comparisons along continental margins, estimates of divergence time included zero, and the upper confidence limits for gene flow exceeded 10 individuals per generation (Table 3). In contrast, populations separated by expanses of deep ocean exhibited strong genetic differentiation. This pattern was evident whether comparisons were between a mid-oceanic island population (Hawaii) and continental coastal populations, or between samples collected from the eastern and western margins of ocean basins.

Tagging data indicate that scalloped hammerhead sharks use offshore oceanic habitat, but do not regularly roam across large distances. The median distance between mark and recapture of adults along the eastern USA from a total of 3278 tagged individuals at liberty from 0 to 9.6 years (mean = 2.3 years) was less than 100 km (Kohler & Turner 2001). These sharks are most often encountered over continental or island shelves; it is unusual to capture a hammerhead in the open ocean. However, there is a record of an individual traversing 1600 km over deep water (Kohler & Turner 2001), indicating that contemporary movement

across oceans, or at least between continental margins and mid-oceanic islands, is possible. Therefore, tagging and genetic data are concordant; individuals appear to disperse readily across continuous habitat (continental shelves) and rarely across open oceans.

Natal homing as a mechanism for population subdivision

A growing body of data indicates significant population genetic differentiation in elasmobranchs, at least in maternal lineages (Gardner & Ward 1998; Pardini *et al.* 2001; Keeney *et al.* 2003, 2005; Schrey & Heist 2003). Philopatry may be an important component of elasmobranch behaviour (Hueter 1998; Hueter *et al.* 2002). However, because of the relatively low DNA mutation rate in elasmobranchs (Martin *et al.* 1992; Martin 1993), the logistic challenges in acquiring genetic samples, and the difficulty in verifying philopatric behaviour through tagging studies, natal homing in elasmobranchs has remained largely untested.

The Φ_{ST} values for *S. lewini* were high for analyses of neonate sharks in their nursery areas ($\Phi_{ST} = 0.525$ for hierarchical analysis of populations within oceans). However, this comparison is driven largely by differences between geographically distant nurseries. Keeney *et al.* (2003, 2005) found a similar trend of isolation by distance among blacktip sharks in the Gulf of Mexico and Atlantic Ocean. Over spatial scales of 100–250 km they found little evidence of population differentiation, but between South Carolina, Florida, Texas, and Yucatan they found significant structure (Φ_{ST} as high as 0.886 between the western Atlantic and the Yucatan). Although we cannot rule out a role for fidelity by adult hammerheads when they encounter and return to suitable nursery habitat (that is not necessarily their place of birth), mtDNA evidence argues against strong natal homing behaviour; scalloped hammerheads appear to stray between proximal nursery areas. Indeed, our genetic findings are consistent with anecdotal observations; scalloped hammerhead sharks use artificially enlarged estuaries as nursery grounds in Hawaii (Kahului Harbor, Maui; Hawaii Kai Marina, O'ahu; K. Duncan, unpublished), where the recently enlarged nursery habitats are 100–600 km from established nurseries. Thus, the most extreme version of site fidelity (natal homing) is not recorded in the maternal genetic architecture of *S. lewini*.

Phylogeography and species history

Coalescence analyses indicate that the oldest extant *S. lewini* populations are in the central Indo-West Pacific region (Australia and the Philippines). Based on genetic distances and the relationships of haplotypes (Fig. 3), there were several dispersal events from this region. The deepest genetic divergences within the Indian and Pacific Oceans

are $d = 3.11\%$ and $d = 2.56\%$, respectively. On the other hand, maximum divergences within the Atlantic Ocean ($d = 0.77\%$) and East Pacific ($d = 0.88\%$) are markedly less. Although no haplotypes are shared between the Atlantic Ocean and the Indian or Pacific, haplotypes from the Indo-West Pacific are separated from the Atlantic by as little as 0.18% , indicating a late Pleistocene divergence. This pattern is consistent with the centrifugal hypotheses for species distributions, where the highest species diversity (and genetic diversity) is in the core of the Indo-West Pacific (Briggs 1999, 2000).

If *S. lewini* had a circumtropical distribution prior to the closing of the Isthmus of Panama, we envision two possible scenarios to explain the pattern of haplotype distribution between the Atlantic and the Indo-West Pacific Oceans: (i) The Atlantic populations (*sensu stricto*) were isolated by the Isthmus closure, and the Indo-West Pacific haplotypes most closely related to the Atlantic lineage (*sensu stricto*) are the product of recent dispersal from the Atlantic into the Indo-Pacific (gene flow west to east). (ii) The cryptic Atlantic lineage (from Abercrombie *et al.* 2005 and Quattro *et al.* 2006) was isolated by the Isthmus closure, and the more closely related Atlantic populations are the result of recent colonization of the Atlantic from the Indo-Pacific (gene flow east to west). Both scenarios invoke dispersal around southern Africa, possibly during warm interglacials (see Peeters *et al.* 2004).

We support the west to east scenario for three reasons. First, the cryptic Atlantic species is much more divergent than the Atlantic-Pacific divergence in *S. lewini* (*sensu stricto*); $d = 6.5\%$ vs. $d = 2.7\%$. Transisthmian divergence based on the cryptic Atlantic lineage would generate a mutation rate similar to that of mtDNA coding regions in bony fishes ($2\%/Myr$, Bowen *et al.* 2001), which we regard as unrealistic given the slow mutation rates documented for sharks (Martin *et al.* 1992; Martin 1993; J. Schultz, unpublished). Second, the sequence divergence between the closely related Indo-West Pacific and Atlantic haplotypes (SL17–20, Fig. 2) is much too shallow for this separation to have been caused by the Isthmus closure (by any molecular clock). Third, the Indo-Pacific members of this lineage are clearly nested within a greater diversity of Atlantic lineages in phylogenetic analyses. The hypothesis of dispersal from the Atlantic to the Indo-Pacific is an exception to the widely accepted transfer of organisms from the Indian to the Atlantic (Edwards 1990; Bowen *et al.* 2001; Rocha *et al.* 2005). However, hammerhead sharks are active swimmers from birth whereas the examples of east to west dispersal involve passive larval transport — a crucial distinction between the phylogeographic patterns of bony fishes and elasmobranchs.

Like the eastern Atlantic, the tropical eastern Pacific is regarded as an isolated biogeographical province (Briggs 1974, 2003). Within the eastern Pacific Ocean, we observed

five haplotypes (SL1–4, 7, Fig. 2). Two of these (SL1 and 2) are shared with Hawaii, and the other three are distinguished from the common widespread haplotype by single mutations. This indicates a population recently derived from the Indo-West Pacific, concordant with coalescent analysis (~100 000 years ago, Table 1). Under this scenario of recent dispersal, Hawaii and perhaps the archipelagos of the South Pacific may be stepping-stones for colonization into the eastern Pacific.

Populations within the Atlantic also have shallow coalescence times, but in this case, coalescence occurs among haplotypes at the terminus of a divergent lineage, indicating an Atlantic lineage with millions of years of isolation from the Indo-West Pacific. Although tropical Atlantic habitats do not suffer the drastic climate shifts characteristic of the eastern Pacific (e.g. El Niño events), the genetic architecture of *S. lewini* within the Atlantic is also probably influenced by large scale environmental changes (e.g. range contraction during glacial maxima). Moreover, dependence on estuarine nursery areas and shallow bays makes these sharks vulnerable to smaller scale ecological shifts as well.

Comparison with other globally distributed marine animals

Analysis of two other globally distributed sharks, the shortfin mako (*Isurus oxyrinchus*) and the soupfin (*Galeorhinus galeus*), revealed markedly less population structure than is evident in *S. lewini* (RFLP mtDNA, Heist *et al.* 1996; Ward & Gardner 1997; microsatellites, Schrey & Heist 2003). This is not unexpected considering that both the shortfin mako and the soupfin are more oceanic species than *S. lewini*. However, the global pattern of genetic diversity in these species was similar to what we discovered for *S. lewini*: low diversity within the Atlantic Ocean and significant differences in haplotype frequencies between ocean basins.

Many large teleosts show ancient separations between the Atlantic and Indo-Pacific Oceans (Graves 1998). For example, mtDNA markers in the bigeye tuna (*Thunnus obesus*) show a global pattern similar to (but shallower than) what we observe in hammerheads. However, tuna haplotypes detected in South Africa were also observed in the northwestern Atlantic (Chow *et al.* 2000), a pattern not evident for the hammerheads. This may be due to habitat differences that influence the distribution of genetic markers; tuna are predominantly oceanic, whereas hammerheads are dependent on shallow water, near continental shelves or around oceanic islands for part of their life history.

Perhaps a more appropriate comparison is with cetaceans. Like hammerhead sharks, cetaceans are viviparous, and dispersal is nektonic. A study of the widely distributed

dusky dolphin (*Lagenorhynchus obscurus*) revealed high dispersal along continental shelves but little transoceanic gene flow (Cassens *et al.* 2005), a pattern similar to *S. lewini*. Results from another widespread species, the bottlenose dolphin (*Tursiops* spp.), revealed strong population genetic differentiation between pelagic and nearshore populations, as well as between populations sampled along continental margins (Natoli *et al.* 2004), indicating more limited dispersal than in hammerheads. Such a difference may reflect social behaviours influencing cetacean migration (Amos *et al.* 1993) which are not observed in sharks. Taken together, these comparisons across taxa suggest that hammerhead population structure is more similar to patterns observed in cetaceans than to bony fishes.

Insight into speciation?

Since the rise of the Isthmus of Panama and the severing of circumglobal tropical seas, the western Atlantic and eastern Pacific are geological ages apart. High genetic diversity of the Indo-West Pacific populations and low genetic diversity in areas remote from the Indo-West Pacific (the Atlantic and the eastern Pacific), coupled with evidence for limited trans-oceanic gene flow, indicate that speciation in hammerheads (and other tropical and subtropical sharks) probably occurs by peripatric mechanisms envisaged by Mayr (1954). The Indo-West Pacific is a centre of diversity for tropical sharks (like *S. lewini*) and, for widespread species, the distribution margins may be areas where unique haplotypes emerge and increase to high frequency in the absence of migration. Given sufficient time, such isolation may result in speciation. Assuming the Indo-West Pacific is the centre of origin for hammerheads, this peripheral isolation model may explain the restricted distribution of the four diminutive *Sphyrna* species in the eastern Pacific and western Atlantic.

Novel lineages that emerged at the range margins may also disperse back into the centre of the species' distribution, a centripetal pattern documented for other groups of tropical marine organisms (Briggs 2000, 2003; Meyer 2003). Our mapping of geography onto a cladogram provided evidence of this process in one case; haplotypes from the western Indian Ocean (South Africa) are deeply nested within the Atlantic lineage.

Conservation of shark populations

While individual nurseries may not be genetically distinct, they are nonetheless essential to population viability (Branstetter 1990; see Bowen *et al.* 2005). Here we have demonstrated strong population structure overall. In some cases, populations are isolated and have a limited number of nursery habitats (e.g. Hawaii). In other cases, there is higher connectivity (i.e. along continental margins). However,

genetic connectivity among marine populations may not be sufficient to rebuild depleted populations, and populations connected by a few migrants per generation can still be isolated in terms of biomass (Waples 1998). Furthermore, recent evidence indicates that juvenile scalloped hammerhead sharks reside within nursery habitats for extended periods of time (at least one year post parturition, Duncan & Holland 2006). Prudent management for scalloped hammerhead sharks (and other sharks with similar nursery use behaviour) must therefore include not only population-specific protection in the adult phase, but also access to regional nurseries (see Bowen & Roman 2005). The overall strong population structure in *S. lewini* indicates that regional populations, if depleted, will not recover swiftly through immigration, but slowly through reproduction.

Acknowledgements

The most essential part of this work was the collection of samples across a wide geographical range. For help in sample collection, we gratefully thank A. Amorim, G. Burgess, J. Carlson, C. Caraguel, D. Chapman, J. Chidlow, S. Clarke, G. Cliff, L. Dagorn, S. Fennessey, M. Francis, J. Garmin, J. Grady, A. Guadiano, E. Heist, D. Itano, J. Loefer, F. Marquez, F. Magana, R. McAuley, G. Naylor, B. Nishimoto, R. Nu'u, A. Piercy, K. Priede, J. Quattro, W. Robbins, R. Robertson, J. Romine, D. Rowat, P. Santos, G. Sedberry, and J. Schultz. We also thank the South African Natal Shark Board, Seaworld South Africa, Maui Ocean Center, Hilo Division of Aquatic Resources and the Seychelles Bureau of Standards. For laboratory, analytical, and logistic support we thank R. Cann, A. Faucci, J. Grady, E. Heist, K. Holland, S. Karl, D. Keeney, P. Lee, E. Lewallen, F. Moeretzsohn, J. Parrish, and R. Toonen. For use of their unpublished data we thank J. Patton and J. Schultz. We thank J. Grady and T. Trejo for their critical reviews of the manuscript. This research was funded by the American Elasmobranch Society, the Hawaii Institute of Marine Biology, the NSF Graduate K-12 program grant to EECB, a NSF predoctoral fellowship, NSF Grant OCE-0453167, Shark Trust, Sigma Xi, and Seagrant Hawaii.

References

- Abercrombie DL, Clarke SC, Shivji MS (2005) Global-scale genetic identification of hammerhead sharks: application to assessment of the international fin trade and law enforcement. *Conservation Genetics*, **6**, 775–788.
- Amos B, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. *Report of the International Whaling Commission, Special Issue*, **13**, 99–103.
- Amos W, Schloetterer C, Tautz D (1993) Social structure of pilot whales revealed by analytical DNA profiling. *Science*, **260**, 670–672.
- Anislado-Tolentio V, Robinson-Mendoza C (2001) Age and growth for the scalloped hammerhead shark, *Sphyrna lewini* (Griffin and Smith, 1834), along the central Pacific coast of Mexico. *Scientia Marinas*, **27**, 501–520.
- Bowen BW, Roman J (2005) Gaia's handmaidens: the Orlog model for conservation. *Conservation Biology*, **19**, 1037–1043.

- Bowen BW, Bass AL, Rocha LA, Grant WS, Robertson DR (2001) Phylogeography of the trumpETFish, genus *Aulostomus*: ring species complex on a planetary scale. *Evolution*, **55**, 1029–1039.
- Bowen BW, Bass AL, Soares L, Toonen RJ (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). *Molecular Ecology*, **14**, 2389–2402.
- Branstetter S (1987) Age, growth and reproductive biology of the silky shark, *Carcharhinus falciformis*, and the scalloped hammerhead, *Sphyrna lewini*, from the northwestern Gulf of Mexico. *Environmental Biology of Fishes*, **19**, 161–173.
- Branstetter S (1990) Early life-history implications of selected carcharhinoid and lamnoid sharks of the northwest Atlantic. In: *Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries* (eds Pratt HL, Gruber SH, Taniuchi T). NOAA Technical Report. NMFS '90, pp. 17–28.
- Briggs JC (1974) *Marine Zoogeography*. McGraw-Hill, New York.
- Briggs JC (1999) Extinction and replacement in the Indo-West Pacific Ocean. *Journal of Biogeography*, **26**, 777–783.
- Briggs JC (2000) Centrifugal speciation and centers of origin. *Journal of Biogeography*, **27**, 1183–1188.
- Briggs JC (2003) Marine centres of origin as evolutionary engines. *Journal of Biogeography*, **30**, 1–18.
- Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and Human Evolution. *Nature*, **325**, 31–36.
- Cassens I, Van Waerebeek K, Best PB *et al.* (2005) Evidence for male dispersal along the coasts but no migration in pelagic waters in dusky dolphins (*Lagenorhynchus obscurus*). *Molecular Ecology*, **14**, 107–121.
- Castro JI (1993) The shark nursery of Bulls Bay, South Carolina, with a review of the shark nurseries of the southeastern coast of the United States. *Environmental Biology of Fishes*, **38**, 37–48.
- Chen CT, Leu TC, Joung SJ, Lo NCH (1990) Age and growth of the scalloped hammerhead, *Sphyrna lewini*, in northeastern Taiwan waters. *Pacific Science*, **44**, 156–170.
- Chow S, Okamoto H, Miyabe N, Hiramatsu K, Barut N (2000) Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology*, **9**, 221–227.
- Clarke TA (1971) The ecology of the scalloped hammerhead shark, *Sphyrna lewini*, in Hawaii. *Pacific Science*, **25**, 133–144.
- Clement M, Posada D, Crandall K (2000) rcs: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.
- Coates AG, Obando JA (1996) The geologic evolution of the Central American isthmus. In: *Evolution and Environment in Tropical America* (eds Jackson J, Budd AF, Coates AG), pp. 21–56. University of Chicago Press, Chicago.
- Coates AG, Collins LS, Aubry MP, Berggren WA (2004) The geology of the Darien, Panama, and the late Miocene-Pliocene collision of the Panama arc with northwestern South America. *GSA Bulletin*, **116**, 1327–1344.
- Compagno LJV (1984) FAO Species Catalogue. Vol. 4. Parts 1 & 2, Sharks of the world. *FAO Fisheries Synopsis*, p. 125.
- Compagno LJV (1988) *Sharks of the Order Carcharhiniformes*. Princeton University Press, Princeton.
- Cortes E (2004) Incorporating uncertainty into demographic modeling: application to shark populations and their conservation. *Conservation Biology*, **16**, 1048–1062.
- Duncan KM, Holland KN (2006) Habitat use, growth rates and dispersal patterns of juvenile scalloped hammerhead sharks (*Sphyrna lewini*) in a nursery habitat. *Marine Ecology Progress Series*, in press.
- Edwards A (1990) *Fish and Fisheries of Saint Helena Island*. Center for Tropical coastal Management Studies, University of Newcastle upon Tyne, Newcastle.
- Excoffier L, Smouse PE, Quattro JM (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 (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.
- Fu YX (1996) Estimating the age of the common ancestor of a DNA sample using the number of segregating sites. *Genetics*, **144**, 829–838.
- Gardner MG, Ward RD (1998) Population structure of the Australian gummy shark (*Mustelus antarcticus* Gunther) inferred from allozymes, mitochondrial DNA and vertebrae counts. *Marine and Freshwater Research*, **49**, 733–745.
- Gilbert CR (1967) A revision of the hammerhead sharks (family Sphyrnidae). *Proceedings of the United States National Museum*, **119**, 1–88.
- Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from the sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**, 415–426.
- Grant WS, Waples RS (2000) Spatial and temporal scales of genetic variability in marine and anadromous species: implications for fisheries oceanography. In: *Fisheries Oceanography: an Integrative Approach to Fisheries Ecology and Management* (eds Harrison P, Parsons TR), pp. 61–93. Blackwell Science Ltd, Oxford.
- Graves JE (1998) Molecular insights into the population structure of cosmopolitan marine fishes. *Journal of Heredity*, **89**, 427–437.
- Harpending RC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, **66**, 591–600.
- Heist EJ (2004) Genetics of sharks, skates, and rays. In: *Biology of Sharks and Their Relatives* (eds Carrier JC, Musick JA, Heithaus MR), pp. 471–485. CRC Press, New York.
- Heist EJ, Musick JA, Graves JE (1996) Genetic population structure of the shortfin mako (*Isurus oxyrinchus*) inferred from restriction fragment length polymorphism analysis of mitochondrial DNA. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 583–588.
- Hueter RE (1998) Philopatry, natal homing and localized stock depletion in sharks. *Shark News*, **12**, 1–2.
- Hueter RE, Heupel MR, Heist EJ, Keeney DB (2002) The implications of philopatry in sharks for the management of shark fisheries. *Northwest Atlantic Fisheries Organization Scientific Council Meeting*, Serial no. N4744, NAFO SCR Doc. 02/122. From the IUCN/SSC Shark Specialist Group, Nature Conservation Bureau Ltd, Berkshire, UK.
- Kajura SM, Holland KN (2002) Electroreception in juvenile scalloped hammerhead and sandbar sharks. *Journal of Experimental Biology*, **205**, 3609–3621.
- Keeney DB, Heupel M, Hueter RE, Heist EJ (2003) Genetic heterogeneity among blacktip shark, *Carcharhinus limbatus*, continental nurseries along the U.S. Atlantic and Gulf of Mexico. *Marine Biology*, **143**, 1039–1046.
- Keeney DB, Heupel MR, Hueter RE, Heist EJ (2005) Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. *Molecular Ecology*, **14**, 1911–1923.

- Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–576.
- Klimley AP (1993) Highly directional swimming by scalloped hammerhead sharks, *Sphyrna lewini*, and subsurface irradiance, temperature, bathymetry, and geomagnetic field. *Marine Biology*, **117**, 1–22.
- Klimley AP, Butler SB (1988) Immigration and emigration of a pelagic fish assemblage to seamounts in the Gulf of California related to water mass movements using satellite imagery. *Marine Ecology Progress Series*, **49**, 11–20.
- Klimley AP, Nelson DR (1981) Schooling of the scalloped hammerhead shark, *Sphyrna lewini*, in the Gulf of California. *Fishery Bulletin*, **79**, 356–360.
- Klimley AP, Nelson DR (1984) Diel movement patterns of the scalloped hammerhead shark (*Sphyrna lewini*) in relation to El Bajo Espíritu Santo: a refuging central-position social system. *Behavioral Ecology and Sociobiology*, **15**, 45–54.
- Knowlton N, Weigt LA (1998) New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **265**, 2257–2263.
- Kohler NE, Turner PA (2001) Shark tagging: a review of conventional methods and studies. *Environmental Biology of Fishes*, **60**, 191–223.
- Liu KM, Chen CT (1999) Demographic analysis of the scalloped hammerhead, *Sphyrna lewini*, in the northwestern Pacific. *Fisheries Science*, **65**, 218–223.
- Lubbock R (1980) The shore fishes of Ascension Island. *Journal of Fish Biology*, **17**, 283–303.
- Lund R (1990) Chondrichthyan life history styles as revealed by the 320 million years old Mississippian of Montana. *Environmental Biology of Fishes*, **27**, 1–19.
- Maddison WP, Maddison DR (1992) *MACCLADE: Analysis of Phylogeny and Character Evolution, Version 3.3*, pp. 98. Sinauer Associates, Sunderland.
- Martin A (1993) Hammerhead shark origins. *Nature*, **364**, 494.
- Martin AP, Naylor GJP, Palumbi SR (1992) Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature*, **357**, 153–155.
- Mayr E (1954) Change of genetic environment and evolution. In: *Evolution as a Process* (eds Huxley J, Hardy AC, Ford EB), pp. 157–180. Allen & Unwin, London.
- Meyer CP (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society*, **79**, 401–459.
- Meyer CL, Holland KN, Papastamatiou YP (2005) Sharks can detect changes in the geomagnetic field. *Journal of the Royal Society Interface*, **2**, 129–130.
- Montgomery JC, Walker MM (2001) Orientation and navigation in elasmobranchs: which way forward? *Environmental Biology of Fishes*, **60**, 109–116.
- Natoli A, Peddemors VM, Hoelzel AR (2004) Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *Journal of Evolutionary Biology*, **17**, 363–375.
- Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics*, **158**, 885–896.
- Pardini AT, Jones CS, Noble LR *et al.* (2001) Sex-biased dispersal in great white sharks. *Nature*, **412**, 139–140.
- Peeters JC, Acheson R, Brummer G-JA *et al.* (2004) Vigorous exchange between the Indian and Atlantic oceans at the end of the past five glacial periods. *Nature*, **430**, 661–665.
- Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.
- Quattro JM, Stoner DS, Driggers WB *et al.* (2006) Evidence of cryptic speciation within hammerhead sharks (genus *Sphyrna*). *Marine Biology*, **148**, 1143–1155.
- Ricou LE (1987) The Tethyan oceanic gates: a tectonic approach to major sedimentary changes within Tethys. *Geodynamica Acta*, **1**, 225–232.
- Rocha LA, Roberston DR, Rocha CR, Van Tassell JL, Craig MT, Bowen BW (2005) Recent invasion of the tropical Atlantic by an Indo-Pacific coral reef fish. *Molecular Ecology*, **14**, 3921–3928.
- 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.
- Simpfendorfer CA, Milward NE (1993) Utilization of a tropical bay as a nursery area by sharks of the families Carcharhinidae and Sphyrnidae. *Environmental Biology of Fishes*, **37**, 337–345.
- Snelson FF, Williams SE (1981) Notes on the occurrence, distribution, and biology of elasmobranch fishes in the Indian River Lagoon system, Florida. *Estuaries*, **4**, 110–120.
- Springer S (1967) Social organization of shark populations. In: *Sharks, Skates and Rays* (eds Gilbert PW, Matheswon RF, Rall DP), pp. 149–174. Johns Hopkins University Press, Baltimore.
- Swofford DL (2003) *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotype associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.
- Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438–450.
- Waples RS, Gustafson RG, Weitkramp LA *et al.* (2001) Characterizing diversity in salmon from the Pacific Northwest. *Journal of Fish Biology*, **59**, 1–41.
- Ward RD, Gardner MG (1997) *Stock Structure and Species Identification of School and Gummy Sharks in Australian Waters*. CSIRO, Hobart, Tasmania.

This work is part of a larger study of nursery use behaviour of scalloped hammerhead sharks, and by comparison the importance of nursery areas for other large, wide-roaming shark species. Kanesa Duncan has a recent PhD studying ecology, behaviour and evolution of marine fishes (University of Hawaii at Mānoa). Andrew Martin studies the ecology and evolution of organisms ranging from bacteria to sharks and conservation biology of organisms and genes (University of Colorado at Boulder). Brian Bowen studies marine evolutionary processes and conservation genetics (Hawaii Institute of Marine Biology, University of Hawaii at Mānoa). Heinz Gert de Couet studies the function and evolution of genetic networks responsible for the cytoskeleton and neuromuscular systems of animals (University of Hawaii at Mānoa).

Appendix I

Sphyrna lewini and cryptic Atlantic lineage. Polymorphic nucleotide positions for 24 haplotypes of *S. lewini* ($n = 271$) and one cryptic Atlantic haplotype ($n = 7$). Haplotype numbers, location, and number of individuals with a given haplotype are listed in the left columns. The positions of polymorphic base pairs are listed across the top row. The nucleotide at each position is given for haplotype 1. Only nucleotides different from haplotypes 1 are given for all other haplotypes. Nucleotides identical to haplotype 1 are indicated with periods (.) and deletions are indicated with dashes (-). Complete haplotypes sequences are deposited in GenBank (Accession nos: DQ438148–DQ438172).

Haplotype number	Location	<i>n</i>	6 6 7 9 0 0 3 7 8 1 4 2 9 8 5 7 7 9 1 2 4 9 0 4 6 8 9 5 3 6 1 2 2 4 9 0 1 4 5 6 7 2 3 4																																																					
			1 4 4 4 5 2 3 3 3 4 4 7 7 1 2 3 5 5 6 6 6 7 8 8 8 8 0 4 5 5 6 9 2 2 3 4 4 7 7 7 9 9 1 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 5																																																					
1	Indo-Pac	105	C	T	A	T	A	A	C	T	A	T	T	A	T	A	C	A	T	G	T	C	C	T	C	A	C	A	C	A	A	C	G	A	T	A	T	A	A	C	C	T	A	T	A	C										
2	Pacific	28	T						
3	Pacific	1	C						
4	Pacific	1	T	T				
5	Pacific	2	T				
6	Pacific	2	C				
7	Pacific	1	T				
8	Indo-Pac	4	T			
9	Indo-Pac	15	T		
10	Atlantic	1	A	.	A	.	T	.	T	T	G	C	A	-	.	C	T	A		
11	Atlantic	1	A	.	A	.	T	.	T	T	G	C	A	-	.	C	T	A		
12	Atlantic	2	A	.	A	.	T	.	T	.	G	C	A	-	.	C	T	A	
13	Atlantic	1	A	.	A	.	T	.	T	.	G	C	A	-	.	C	T	A	
14	Atlantic	5	A	.	A	.	T	.	T	.	G	C	A	-	.	C	T	A	
15	Atlantic	26	A	.	A	.	T	.	T	.	G	C	A	-	.	C	T	A
16	Atlantic	7	A	.	A	.	T	.	T	.	G	C	A	-	.	C	T	A	
17	Indian	1	G	A	.	A	.	.	.	T	.	G	C	A	-	.	C	T	A	
18	Indian	1	A	.	A	.	.	.	T	.	G	C	A	-	.	C	T	A	
19	Indo-Pac	36	A	.	A	.	.	.	T	.	G	C	A	-	.	C	T	A	
20	Indian	1	A	.	A	.	.	.	T	.	G	C	A	-	.	C	T	A	
21	Indian	1	A	.	A	G	C	A	-	.	C	T	A	
22	Indian	3	A
23	Indian	3	A
24	Indo-Pac	23	A
25	Cryptic Atlantic	7	A	
Total	<i>n</i>	278	.	A	T	G	T	C	.	A	T	C	.	.	.	C	.	T	.	.	.	A	C	T	.	C	T	T	.	.	.	T	G	T	A	.	C	T	C	G	G	A	T	C	T	C	G	.								