



Oceanic dispersal in a sedentary reef shark (*Triaenodon obesus*): genetic evidence for extensive connectivity without a pelagic larval stage

Nicholas M. Whitney^{1*}, William D. Robbins^{2,3}, Jennifer K. Schultz^{1,4}, Brian W. Bowen¹ and Kim N. Holland¹

¹Hawaii Institute of Marine Biology and Zoology Department, University of Hawaii at Manoa, Honolulu, HI 96744, USA, ²School of Marine and Tropical Biology, James Cook University, Townsville, Qld 4811, Australia, ³Wildlife Marine, Perth, WA 6020, Australia, ⁴Office of Protected Resources, National Marine Fisheries Service, Silver Spring, MD 20910, USA

ABSTRACT

Aim Most reef fishes are site-attached, but can maintain a broad distribution through their highly dispersive larval stage. The whitetip reef shark (*Triaenodon obesus*) is site-attached, yet maintains the largest Indo-Pacific distribution of any reef shark while lacking the larval stage of bony (teleost) fishes. Here we use mitochondrial DNA (mtDNA) sequence data to evaluate the enigma of the sedentary reef shark that maintains a distribution across two-thirds of the planet.

Location Tropical Pacific and Indian Oceans.

Methods We analysed 1025 base pairs of the mtDNA control region in 310 individuals from 25 locations across the Indian and Pacific Oceans. Phylogeographic and population genetic analyses were used to reveal the dispersal and recent evolutionary history of the species.

Results We resolved 15 mtDNA control region haplotypes, but two comprised 87% of the specimens and were detected at nearly every location. Similar to other sharks, genetic diversity was low ($h = 0.550 \pm 0.0254$ and $\pi = 0.00213 \pm 0.00131$). Spatial analyses of genetic variation demonstrated strong isolation across the Indo-Pacific Barrier and between western and central Pacific locations. Pairwise Φ_{ST} comparisons indicated high connectivity among archipelagos of the central Pacific but isolation across short distances of contiguous habitat (Great Barrier Reef) and intermittent habitat (Hawaiian Archipelago). In the eastern Pacific only a single haplotype (the most common one in the central Pacific) was observed, indicating recent dispersal (or colonization) across the East Pacific Barrier.

Main conclusions The shallow haplotype network indicates recent expansion of modern populations within the last half million years from a common ancestor. Based on the distribution of mtDNA diversity, this began with an Indo-West Pacific centre of origin, with subsequent dispersal to the Central Pacific and East Pacific. Genetic differences between Indian and Pacific Ocean populations are consistent with Pleistocene closures of the Indo-Pacific Barrier associated with glacial cycles. Pairwise population comparisons reveal weak but significant isolation by distance, and notably do not indicate the high coastal connectivity observed in other shark species. The finding of population structure among semi-contiguous habitats, but population connectivity among archipelagos, may indicate a previously unsuspected oceanic dispersal behaviour in whitetip reef sharks.

Keywords

Control region, dispersal, East Pacific Barrier, elasmobranch, Indo-Pacific Barrier, marine biogeography, marine fish, mtDNA, phylogeography.

*Correspondence: Nicholas M. Whitney, Center for Shark Research, Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34203, USA.
E-mail: nwhitney@mote.org

INTRODUCTION

The dispersal of marine organisms, and the corresponding impacts on species range and evolution, have been controversial topics with few clear patterns identified to date (Bohonak, 1999; Cowen *et al.*, 2006; Lester *et al.*, 2007; Rocha *et al.*, 2007). Most attention has been focused on pelagic (oceanic) larvae, providing little insight into the phylogeography of elasmobranchs (sharks and rays), which lack a pelagic larval stage. The relationship between dispersal ability and population structure in sharks has been explored in relatively few species, in part because of the difficulty in acquiring adequate sample sizes and range-wide coverage (Heist, 1999, 2005). Many large, pelagic sharks (e.g. the shortfin mako, *Isurus oxyrinchus*; whale shark, *Rhincodon typus*; and white shark, *Carcharodon carcharias*) undergo long oceanic movements (Kohler *et al.*, 1998, 1999; Eckert & Stewart, 2001; Bonfil *et al.*, 2005) with little genetic structure across much of their range (Schrey & Heist, 2003; also Hoelzel *et al.*, 2006; Castro *et al.*, 2007; Jorgensen *et al.*, 2010; but see Chabot & Allen, 2009). Coastal species, such as the scalloped hammerhead, *Sphyrna lewini* (Duncan *et al.*, 2006) and blacktip shark, *Carcharhinus limbatus* (Keeney & Heist, 2006), exhibit connectivity along coastlines but significant structure across oceanic barriers. Similarly, lemon sharks (*Negaprion brevirostris* and *Negaprion acutidens*) exhibit significant isolation by oceanic distance, with the eastern Pacific Ocean serving as an impassable oceanic barrier (Schultz *et al.*, 2008). Although a few shark species have shown genetic structure over relatively short distances, these species have limited coastal ranges, which is consistent with low vagility (Pacific angel shark, *Squatina californica*, and leopard shark, *Triakis semifasciata*) and in some cases are insular shelf species apparently isolated by deep channels or strong regional currents (zebra shark, *Stegostoma fasciatum*; Pacific angel shark, *Squatina californica*; and spot-tail shark, *Carcharhinus sorrah*) (Gaida, 1997; Lewallen *et al.*, 2007; Dudgeon *et al.*, 2009; Ovenden *et al.*, 2009).

The whitetip reef shark, *Triaenodon obesus* (Rüppell, 1837), represents a striking contrast between individual dispersal ability and species distribution. Strongly attached to coral reef habitat, these medium-sized (c. 1.8 m total length) sharks remain motionless in caves, resting for hours during the day, and emerging at night to feed on benthic prey amongst reef holes and crevices (Randall, 1977; Nelson & Johnson, 1980). The few studies of their movements indicate small daily home ranges of c. 1 km² (Nelson & Johnson, 1980), with maximum dispersal distances of 9–24 km over several years (Nelson & Johnson, 1980; Whitney *et al.*, 2012). The site fidelity of *T. obesus* is also recognized in traditional Hawaiian culture, and may have given rise to the belief in 'aumākua, the guardian spirits of ancestors that abide near their descendants (Taylor, 1993). Although usually not directly targeted by commercial fisheries, *T. obesus* populations may be highly vulnerable to recreational fishing (Whitney *et al.*, 2012), with populations along the Great Barrier Reef declining at 7% year⁻¹ (Robbins *et al.*, 2006).

Triaenodon obesus is the only requiem shark (family Carcharinidae) that is both benthic and coastal, two additional factors that are associated with limited dispersal and a small species range (Musick *et al.*, 2004). Despite the apparent lack of movement, *T. obesus* is the most broadly distributed reef shark, recorded in the Red Sea and Western Indian Ocean, throughout the tropical Indo-Pacific, and into the Central and Eastern Pacific Oceans, including some of the most isolated island groups in the world (Randall, 1977; Compagno, 1984). Paradoxically, this species exhibits a level of site-fidelity comparable to that of many coral reef teleosts (bony fishes), but has a distribution spanning nearly 70% of the Earth's circumference. The few teleost reef fishes that span this range have highly dispersive oceanic larvae (Craig *et al.*, 2007; Horne *et al.*, 2008), while the whitetip reef shark is viviparous, giving birth to swimming progeny (Randall, 1977; Robbins, 2006a).

Herein, we present the phylogeography of the world's most broadly distributed reef shark. We use mitochondrial DNA (mtDNA) analyses to reconcile the species' broad distribution with the well-documented site-fidelity. These data will also illuminate the evolutionary history and patterns of dispersal, and the impact of biogeographic barriers to gene flow in a species that may be experiencing significant population declines (Friedlander & Demartini, 2002; Robbins *et al.*, 2006; Heupel *et al.*, 2009). To accomplish this goal, we sample across most of the species' range, and embed fine-scale sampling in the Great Barrier Reef and the Hawaiian Archipelago. The latter provides intermittent habitats along an isolated transect of 2600 km, an excellent platform for testing the limits of dispersal in a site-attached reef species.

MATERIALS AND METHODS

Sampling

A total of 310 *T. obesus* specimens were collected between 2002 and 2008 from 25 locations across the tropical Indian and Pacific Oceans (Fig. 1), including nine locations within the Hawaiian Archipelago (Fig. 2) and three locations along the Great Barrier Reef (GBR). Samples were obtained from free-swimming animals using biopsy probes (Robbins, 2006b) attached to an underwater speargun, or using livestock ear-notchers to clip tissue from the fins of resting sharks. Samples were also obtained from bycatch along the GBR, from lobster (funnel) traps in the Northwestern Hawaiian Islands, and from a few locally caught captive animals in public aquaria in Guam, Hong Kong and Singapore. Additional specimens were collected by hand, spear, or on hook and line in Okinawa, Fiji, Osprey Reef, GBR and Palmyra. Note that the island of Hawaii is referred to as the Big Island throughout so as to distinguish it from the Hawaiian Archipelago.

Laboratory procedures and analysis

Tissue samples were preserved in 80–95% ethanol (EtOH) or saturated NaCl solution (Seutin *et al.*, 1991) and stored at

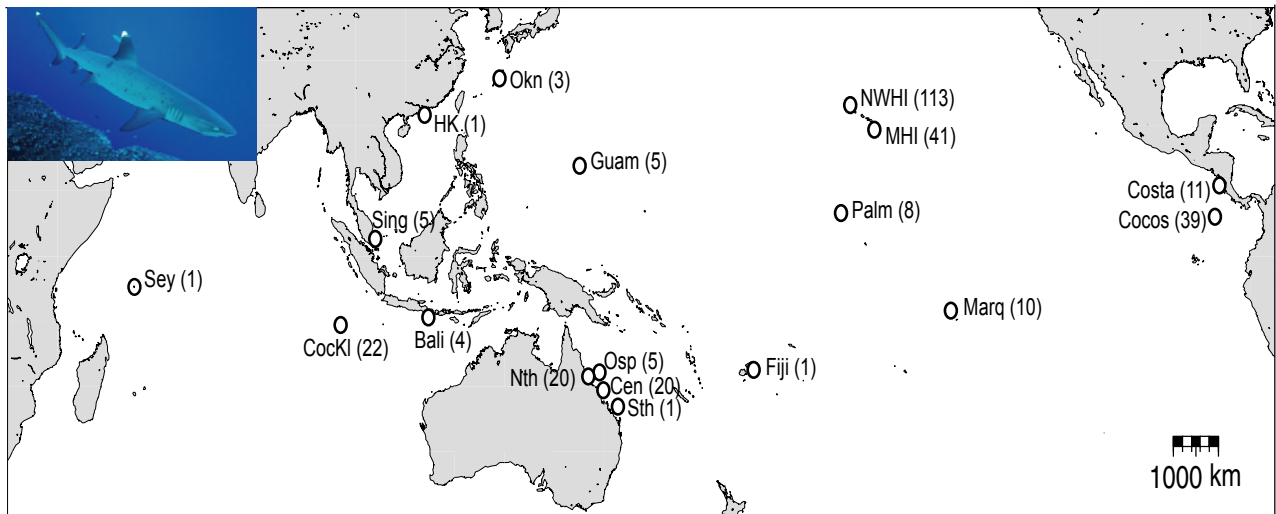


Figure 1 Locations and sample sizes (in parentheses) of *Triaenodon obesus* populations sampled throughout the tropical Pacific and Indian Oceans. Sey, Seychelles; CoclI, Cocos (Keeling); Sing, Singapore; HK, Hong Kong; Okn, Okinawa; Osp, Osprey Reef; Nth, North Great Barrier Reef; Cen, Central Great Barrier Reef; Sth, South Great Barrier Reef; NWHI, Northwestern Hawaiian Islands; MHI, Main Hawaiian Islands; Palm, Palmyra; Marq, Marquesas; Costa, Costa Rica; Cocos, Cocos Islands, Costa Rica.

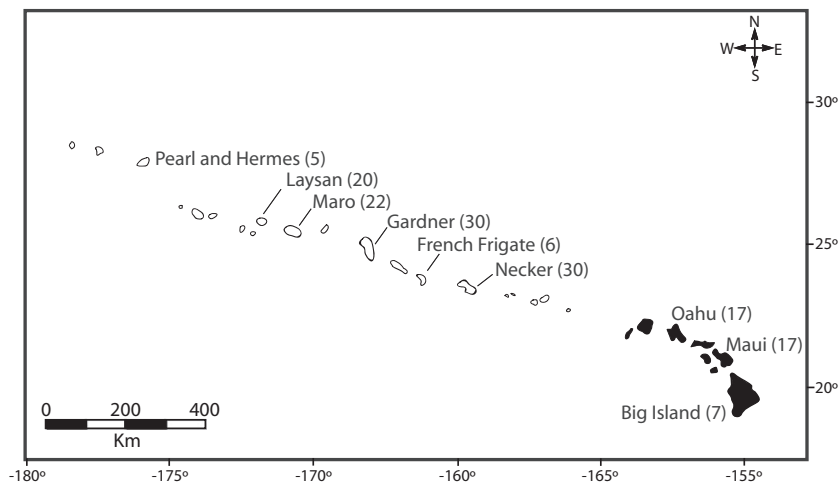


Figure 2 Locations and sample sizes (in parentheses) of *Triaenodon obesus* populations sampled within the Hawaiian Archipelago. Note that the island of Hawaii is referred to as the Big Island throughout to distinguish it from the Hawaiian Archipelago as a whole.

room temperature. Total genomic DNA was extracted from all specimens using DNeasy Tissue Kits (Qiagen, Inc., Valencia, CA, USA) following the manufacturer's protocol for animal tissues, and stored at $-20\text{ }^{\circ}\text{C}$. We amplified the entire mitochondrial control region (c. 1100 bp) using the light strand primer Pro-L and the heavy strand primer 282-H following the protocol of Keeney *et al.* (2003). Reactions were carried out in 25 μL aliquots, containing 1 \times *Taq* buffer (50 mM KCL, 10 mM Tris-HCL, 0.1% Triton X-100, pH 9.0), 1.5 mM MgCl₂, 130 μM each dNTP, 0.5 μM each primer, 1.5 units *Taq* DNA polymerase, and c. 20 ng template DNA. Reaction conditions included an initial denaturing step at 94 $^{\circ}\text{C}$ for 2 min, followed by 30 cycles of 30 s denaturing at 94 $^{\circ}\text{C}$, 30 s annealing at 53 $^{\circ}\text{C}$, and 1 min extension at 72 $^{\circ}\text{C}$.

A final 10 min extension was conducted at 72 $^{\circ}\text{C}$. Amplification products were purified using 0.75 units of Exonuclease I, 0.5 units of Shrimp Alkaline Phosphatase (ExoSAP; USB, Cleveland, OH, USA) per 7.5 μL of PCR products at 37 $^{\circ}\text{C}$ for 60 min, followed by deactivation at 80 $^{\circ}\text{C}$ for 10 min. DNA sequencing was performed with fluorescently labelled dideoxy terminators on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Hawaii Institute of Marine Biology EPSCoR Sequencing Facility and by MacroGen (<http://www.macrogen.com>), using BigDyeTM nucleotide terminators with the primers Pro-L, 282, and a third internal light strand primer (Rf45: 5'-TACGGTTTGTGGTACATTAC-3') (Robbins, 2006a). DNA sequences were edited using SEQUENCHER 4.52b (Gene Codes Corporation, Ann Arbor, MI, USA).

Edited mitochondrial DNA sequences (1025 bp) were used to construct a statistical parsimony network in TCS 1.21 (Clement *et al.*, 2000) using the default settings. Haplotype diversity (h), nucleotide diversity (π) and genetic distances (d) were calculated in ARLEQUIN 3.1 (Excoffier *et al.*, 2005). Pairwise population comparisons (Φ_{ST}) and Mantel tests for isolation by distance were also conducted in ARLEQUIN (16,000 permutations) for the 18 samples with five or more individuals.

To identify barriers to maternal gene flow across the range of the species, we plotted average pairwise distances on a map created via Delaunay triangulation and Voronoi tessellation in BARRIER 2.2 (Manni *et al.*, 2004). Because no obvious criterion exists to group populations, we used SAMOVA 1.0 (Dupanloup *et al.*, 2002) to define maximally differentiated groups in a spatial analysis of molecular variance (Φ_{ST} , AMOVA; Weir & Cockerham, 1984). SAMOVA employs a simulated annealing procedure and uses allele frequency data along with geographic coordinates of the sample populations to identify groups of populations that exhibit close genetic relationships. This is done by maximizing the proportion of the total genetic variance between groups (indicated by the largest F_{CT} value). Because small sample sizes may not accurately represent the genetic diversity within a population, only the 18 samples consisting of five or more specimens were included in BARRIER and SAMOVA analyses.

We constructed and assessed mismatch distributions in ARLEQUIN (using 16,000 replicates), testing population-level expansion, where $\tau = 2\mu T$ and $\theta = 2N_f\mu$, where N_f is the female effective population size and μ is the divergence rate per locus per year (Rogers & Harpending, 1992; Schneider & Excoffier, 1999), and applying a generation time (T) of 12 years, given that females mature at about 8 years of age and can live to at least 19 years old (Robbins, 2006a). We assumed a range of mutation rates based upon the transisthmian divergence of other shark species, including blacktip sharks (0.43% Myr⁻¹; Keeney & Heist, 2006), lemon sharks (0.7% Myr⁻¹; Schultz *et al.*, 2008), and hammerhead sharks (0.8% Myr⁻¹; Duncan *et al.*, 2006). We were unable to calibrate a mutation rate using *T. obesus* sequence data because this species does not occur across a known biogeographic break such as the Isthmus of Panama. We present provisional coalescence times using the rate of 0.7% Myr⁻¹ with 95% lower and upper confidence intervals.

RESULTS

Our efforts resolved 1025 base pairs of the mitochondrial control region from 310 *T. obesus* and revealed 20 polymorphic sites and 15 haplotypes. These haplotypes (hap 1–15) are available in GenBank (<http://www.ncbi.nlm.nih.gov>; accession numbers HQ833003–HQ833017). Six of the fifteen haplotypes were observed in more than one individual. Variable nucleotide positions consisted of 15 transitions, 4 transversions and 1 indel (see Appendix S1 in the Supporting Information). Overall haplotype diversity and nucleotide diversity across the

species ($h = 0.550 \pm 0.0254$ and $\pi = 0.00213 \pm 0.00131$) did not reveal any consistent geographic trends except for low diversity in the eastern Pacific and at some Hawaiian locations (Table 1). The most common haplotype (hap 1) was shared by 192 individuals and observed in every sample with four or more specimens (Figs 3 & 4). Hap 3 was shared by 79 individuals and observed in every sample with five or more specimens, except for the Big Island of Hawaii and the two East Pacific samples, which were fixed for hap 1 (Fig. 3). Haplotypes 1 or 3 were found in 87% of Australian specimens (total $n = 46$) and 99% of Hawaiian specimens (total $n = 154$), with only two Hawaiian individuals showing unique haplotypes. Cocos (Keeling) (Indian Ocean, $n = 22$) specimens were predominantly hap 4 (observed in only four individuals at two other locations: northern GBR and Seychelles), hap 5 (observed in only one other individual at the Marquesas), and hap 6 (observed at no other sampled locations; Fig. 3). Based on the six haplotypes observed in more than one individual, haplotypes (haps 1–3) that predominate the Pacific Ocean are rare in our limited Indian Ocean samples, and the haplotypes (haps 4–6) that predominate the Indian Ocean samples are rare in the Pacific (Fig. 4).

Most samples with multiple haplotypes met expectations for population expansion, with the exception of Marquesas, Laysan and Gardner, which showed significant deviation from a unimodal mismatch distribution ($\alpha = 0.05$). The earliest population expansion estimate is for Cocos (Keeling) at *c.* 466 ka (thousand years ago), and other population expansions occurred 383–167 ka (Table 1).

Analyses performed on samples with five or more individuals in SAMOVA (Table 2) and BARRIER (Fig. 4) provided mostly concordant results in identifying barriers to maternal gene flow. The strongest barrier is between Cocos (Keeling) (our only sample with ≥ 5 specimens in the Indian Ocean) and all other locations, consistent with a partition at the Indo-Pacific Barrier (IPB). Dividing the species into two groups, Cocos (Keeling) and all other locations, accounts for 39% of molecular variance (Table 2). There are additional barriers in the western Pacific ($\Phi_{ST} = 0.32$ –0.68), and between the western and central Pacific ($\Phi_{ST} = 0.32$ –0.45), but the barrier between the central and eastern Pacific was ambiguous due to low haplotype diversity (Fig. 4; Table 3a).

Barriers were also identified over short distances: between the central GBR and northern GBR ($\Phi_{ST} = 0.25$, $P < 0.001$); and between Necker and other locations in Hawaii ($\Phi_{ST} = 0.00$ –0.69; Fig. 4; Table 3). Overall there was weak but significant genetic structure among Hawaiian Islands ($\Phi_{ST} = 0.002$, $P = 0.008$) but somewhat stronger partitions at the Big Island ($\Phi_{ST} = 0.07$ –0.40, $P < 0.001$ –0.48) and Necker ($\Phi_{ST} = -0.07$ to 0.37, $P < 0.001$ –0.99), where samples were fixed and nearly fixed, respectively, for hap 1 (Table 3b). Cocos Island and Costa Rica in the eastern Pacific were also fixed for this haplotype. Island groups in the Central Pacific as a whole showed little evidence of population structure, with Palmyra (Line Islands) and Marquesas exhibiting haplotype frequencies similar to the Hawaiian Islands

Table 1 Genetic diversity indices for populations of *Triacodon obesus* in the Indo-Pacific region with a sample size > 2. The sample size is given in parentheses. Consecutive columns indicate number of haplotypes (Hap), haplotype diversity (h), and nucleotide diversity (π). Tau (τ), years since population expansion (based on a mutation rate of 0.7% Myr⁻¹ with 95% confidence intervals), confidence interval (CI), initial theta (Θ_0), initial female effective population size (N_{f0}), contemporary theta (Θ_1), and contemporary female effective population size (N_{f1}) are shown for populations that met the assumption of mismatch distribution ($\alpha = 0.05$). A value of infinity (∞) indicates the maximum value provided by ARLEQUIN.

	Hap	h	π	τ	Years ago (95% CI)	Θ_0	N_{f0}	Θ_1	N_{f1}
Cocos Keeling (22)	4	0.61	0.003	6.7	466,000 (0–6,660,000)	0	–	4.26	24,700
Singapore (5)	2	0.60	0.001	2.4	167,000 (0–351,000)	0	–	3.82	22,200
Okinawa (3)	2	0.67	0.002	–	–	–	–	–	–
Guam (5)	2	0.40	0.002	3.2	223,000 (36,600–267,000)	0	–	0.49	2800
Bali (4)	4	1	0.004	–	–	–	–	–	–
Australia (46)									
Osprey Reef (5)	2	0.40	0.002	3.2	223,000 (36,900–267,000)	0	–	0.49	2800
North GBR (20)	4	0.69	0.002	5.5	383,000 (0–6,580,000)	0.004	20	3.89	22,600
Central GBR (20)	4	0.43	0.002	0	0 (0–37,900)	0	–	∞	∞
Marquesas (10)	3	0.64	0.003	–	–	–	–	–	–
Palmyra (8)	2	0.43	0.002	0	0 (0–39,700)	0	–	∞	∞
Hawaii (154)	4	0.46	0.002	0	0 (0–38,000)	0	–	∞	∞
Pearl and Hermes (5)	2	0.40	0.002	3.2	223,000 (0–267,000)	0	–	0.49	2800
Laysan (20)	2	0.52	0.002	–	–	–	–	–	–
Maro (22)	2	0.45	0.002	0	0 (0–38,000)	0	–	∞	∞
Gardner (30)	2	0.51	0.002	–	–	–	–	–	–
French Frigate (6)	2	0.53	0.002	5.2	362,000 (0–5,349,000)	0.002	10	∞	∞
Necker (30)	2	0.19	0.001	3.0	209,000 (33,400–244,000)	0	–	0.12	700
Oahu (17)	2	0.49	0.002	0	0 (0–34,600)	0	–	∞	∞
Maui (17)	4	0.60	0.003	5.4	376,000 (30,100–636,000)	–	–	–	–
Big Island (7)	1	0	0	–	–	–	–	–	–
Cocos Island (39)	1	0	0	–	–	–	–	–	–
Costa Rica (11)	1	0	0	–	–	–	–	–	–
Overall (310)	15	0.55	0.002	80	–	1.225	–	1.909	–

($\Phi_{ST} = -0.03$ to 0.01, N.S.; Table 3a). Overall we found weak but significant support for isolation by distance ($r^2 = 0.32$; $P < 0.001$), with a broad range of pairwise genetic distances frequently occurring in populations separated by < 10,000 km (Fig. 5).

DISCUSSION

Population genetic analyses (on samples with $n \geq 5$) of the broadly distributed whitetip reef shark indicate that the strongest barriers to maternal gene flow occur between the Pacific and Indian Oceans. Secondary barriers are apparent between reef habitats separated by more than a few thousand kilometres of open ocean; however, these population-level distinctions are based on frequency shifts in a few common haplotypes, indicating that oceanic dispersal is not sufficient to homogenize regional populations, but has occurred recently and/or sporadically in this sedentary reef species.

Prior to dissecting these results, we note two limitations based on sample sizes. Despite 7 years of sampling by authors, and the generous contributions of those recognized in acknowledgments, seven of our 15 locations are characterized by fewer than five specimens. These small samples (< 5 individuals) were not included in most analyses.

Although small samples are useful for resolving phylogeographic (and molecular evolutionary) patterns, they are less useful for population genetic analyses (which depend on accurately categorizing the distribution of haplotypes). For the remaining sample sizes (5–39 individuals), we assume that these samples accurately represent the genetic diversity within populations. We feel that this is appropriate because of a strong geographic signal: haps 1 and 3 predominate in the Pacific, while haps 4–6 predominate in the Indian Ocean. Because we base corresponding conclusions on the predominance of different haplotypes, additional sampling is unlikely to alter these results. Second, our characterization of the Indian Ocean is limited to Cocos (Keeling) near the Coral Triangle ($n = 22$) and Seychelles in the western equatorial region ($n = 1$). More work is needed to examine biogeographic affiliations of the western Indian Ocean, although the single individual from Seychelles had the most common haplotype (hap 4) found at Cocos (Keeling). Other reef-associated vertebrates have shown high connectivity across the region between Cocos (Keeling) and Seychelles (Craig *et al.*, 2007; Horne *et al.*, 2008; Gaither *et al.*, 2010; Reece *et al.*, 2010), so a major evolutionary partition within *T. obesus* seems unlikely across this region, but further investigation is needed to confirm this.

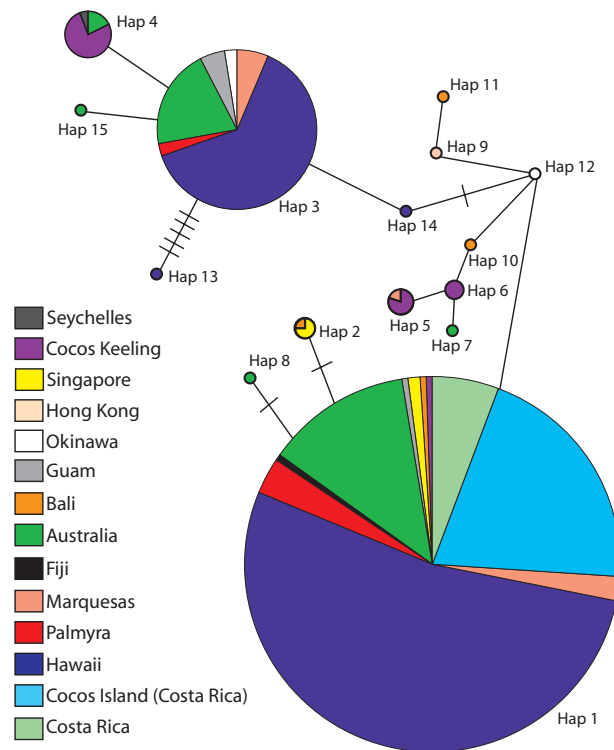


Figure 3 Spanning haplotype network of *Triaenodon obesus* for all sampled locations with pooled Australia and pooled Hawaii locations, respectively. Circle sizes correspond to the number of individuals with each haplotype. Hash marks represent the number of base pair differences between haplotypes that differ by more than one base pair.

Phylogeography and species history

An ongoing debate in biogeography concerns whether the biodiversity hotspot in the Coral Triangle is a centre of speciation, or a centre of accumulation (Jokiel & Martinelli, 1992; Briggs, 1999, 2005). Mora *et al.* (2003) concluded that the distributions of reef fishes indicate an expansion out of the central Indo-Pacific, consistent with a centre of speciation. Intraspecific patterns of mtDNA diversity can also indicate an expansion from the central Indo-Pacific (Bowen *et al.*, 1998). The earliest expansion of contemporary *T. obesus* populations (c. 466 ka) is observed at Cocos/Keeling adjacent to the Coral Triangle. Although haplotype diversity is low and coalescence time estimates have large confidence intervals, they indicate an eastward dispersal pattern to the central and eastern Pacific, a pattern recognized in numerous marine taxa (Briggs, 1966; Mora *et al.*, 2003). Colonization by a few individuals and subsequent population expansion are also supported by dramatic increases in the female effective population size (N_f) and genetic diversity (Θ) over time (Table 1). The lack of population structure between central Pacific locations and the central GBR in the western Pacific may indicate a southern route of species expansion, although a northern route (e.g. colonization from Japan or western Pacific via the Kuroshio Current) has also been proposed to explain the colonization of the Hawaiian Islands (Hourigan & Reese, 1987; Craig *et al.*, 2010). Further sampling in the proposed centre of origin and

from possible colonization sites are required to resolve Pacific colonization routes.

The presence of a single haplotype (hap 1) in the eastern Pacific is consistent with a Pleistocene colonization event. We cannot date a population expansion in the eastern Pacific (due to the uniformity of mtDNA haplotypes), but haplotypes shared with the central Pacific indicate a connection in the last few hundred thousand years, and possibly as recently as the current interglacial period. This uncertainty is due to hap 1 fixation in all eastern Pacific samples which, when combined with low haplotype diversity in the central Pacific, provided little power to assess the EPB in this species. Climate is probably the driving force behind these recent colonizations into the eastern Pacific, as this region was inhospitable to tropical fauna at the height of Pleistocene glaciations (Bowen & Karl, 2007). Notably, the range expansion by *T. obesus* may be ongoing: the first record of this species in the Revillagigedo Archipelago (Mexico) is from the 1990s (Ochoa-Lopez *et al.*, 1997) and reports by fishermen indicate a parallel expansion to the Baja California Peninsula also in recent decades (J. Ketchum, UC Davis, pers. comm.).

Whether *T. obesus* ever inhabited the Atlantic Ocean has been a source of debate. Musick *et al.* (2004) reported that the species has gone extinct in the Atlantic, based on fossil teeth from North Carolina (Purdy *et al.*, 2001). However, these teeth had previously been assigned to the extinct lamnid *Lamna cattica* (Leriche, 1910; Cappetta, 1987), and more recent work

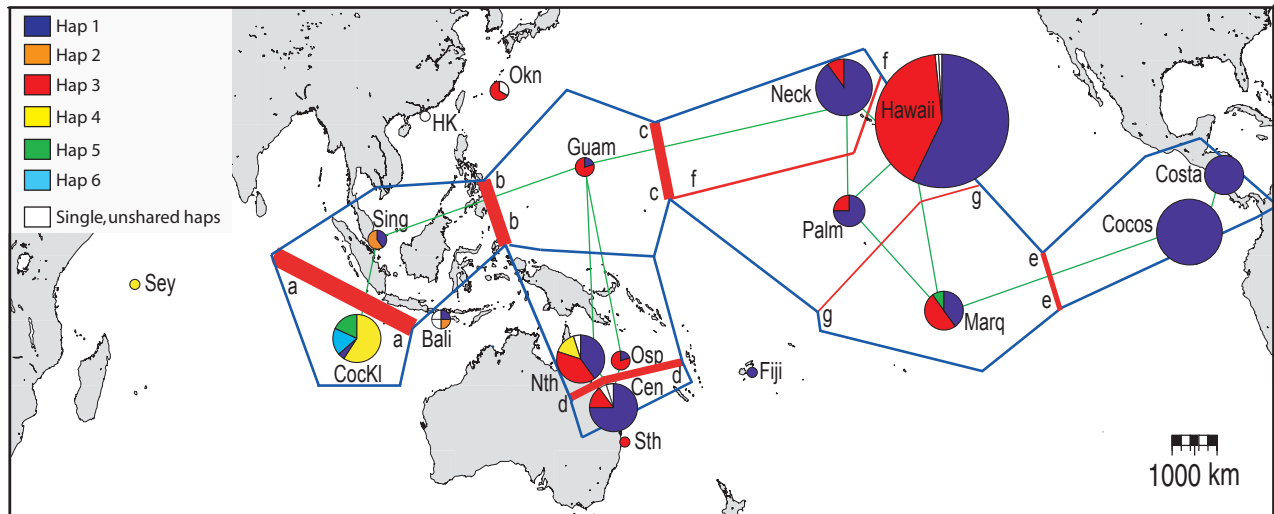


Figure 4 Genetic barriers (red lines) of *Triaenodon obesus* populations ranked in order of impermeability (indicated by line thickness and barrier letters a–g) using Delaunay triangulation and Voronoi tessellation as implemented in BARRIER. Haplotype distribution is indicated by colour at each location, with circle size corresponding to sample size. All abbreviations and numerical sample sizes are defined in Fig. 1. Locations with fewer than five specimens were excluded from BARRIER analysis and are shown here outside of BARRIER boundaries. Hawaiian locations were pooled except for Necker. As BARRIER delineates boundaries between all geographic locations, a weak boundary between Cocos and Costa (both fixed for hap 1) and two weaker boundaries have been omitted for clarity.

by Ward & Bonavia (2001), Reinecke *et al.* (2005), and J. Bourdon & B. Heim (<http://www.Elasmo.com>, pers. comm.) support their lamniform origin. Therefore current evidence indicates that *T. obesus* never colonized the Atlantic, and the history of this species is probably limited to the Indo-Pacific.

Concordant barriers to gene flow for Indo-Pacific sharks

The IPB is a well-known biogeographic boundary (Briggs, 1974; Rocha *et al.*, 2007) caused by Pleistocene sea level changes resulting in the periodic emergence of the Sunda Shelf connecting the Asian and Australian continents (Fleminger, 1986; Voris, 2000; Bellwood & Wainwright, 2002). Population genetic separations are observed in this region for faunas as diverse as crustaceans and sea turtles (Barber *et al.*, 2006; Dethmers *et al.*, 2006). Many reef fishes exhibit strong genetic structure across this barrier (e.g. Nelson *et al.*, 2000; Lourie & Vincent, 2004; Craig *et al.*, 2007; Gaither *et al.*, 2010, 2011), whereas oceanic species often do not (Castro *et al.*, 2007; Theisen *et al.*, 2008). The phylogeography of three other broadly distributed coastal shark species supports isolation across this barrier. No haplotypes are shared between blacktip shark (*Carcharhinus limbatus*) populations in the Indian and Pacific Oceans (Keeney & Heist, 2006), and strong genetic structure ($\Phi_{ST} = 0.397$) is observed between Pacific and Indian Ocean populations of scalloped hammerhead (*Sphyrna lewini*; Duncan *et al.*, 2006). Similar genetic structure exists for the sicklefin lemon shark (*Negaprion acutidens*), with no sharing of haplotypes between Taiwan and Australia, indicating north–south isolation across the Sunda Shelf (Schultz *et al.*, 2008).

We conclude that this biogeographic barrier, originally identified in reef (teleost) fishes and invertebrates, is equally effective for coastal sharks.

Table 2 Fixation indices for various population group compositions of *Triaenodon obesus* in the Indo-Pacific region as calculated using an analysis of molecular variance (AMOVA) in SAMOVA. All values are significant at $P < 0.001$. Abbreviations are defined in Fig. 1.

No. of groups	No. of samples	Group composition	F_{CT}
2	12	CocKl Others	0.394
3	12	CocKl Osp/Guam Others	0.387
4	12	CocKl Osp Guam Others	0.37
5	12	CocKl Osp Guam Sing Others	0.367
6	12	CocKl Guam Sing Cen/Neck/Cocos/Costa Hawaii/Marq/Palm Nth/Osp	0.336

Table 3 Pairwise Φ_{ST} values for populations of *Trienodon obesus* from (a) all locations and (b) Hawaiian Archipelago locations only. Italics indicate values significant at $P \leq 0.05$, bold italics indicate values significant at $P \leq 0.01$. For (a) Hawaiian locations are combined except for Necker and the Big Island, which showed significant genetic structure from other Hawaiian Islands. Abbreviations are defined in Fig. 1. For (b) abbreviations are as follows: FFS, French Frigate Shoals; GR, Gardner Reef; LN, Laysan; MR, Maro Reef; PHR, Pearl and Hermes Reef; Neck, Necker; BigIs, Big Island (Island of Hawaii).

(a)

	CocKI	Sing	Guam	Osp	Nth	Cen	Marq	Palm	Haw	Neck	BigIs	Cocos	Costa
CocKI	0.00												
Sing	0.57	0.00											
Guam	0.14	<i>0.68</i>	0.00										
Osp	0.14	0.68	-0.25	0.00									
Nth	0.19	0.47	-0.04	-0.04	0.00								
Cen	0.47	0.32	<i>0.48</i>	0.48	0.25	0.00							
Marq	<i>0.16</i>	0.42	-0.03	-0.03	-0.05	0.19	0.00						
Palm	0.38	<i>0.32</i>	0.36	0.36	0.13	-0.07	0.06	0.00					
Haw	0.38	0.36	0.18	0.18	0.06	<i>0.07</i>	0.01	-0.03	0.00				
Neck	0.60	0.45	0.69	0.69	0.41	-0.01	0.39	0.02	0.15	0.00			
BigIs	0.56	<i>0.57</i>	0.79	0.79	0.42	0.02	0.39	0.12	<i>0.15</i>	-0.02	0.00		
Cocos	0.75	0.86	0.94	0.94	0.64	0.18	0.70	0.46	0.28	<i>0.09</i>	0.00	0.00	
Costa	0.60	<i>0.66</i>	0.85	0.85	0.46	0.06	0.46	0.20	0.22	0.01	0.00	0.00	0.00

(b)

	FFS	GR	LN	Maui	MR	Oahu	PHR	Neck	BigIs
FFS	0.00								
GR	0.01	0.00							
LN	-0.09	-0.02	0.00						
Maui	-0.11	0.01	-0.04	0.00					
MR	-0.12	0.08	-0.01	-0.03	0.00				
Oahu	-0.13	0.04	-0.04	-0.05	-0.05	0.00			
PHR	-0.17	0.13	0.00	-0.06	-0.10	-0.09	0.00		
Neck	0.11	0.37	0.26	<i>0.18</i>	<i>0.11</i>	0.15	-0.07	0.00	
BigIs	0.23	0.40	<i>0.29</i>	0.19	0.16	0.20	0.07	-0.02	0.00

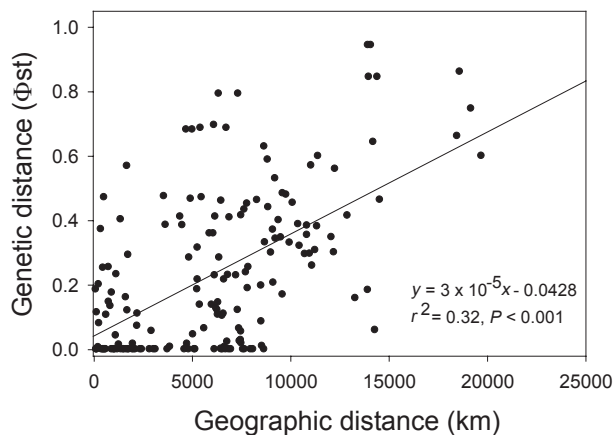


Figure 5 Isolation-by-distance relationship represented by pairwise Φ_{ST} values for all 18 populations with $n \geq 5$ samples versus total geographic (Euclidean) distance between sampled locations of *Trienodon obesus* in the Indo-Pacific region.

Large oceanic distances also appear to be a barrier for *T. obesus*. After the IPB, the strongest barriers occur in the western and central Pacific populations, with strong genetic

structure across the western Pacific ($\Phi_{ST} = 0.32\text{--}0.68$) and between Asia and the Central Pacific ($\Phi_{ST} = 0.32\text{--}0.45$). As noted above, our analyses also indicated weaker structure across the East Pacific Barrier (EPB), a 4000–7000 km stretch of open ocean between Hawaii and the Americas (Briggs, 1974). The EPB is a strong barrier to maternal gene flow in scalloped hammerhead sharks ($\Phi_{ST} = 0.448\text{--}0.629$; Duncan *et al.*, 2006), and functions as an impermeable barrier to gene flow in lemon sharks, separating two sister species (Schultz *et al.*, 2008). It is possible that the EPB is also a strong barrier for *T. obesus*, but was breached in recent evolutionary history by a rare founder event.

Structure along contiguous habitat and connectivity over oceanic divides

We find evidence for site fidelity, described in movement studies of this species (Randall, 1977; Nelson & Johnson, 1980; Whitney *et al.*, 2012), paradoxically coupled with connectivity across stretches of open ocean in some cases. Unlike the coastal sharks discussed above, *T. obesus* exhibits genetic structure along stretches of contiguous reef habitat. The significant

structure observed between the north and central GBR, separated by < 550 km, represents unprecedented genetic isolation in carcharhinid sharks.

The thorough survey of Hawaiian locations is motivated by management concerns for the Papahānaumokuākea Marine National Monument, 2000 km of uninhabited reef in the Northwestern Hawaiian Islands. However, the Hawaiian Archipelago also provides a linear array of islands that are unparalleled for examining fine-scale population structure in the sea (Ramon *et al.*, 2008; Eble *et al.*, 2009; Craig *et al.*, 2010; Toonen *et al.*, 2011). For *T. obesus* the islands of Necker and the Big Island show evidence of genetic isolation despite homogeneity throughout the rest of the Hawaiian Archipelago. The Big Island is the largest and youngest (*c.* 0.43 Ma; Clague & Dalrymple, 1987; Carson & Clague, 1995) of the Hawaiian Islands, and the fixation of hap 1 there may reflect a relatively recent colonization compared to the other islands, or may simply be a product of small sample size ($n = 7$). Necker, however, is located near the centre of the sampled Hawaiian locations and has a large sample size ($n = 30$) but still exhibits near fixation for hap 1.

It remains unclear why Necker should be genetically isolated when islands further apart show no significant differences, or why there should be significant structure between North and Central GBR populations. However, genetic differentiation is based primarily on the ratio of just two haplotypes. Non-random sampling could lead to the inclusion of related individuals, but this seems unlikely given the small litter size of this species (a mean of 2.2 pups; Robbins, 2006a). Conversely, no structure was found between island groups in the Central Pacific. Although sample sizes were low in some cases, all populations were dominated by hap 1 and hap 3 in similar ratios, indicating connectivity over thousands of kilometres of open ocean.

Dispersal mechanisms and behaviour

We find weak (but significant) support for isolation by distance, a relationship probably reduced by the strong genetic structure over short stretches of continuous or intermittent habitat, combined with less differentiation across some oceanic divides. These seemingly contradictory findings may be explained by a combination of behaviour and geography. Individuals of *Triaenodon obesus* in Hawaii make periodic movements of several kilometres (up to 24 km; Whitney *et al.*, 2012). Assuming similar movements throughout their species range, a dispersing animal in an insular, shallow area (such as the South China Sea) or an area of expansive available habitat (such as the GBR) is likely to encounter suitable alternative habitat over a much shorter distance than an individual dispersing from an oceanic island. While the accumulation of these short movements over time should diminish genetic differences between areas such as the northern and central GBR, such movements may be infrequent, centred around a stable home range, or coupled with some degree of natal homing for reproduction. High levels of site fidelity can

increase both the resource familiarity and foraging success of marine animals (Bradshaw *et al.*, 2004), so the long-term ecological benefits of this may outweigh the advantages of dispersal and genetic exchange.

Neither short movements nor natal homing are likely for sharks dispersing from isolated oceanic islands, as these movements would need to cover a much greater distance than coastal dispersal before the animal reached suitable habitat. However, density-dependent factors that serve as motivation for dispersal may also be greater, potentially leading to increased rates of dispersal from oceanic islands compared to coastal habitats. For instance, shallow ledges and caves are rare at isolated oceanic pinnacles such as Roca Partida (Revillagigedo Archipelago, Mexico, 386 km south-west of the Baja Peninsula). Scarcity of these resting sites forces multiple *T. obesus* to stack on top of each other and large muraenids in the few ledges available (Whitney, 2009), and the inability to find refuges may be a strong driver of dispersal from this and other oceanic locations.

Such factors would be expected to have a similar effect on other reef sharks such as *Carcharhinus amblyrhynchos* and *Carcharhinus melanopterus*, which overlap with *T. obesus* throughout much of their range and collectively form the three most common reef sharks in the tropical Indo-Pacific (Compagno, 1984). *Triaenodon obesus* is the only one of these species to have crossed the EPB despite being the least suited to long periods of continuous swimming (Randall, 1977), and shows no evidence of the long (> 130 km) repeated trans-oceanic movements observed in *C. amblyrhynchos* (Heupel *et al.*, 2010; C. Meyer, University of Hawaii, pers. comm.). A recent analysis of sandbar shark (*Carcharhinus plumbeus*) phylogeography presents an even more extreme contrast to *T. obesus*. *Carcharhinus plumbeus* is a large coastal species known to migrate several thousand kilometres, yet shows strong mtDNA structure between Hawaii and Australia (Portnoy *et al.*, 2010), where we find little evidence of structure in *T. obesus*.

In evaluating the enigma of broad distribution coupled with no drifting larval stage, two key findings indicate a solution. First, whitetip reef sharks show population structure along continuous or semi-continuous habitat, that other broadly distributed reef sharks are known to transit (Heupel *et al.*, 2010; C. Meyer, pers. comm.). Second, whitetip reef sharks show little or no structure across the central-western Pacific Ocean, whereas other sharks do (Portnoy *et al.*, 2010). That this weak swimmer is more broadly distributed and exhibits more connectivity over oceanic gaps than these other shark species raises the possibility of an entirely different mechanism of dispersal. Whereas *C. melanopterus*, *C. amblyrhynchos* and *C. plumbeus* may disperse primarily via active migration, perhaps *T. obesus* uses a form of passive or semi-passive dispersal. Individuals could be driven offshore by storms or surge (e.g. Heupel *et al.*, 2003), and become associated with drifting debris or flotsam (e.g. Thiel & Gutow, 2005). Their low metabolic rate (Whitney *et al.*, 2007) and ability to rest in place and associate with structure (Randall, 1977) may allow them to survive for much longer periods on the open ocean

than either *C. melanopterus* or *C. amblyrhynchos*. Thus *T. obesus* may have a unique suite of characteristics that paradoxically make the species ill-suited for controlled oceanic migration, but well-suited for surviving lengthy bouts of oceanic existence. Further surveys of species associated with flotsam or translocation telemetry experiments would be required to test this 'poor swimmer, good drifter' hypothesis.

CONCLUSIONS

The whitetip reef shark is a reef predator/scavenger with famously sedentary behaviour, yet a paradoxically broad range. Individuals will remain motionless for hours, and will return to the same resting sites for years (Whitney *et al.*, 2012). Indeed the species name *obesus* is derived from the Latin word for fat or plump, a moniker that aptly describes this sedentary behaviour (Randall, 1977). What are we to conclude, then, about the sedentary shark with closely related populations distributed across half the planet? Clearly this species has a life-history component that allows for occasional or episodic dispersal.

Significant structure is observed between locations on the GBR, and between a minority of islands within the Hawaiian Archipelago, consistent with the sedentary behaviour observed in individuals of the species. However, these results are contradicted by the companion finding of high connectivity throughout most of the Hawaiian Islands and between archipelagos of the Central Pacific. Individuals dispersing across coastal areas may move a few dozen kilometres before finding suitable habitat (Whitney *et al.*, 2012), whereas those dispersing from oceanic islands must move long distances before finding suitable habitat. Lacking the standard 'larval' mechanism of dispersal, we propose that the sedentary behaviour of the whitetip reef shark may be an asset for passive dispersal. When dispersing across oceanic, oligotrophic (low nutrient) waters, a resting stage with reduced metabolic requirements could be an advantage, and may explain the paradox of the sleepy shark with a vast range.

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SUPPORTING INFORMATION

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

Appendix S1 Intra-specific polymorphic nucleotide positions of control region haplotypes in *Triaenodon obesus*.

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BIOSKETCH

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