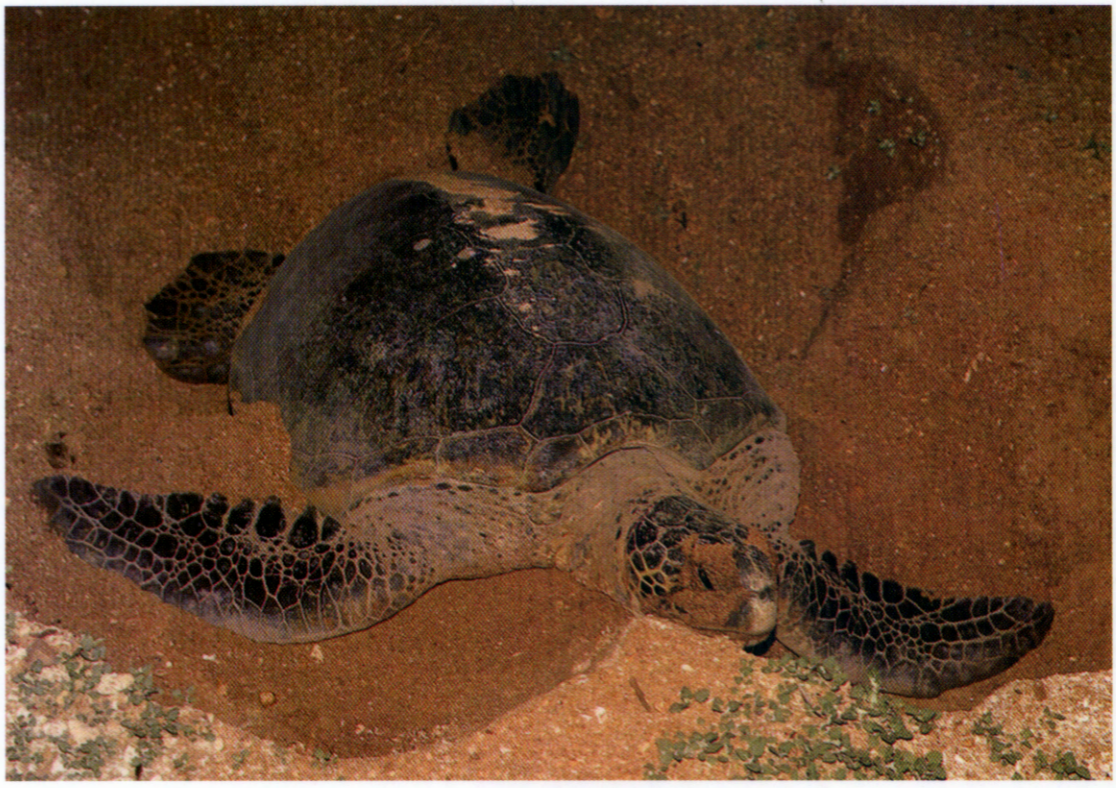
PACIFIC SCIENCE

A Quarterly Devoted to the
Biological and Physical Sciences of the Pacific Region

Volume 68 • Number 4 • October 2014



Official Journal of the Pacific Science Association

Genetic Stock Structure of Green Turtle (*Chelonia mydas*) Nesting Populations across the Pacific Islands[1]

Peter H. Dutton,[2,9] *Michael P. Jensen*,[2] *Karen Frutchey*,[3] *Amy Frey*,[2] *Erin LaCasella*,[2] *George H. Balazs*,[4] *Jennifer Cruce*,[5] *Alden Tagarino*,[6] *Richard Farman*,[7] *and Miri Tatarata*[8]

Abstract: More than two decades have passed since the first studies documenting genetic population structure of green turtles (*Chelonia mydas*) were published. Since then many more have followed and characterization of the genetic structure of green turtle rookeries now covers most of the global distribution of the species, benefitting conservation of this threatened species worldwide. However, important data gaps still exist across a large part of the western and central Pacific Ocean (WCPO). This large area is made up of hundreds of scattered islands and atolls of Micronesia, Melanesia, and Polynesia, most of which are remote and difficult to access. In this study, we assessed stock structure of green turtles throughout the WCPO using mitochondrial (mt) DNA from 805 turtles sampled across 25 nesting locations. We examined whether sequencing longer fragments (770 bp) of the control region increases resolution of stock structure and used genetic analysis to evaluate level of demographic connectivity among island nesting populations in the WCPO. We identified a total of 25 haplotypes characterized by polymorphism within the 770 bp sequences, including five new variants of haplotypes that were indistinguishable with shorter 384 bp reads from previous studies. Stock structure analysis indicated that rookeries separated by more than 1,000 km were significantly differentiated from each other, but neighboring rookeries within 500 km showed no genetic differentiation. Results presented in this paper establish that sequencing of longer fragments (770 bp) of the control region does in some cases increase resolution and that there are at least seven independent stocks in the region.

The green turtle (*Chelonia mydas*) is one of the most common and widely distributed species of marine turtles. They nest and forage throughout tropical and subtropical waters around the world (Hirth 1997). Despite their global distribution, several populations have been extirpated or depleted as a result of overharvest, habitat degradation, and incidental take in fisheries (National Marine Fisheries Service and U.S. Fish and Wildlife Service 2007), and green turtles are listed as Threatened and Endangered globally under

[1] Manuscript accepted 3 January 2014.

[2] Marine Mammal and Turtle Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 8901 La Jolla Shores Drive, La Jolla, California 92037.

[3] Joint Institute for Marine and Atmospheric Research, University of Hawai'i at Mānoa, 1000 Pope Road, MSB 312, Honolulu, Hawai'i 96822.

[4] Marine Turtle Research Program, Pacific Islands Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 1845 Wasp Blvd., Honolulu, Hawai'i 96818.

[5] Guam National Wildlife Refuge, U.S. Fish and Wildlife Service, P.O. Box 8134, MOU-3 Dededo, Guam 96912.

[6] Department of Marine and Wildlife Resources, American Samoa Government, P.O. Box 3730, Pago Pago, American Samoa 96799.

[7] Aquarium des Lagon, B.P. 8185 Nouméa, 98807 Nouvelle-Calédonie.

[8] Direction de l'Environnement, B.P. 4562, 98713 Papeete-Tahiti.

[9] Corresponding author (e-mail: Peter.Dutton@noaa.gov).

Pacific Science (2014), vol. 68, no. 4:451–464
doi:10.2984/68.4.1 (Includes online supplement)
© 2014 by University of Hawai'i Press
All rights reserved

the U.S. Endangered Species Act (ESA) and as Endangered under the IUCN Red List of endangered species, with the exception of the Hawaiian subpopulation, which has recently been listed as of Least Concern (Pilcher et al. 2012). Several populations have increased in recent years (Chaloupka et al. 2008), although the status of many populations, particularly in the Indian and Pacific Oceans, remains unclear and highlights the continued interest in the biology and conservation of this species (Wallace et al. 2011).

The life history of green turtles is characterized by oceanic dispersal of posthatchling turtles that are transported by ocean currents for several years (Bolten 2003). As they approach larger body sizes they become less susceptible to predators and eventually (after approx. 5–10 yr) recruit into neritic feeding habitats (Musick and Limpus 1997, Bolten 2003), where young turtles often move through a succession of developmental habitats over a period of 20 yr or more before settling into one area upon reaching sexual maturity (Musick and Limpus 1997, Jensen et al. 2013; however see Limpus and Walter 1980, Limpus et al. 1994). Once turtles have settled into feeding grounds they generally show strong fidelity to specific areas (Lopez-Castro et al. 2010, Senko et al. 2010*a*, *b*). After reaching sexual maturity both females and males tend to migrate back to their natal rookery to mate and breed (FitzSimmons et al. 1997, Lopez-Castro et al. 2010), resulting in demographic isolation and significant genetic structuring among rookeries or nesting populations (Jensen et al. 2013). Nuclear gene flow may occur if turtles from different nesting stocks interbreed on foraging grounds or along migration corridors (Bowen et al. 2005), but the extent to which this male-mediated gene flow occurs is unclear (Dutton et al. 2013, Roden et al. 2013). Maternally inherited mitochondrial DNA (mtDNA) markers are particularly useful for assessing genetic population structure of regional sea turtle rookeries, given that female natal homing defines reproductive population boundaries. Genetically isolated rookeries (or Management Units [MU] [Moritz 1994]) essentially represent breeding habitats with little or no immigration by reproductive females from neighboring rookeries. This means that such populations are not likely to be recolonized in the case of population decline or extinction and highlights the importance of identifying rookeries in most need of protection (Bowen et al. 2005). Furthermore, characterizing genetically distinct rookeries provides a reference data set that can be used to trace back the natal origin of turtles sampled in-water (e.g., foraging grounds, migratory corridors, fisheries bycatch, harvest, or stranding) (Jensen et al. 2013). Combined, this information can provide a more complete picture of the geographical scale of green turtle stocks that will allow for better assessments of threats to different populations at nesting beaches and in the marine environment, ultimately allowing for improved conservation and management of this species.

It is now more than two decades since the first studies documented the genetic population structure of green turtles (Bowen et al. 1992, Lahanas et al. 1994, Encalada et al. 1996). Since then many more have followed, and characterization of the genetic structure of green turtle rookeries now covers most of the global distribution of the species (Encalada et al. 1996, Dethmers et al. 2006, Formia 2006, Bourjea et al. 2007; P.H.D., M.P.J., A.F., E.L., G.H.B., P. Zárate, O. Chassin-Noria, A. Laura Sarti-Martinez, E. Velez, unpubl. data). However, important data gaps still exist in some areas of the Indo-Pacific and in particular among many of the islands in the western and central Pacific Ocean. This large area is made up of hundreds of scattered islands and atolls, most of which are remote and inaccessible. To date, only the French Frigate Shoals rookery in Hawai'i (Dutton et al. 2008; P.H.D. et al., unpubl. data), five rookeries in the eastern Pacific (P.H.D. et al., unpubl. data), and three atoll rookeries in the Federated States of Micronesia (FSM) (Dethmers et al. 2006) have been genetically characterized, whereas Australia and large parts of Southeast Asia have been extensively sampled (Dethmers et al. 2006). French Frigate Shoals has an increasing population and showed strong genetic isolation of this central North Pacific rookery for both foraging and nesting green turtles (Chaloupka et al. 2008, Dutton et al. 2008). Recent analyses also show that

the central North Pacific stock, represented by French Frigate Shoals, is more closely related to eastern Pacific green turtle rookeries than to those of the western Pacific (P.H.D. et al., unpubl. data). In the western Pacific, Dethmers et al. (2006) showed with limited sampling of the Pacific islands that three rookeries from the FSM were distinct from green turtle rookeries in Australia and Southeast Asia. Moreover, they identified a strong genetic barrier isolating Pacific rookeries (including rookeries along the Great Barrier Reef, the Coral Sea, and New Caledonia) from those in Southeast Asia and the Indian Ocean. However, their study also showed that although these rookeries were located more than 1,000 km from each other they did not appear to be genetically distinct from each other because they shared common and widespread haplotypes identified from a 384 base pair (bp) fragment of the mtDNA control region. Since then, new primers have been developed that target a longer 770 bp fragment of the control region and have uncovered additional variation, enabling improvements in detection of fine-scale stock structure in hawksbills, loggerheads, and leatherback turtles (Monzón-Argüello et al. 2010, LeRoux et al. 2012, Shamblin et al. 2012, Dutton et al. 2013). These have yet to be applied in a comprehensive way for green turtles.

Many of the Oceania rookeries are difficult to get to and most populations are relatively small (Maison et al. 2010, Trevor 2010). The objective of the study reported here was to assess the stock structure of green turtles across the Pacific islands using mtDNA from samples collected from as many nesting populations as possible to provide important information needed to inform regional management of green turtles. We examined whether sequencing longer fragments (770 bp) of the control region increases resolution of stock structure.

MATERIALS AND METHODS

Sampling

A total of 805 samples was collected from 25 different green turtle nesting locations across the western and central Pacific Ocean (Figure 1; see also Table 1). Samples consisted of tissue from dead hatchlings and skin biopsies collected from nesting turtles and stored in vials in 70% ethanol or a saturated salt solution (Dutton 1996). All samples were archived (–20°C) and analyzed at the Marine Turtle Genetics Laboratory at the National Marine Fisheries Service (NMFS) Southwest Fisheries Science Center in La Jolla, California. Samples were collected by regional partners led by the Secretariat of the Pacific Regional Environment Programme (SPREP) in collaboration with National Oceanic and Atmospheric Administration (NOAA)-NMFS from regional rookeries (see Table 1) across Micronesia (including the Republic of Palau, the Republic of the Marshall Islands [RMI], FSM [Yap State]), and the Mariana Islands (including Guam and the Commonwealth of the Northern Mariana Islands [CNMI]), Melanesia (represented by New Caledonia), and Polynesia (including French Polynesia and American Samoa) (Figure 1).

Laboratory Analysis

Genomic DNA was isolated from samples of tissue using one of the following standard extraction techniques: phenol/chloroform (Sambrook et al. 1989), sodium chloride (Miller et al. 1988), a modified DNEasy Qiagen extraction kit (Qiagen, Valencia, California), or a Corbett CAS-1200 extraction robot (Corbett Robotics, San Francisco, California), following standard laboratory protocols (Dutton et al. 2008). Primers LCM15382 and H950g were used to amplify an ~889 bp fragment at the 5′ end of the control region of the mitochondrial genome using polymerase chain reaction (PCR) methodology (Abreu-Grobois et al. 2006, Dutton et al. 2007). A 25 µl PCR reaction was used with the following composition: 18 µl purified H_2O, 2.5 µl of 10X Mg buffer, 1.5 µl DNTPs, 0.75 µl of each primer, 0.5 µl of Taq polymerase, and 1 µl (20–50 ng) of template DNA. PCR reactions were run with the following profile: initial DNA denaturation at 94°C for 2 min, followed by 36 cycles of (1) DNA denaturation at 94°C for 50 sec,

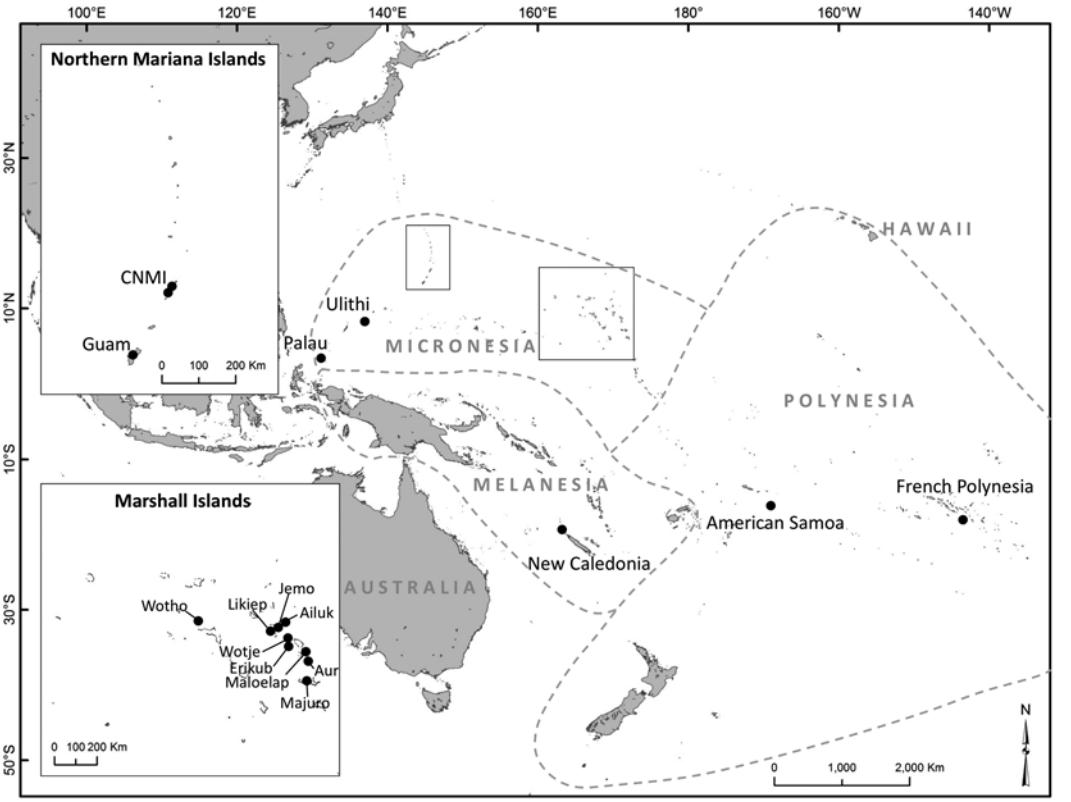


FIGURE 1. Locations of sampled green turtle rookeries in the Pacific islands regions.

(2) annealing of primers at 52°C for 50 sec, and (3) extension of primers at 72°C for 1 min, concluding with a final extension of primers at 72°C for 5 min. Negative controls were included in each PCR reaction to detect contamination. PCR products were purified by combining 5 µl of product with 2 µl of an Exonuclease I and Shrimp Alkaline Phosphatase solution. PCR products were cycle sequenced in both directions using a 12 µl reaction consisting of a 1:1 buffered version of the ABI Big Dye Terminator v 3.1. Labeled extension products were purified using an ethanol precipitation process. Products were analyzed with an Applied Biosystems model 3130 automated DNA sequencer (Applied Biosystems, Foster City, California). All sequences were trimmed to ~770 bp for further analyses because this region contains high-quality sequence and no variation was found outside the 770 bp.

Statistical Analysis

Sequences were analyzed using the program SeqScape v2.5 (Applied Biosystems, Foster City, California). Each sequence was reviewed manually for uncalled and miscalled bases, and all variable positions were confirmed by comparing sequences from the forward and reverse strands. We assigned haplotypes by comparing aligned sequences against a local reference library of published and unpublished green turtle haplotype sequences using Geneious Pro 5.6.3 (Drummond et al. 2011) as well as searching the database on GenBank (http://www.ncbi.nlm.nih.gov). We standardized nomenclature of haplotypes based on these 770 bp alignments, assigning the CmP prefix to numerically sequential names based on the original 384 bp alignments (Dutton et al. 2008; P.H.D., unpubl. data) with a sequential numeric suffix to indicate a variant

resulting from polymorphism in the additional 384 bp region flanking both sides of the old shorter sequence. All unique sequences were then aligned with the CLUSTALW algorithm implemented in Geneious Pro 5.6.3 (Drummond et al. 2011). The alignment of each mtDNA segment was checked and edited by eye separately.

Haplotype ($\hat{h}$) and nucleotide (π) diversity were calculated for each rookery using Arlequin v 3.5.1.2 (Excoffier and Lischer 2010). Haplotype diversity was estimated based on Nei (1987), and nucleotide diversity was calculated assuming the model of Tamura and Nei (1993). We tested for population structure by conducting analysis of molecular variance (AMOVA) (Excoffier et al. 1992), pairwise F_{ST} comparisons, and pairwise exact tests of population differentiation with the software Arlequin v 3.5.1.2 (Excoffier and Lischer 2010), using a probability level of $P < .05$ to infer significance.

A first round of pairwise comparisons tested individual rookeries within regions (rookeries located within 500 km of each other) to determine if these could be considered single or multiple genetic stocks. In cases when sample sizes were inadequate (<10), samples from neighboring rookeries were pooled if these were located within 500 km of each other. Thereafter followed a second set of pairwise comparisons between all identified genetic stocks. Significance values for AMOVA were obtained from 10,000 permutations. In the AMOVA, rookeries were grouped by their identified management units (MU) (stocks [Moritz et al. 1994]) as well as into MUs located within the three regions Micronesia, Melanesia, and Polynesia. Both conventional F_{ST} and sequence-based Φ_{ST} distance measures were used to calculate within- and among-population diversity. Exact tests of population differentiation were conducted with 100,000 permutations and 10,000 dememorization steps (Raymond and Rousset 1995).

RESULTS

We identified a total of 25 haplotypes characterized by polymorphism within the 770 bp mtDNA control region sequences from 805 green turtles (GenBank accession numbers AB819806, AB819808 [T. Hamabata, unpubl. data], and KF311744–KF311766 [this study]). Haplotypes were defined by 55 substitutions (54 transitions and 1 transversion). In total there were 72 polymorphic sites, including a 1 bp, a 3 bp, a 4 bp, and a 10 bp indel. Estimates of nucleotide diversity (π) ranged from 0.0003 (French Polynesia) to 0.0207 (New Caledonia) (Table 1). The number of observed haplotypes per population ranged from 11 for Ulithi Atoll in the FSM (largest sample size) to only two in French Polynesia and the Mariana Islands (small sample sizes). We identified new variants of haplotypes previously defined by the shorter 384 bp sequences based on polymorphism found with the longer 770 bp sequences. This included CmP20 (corresponding to A3, GenBank Accession AY955218, in Dethmers et al. [2006]) and CmP65 (corresponding to CmPo2, GenBank Accession EF555565, in Taquet [2007]). CmP20, which is a common and widespread haplotype throughout the region, further differentiated into four haplotypes based on variation outside the shorter 384 bp reading frame, and CmP65 into two different haplotypes based on the longer sequences (Supplemental Table S1, available online from www.BioOne.org). Two of the new CmP20 haplotypes (CmP20.3 and CmP20.4) were found only in FSM, and one (CmP20.2) only in FSM and Palau (Table 1).

Description of Genetic Stocks

REPUBLIC OF THE MARSHALL ISLANDS (RMI): The 128 samples from RMI were collected from nine different atolls across the 29 atolls and five islands that make up the RMI (Figure 1). The sample sizes from each of the nine locations varied greatly from 1 to 63 (see Table 1). To combine the Marshall Islands samples we first tested for any significant genetic structure across locations that had large enough sample sizes. Three sites were chosen: Jemo ($n = 26$), Ailuk ($n = 17$), and Erikub ($n = 63$). Jemo and Ailuk are located approx. 50 km from each other, Jemo and Erikub approx. 120 km, and Ailuk and Erikub approx. 130 km from each other. The exact test for

TABLE 1

Genetic Diversity Parameters and Haplotype Frequencies for Green Turtle Rookeries Sampled from Different Pacific Island Locations

Location	Latitude	Longitude	Est. Annual Nesting (Nf)	*n*	H	π	SD	h	SD	CmP20								CmP65			CmP44																
										CmP20.1	CmP20.2	CmP20.3	CmP20.4	CmP123.1	CmP22.1	CmP32.1	CmP61.1	CmP65.1	CmP65.2	CmP77.1	CmP40.1	CmP44.1	CmP44.2	CmP56.1	CmP67.1	CmP91.1	CmP173.1	CmP160.1	CmP117.1	CmP47.1	CmP31.1	CmP80.1	CmP85.1	CmP49.1
Marshall Islands			300–400	128	6	0.0014	0.0010	0.4596	0.0467	91	—	—	—	—	22	12	1	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Ailuk	10° 13′ 7.46″ N	169° 58′ 55.59″ E		17	2	—	—	—	—	15	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aur Atoll	10° 13′ 7.46″ N	169° 58′ 55.59″ E		4	3	—	—	—	—	—	—	—	—	—	2	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Erikub	8° 9′ 29.80″ N	171° 10′ 32.48″ E		63	4	—	—	—	—	43	—	—	—	—	8	11	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Jemo Island	9° 0′ 54.25″ N	170° 2′ 41.72″ E		26	3	—	—	—	—	17	—	—	—	—	8	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Likiep Atoll	10° 4′ 45.72″ N	169° 31′ 28.60″ E		2	1	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Majuro Atoll	9° 49′ 58.87″ N	169° 18′ 51.58″ E		1	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Maloelap Atoll	7° 7′ 36.52″ N	171° 11′ 8.11″ E		2	1	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Wotho	10° 10′ 29.59″ N	166° 0′ 37.65″ E		2	2	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Wotje Atoll	9° 26′ 53.11″ N	170° 3′ 12.57″ E		11	2	—	—	—	—	10	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ulithi Atoll, Yap (FSM)			500–1,000	538	11	0.0023	0.0015	0.6765	0.0169	276	2	10	16	2	1	84	76	—	—	68	—	—	—	—	2	1	—	—	—	—	—	—	—	—
Gielop	9° 57′ 14.24″ N	139° 54′ 23.60″ E		461	9	—	—	—	—	230	—	10	11	—	1	78	67	—	—	61	—	—	—	—	2	1	—	—	—	—	—	—	—	—
Loosiep	9° 55′ 4.60″ N	139° 50′ 35.50″ E		77	7	—	—	—	—	46	2	—	5	2	—	6	9	—	—	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Palau			100–500	36	3	0.0027	0.0017	0.3317	0.0879	29	6	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Helen Island	3° 0′ 16.50″ N	131° 7′ 29.36″ E		36	3	—	—	—	—	29	6	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Guam/Commonwealth of the Northern Mariana Islands (CNMI)			<20	48	2	0.0018	0.0013	0.0426	0.0403	47	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Cocos Island (Guam)	13° 14′ 21.30″ N	144° 39′ 9.02″ E		5	1	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Guam	13° 26′ 39.43″ N	144° 47′ 37.32″ E		17	1	—	—	—	—	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Saipan (CNMI)	15° 13′ 46.58″ N	145° 43′ 16.40″ E		19	1	—	—	—	—	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tinian (CNMI)	15° 1′ 39.83″ N	145° 39′ 30.56″ E		6	2	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Rota (CNMI)	14° 6′ 35.53″ N	145° 10′ 16.71″ E		1		—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
American Samoa			30–100	17	4	0.0165	0.0089	0.5128	0.1436	—	—	—	—	—	10	—	—	4	2	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Rose Atoll	14° 32′ 48.00″ S	168° 9′ 7.00″ W		11	4	—	—	—	—	—	—	—	—	—	6	—	—	2	2	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Swains Island	11° 3′ 22.44″ S	171° 4′ 33.58″ W		4	1	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tutuila	14° 15′ 31.63″ S	170° 33′ 43.60″ W		1	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ofu	14° 10′ 20.89″ S	169° 39′ 20.56″ W		1	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
New Caledonia			1,000–2,000	29	9	0.0207	0.0106	0.8399	0.0479	—	—	—	—	—	—	—	—	—	—	—	—	4	2	—	—	—	2	1	1	2	2	5	10	—
Fabre islet	18° 17′ 49.26″ S	163° 0′ 32.51″ E		5	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1			1	2	1	—
Huon Atoll	18° 2′ 25.24″ S	162° 57′ 31.02″ E		19	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	1	—	—	—	1	1	1	2		2	7	—
Leizour islet	18° 17′ 9.92″ S	163° 2′ 35.83″ E		5	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	1	2	—
French Polynesia			300–400	9	2	0.0003	0.0005	0.2500	0.1802	—	—	—	—	—	—	—	—	8	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Mopelia	16° 48′ 40.09″ S	153° 57′ 15.69″ W		9	2	—	—	—	—	—	—	—	—	—	—	—	—	8	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Grand total				800	25					443	8	10	16	2	33	96	77	13	2	69	1	4	2	1	2	1	2	1	1	3	2	5	10	1

Note: Nf, estimated annual number of females at each rookery (Maison et al. 2010); *n*, sample size; H, number of haplotypes; π, nucleotide diversities, and h, haplotype diversities with associated standard deviation (SD).

population differentiation showed no genetic difference between the three rookeries. However, the pairwise F_{ST} result showed significant differentiation between Erikub and Jemo ($F_{ST} = 0.048$, $P < .05$), but all other comparisons remained nonsignificant (data not shown). This is likely due to the haplotype CmP32.1 being found in 17% of samples from Erikub and in only one other individual in Ailuk. Nevertheless, because of the nonsignificant results from the exact test for all comparisons, the much larger sample size for Erikub, and the relatively short distance between those rookeries, they were all pooled for further analysis. Combined, the RMI was dominated by haplotype CmP20.1 (71%), which is common and widespread throughout Micronesia.

FSM (ULITHI ATOLL, YAP STATE): Ulithi Atoll, in Yap State, is the largest green turtle rookery in the FSM and also had the largest sample size ($n = 538$). Samples were collected from two beaches located less than 30 km from each other (Figure 1).

REPUBLIC OF PALAU: Samples from Palau ($n = 36$) were all collected from Helen Island and were mainly represented by haplotype CmP20.1 (81%). Haplotype CmP20.2 was represented in 17% of the samples. The rookery also exhibited haplotype CmP40.1 (2%), which is unique to this rookery (Table 1).

MARIANA ISLANDS (GUAM AND CNMI): The 22 samples from Guam were collected from a number of locations across the island. Samples were collected at Andersen Air Force Base ($n = 4$), Carlos Beach ($n = 1$), Cocos Island ($n = 5$), Ipan beach ($n = 6$), Jinapsan ($n = 1$), Ritidian refuge ($n = 3$), Sea Plane beach ($n = 1$), and Urunao beach ($n = 1$). Because all those locations are found within 50 km of each other and all Guam samples had the same haplotype (CmP20.1), they were pooled for further analysis. Likewise, samples from the CNMI were collected across Tinian ($n = 6$), Saipan ($n = 19$), and Rota ($n = 1$) and also were nearly fixed for CmP20.1 with the exception of one individual at Tinian with haplotype CmP49.1 (Table 1). Samples from these two neighboring territories were grouped together for further analysis.

AMERICAN SAMOA: The samples from American Samoa were collected across four locations that had both low sample sizes ($n = 1–11$) and were a great distance from each other (160–500 km) (see Tuato'o-Bartley et al. 1993). However, these were pooled to represent American Samoa because these sites shared haplotypes (see Table 1).

NEW CALEDONIA: The three sample locations in New Caledonia were all within 30 km of each other and pooled for the analysis. This stock was not characterized by a single haplotype but rather by a high number of haplotypes found at low to intermediate frequency (3%–34%) none of which is shared with any of the other island rookeries in this study (Table 1).

FRENCH POLYNESIA: Samples from French Polynesia were all collected from Mopelia ($n = 9$) and were characterized by the haplotypes CmP65.1 (89%) and CmP56.1 (11%) (Table 1).

Overall Genetic Structure (AMOVA)

The second round of pairwise analysis included the seven genetic stocks defined in the first round of comparisons: (1) RMI; (2) Ulithi Atoll, Yap State, FSM; (3) Helen Island, Palau; (4) CNMI and Guam; (5) New Caledonia; (6) American Samoa; and (7) Mopelia, French Polynesia. Both conventional F_{ST} and exact tests indicated highly significant differentiation between all stocks (Table 2), identifying at least seven independent stocks (MUs) across the region. When grouping the rookeries into the three broad Pacific regions (Micronesia, Melanesia, and Polynesia), the AMOVA hierarchical analysis showed that most of the variation in haplotype frequencies could be attributed to variance among groups when using sequence-based measures (Φ_{ST}: 77.24%, $P < .05$), followed by variation within stocks (Φ_{ST}: 18.71%, $P < .005$), and finally by variation among stocks within groups (Φ_{ST}: 4.05%, $P < .005$) (Table 3).

DISCUSSION

The genetic analysis of green turtle rookeries presented here fills an important information gap in the coverage of green turtle stock structure in the Indo-Pacific. A previous study included three locations in FSM and reported

TABLE 2

Pairwise Comparisons of the Seven Genetic Stocks

Location	Tests	Marshall Is.	Ulithi, Yap	Palau	Guam/ CNMI	Am. Samoa	New Caledonia	French Polynesia
Marshall Is.		0–400 km	>3,000 km	>4,300 km	>2,700 km	>2,800 km	>2,900 km	>4,800 km
Ulithi, Yap	F_{ST}	0.0724**	0–30 km	>1,200 km	>600 km	>5,900 km	>4,000 km	>7,800 km
	Exact test	**						
Palau	F_{ST}	0.0656*	0.1058**	0 km	>1,800 km	>6,500 km	>4,200 km	>8,500 km
	Exact test	**	**					
Guam/ CNMI	F_{ST}	0.1259**	0.1743**	0.1316**	0–250 km	>5,500 km	>4,000 km	>7,500 km
	Exact test	**	**	**				
Am. Samoa	F_{ST}	0.4599**	0.3716**	0.6064**	0.8403**	0–500 km	>2,800 km	>1,500 km
	Exact test	**	**	**	**			
New Caledonia	F_{ST}	0.4021**	0.2631**	0.4272**	0.6210**	0.2991**	0–30 km	>4,500 km
	Exact test	**	**	**	**	**		
French Polynesia	F_{ST}	0.5824**	0.4398**	0.6891**	0.9252**	0.5343**	0.3876**	0 km
	Exact test	**	**	**	**	**	**	

Note: F_{ST} values (conventional haplotype frequencies) and results for the exact test are shown below the diagonal. *P* values are shown as <.05 (*) and <.005 (**). Approximate shortest distance between the stocks is shown above the diagonal. Gray areas show the overall distance between sampling locations within each stock.

TABLE 3

Results from Analysis of Molecular Variance (AMOVA) for Green Turtle Nesting Sites across the Western Pacific Ocean Using Both Conventional Haplotype Frequency F_{ST} and Sequence-Based Φ_{ST} with Tamura and Nei Distance Measure

		Φ_{ST}		Conventional	
Groups	Variance Component[a]	% of Variance	*F*-Statistics[b]	% of Variance	*F*-Statistics[b]
1. Micronesia	AG	77.24%	F_{CT}: 0.7724*	27.63%	F_{CT}: 0.2763*
2. Melanesia	AP/WG	4.05%	F_{SC}: 0.1779**	8.83%	F_{SC}: 0.1220**
3. Polynesia	WP	18.71%	F_{ST}: 0.8129**	63.54%	F_{ST}: 0.3646**

[a] AG is the among-groups component variance; AP/WG is the among-populations/within-group component of variance; WP is the within-population component of variance.

[b] Significance of permutation test (10,000 permutations) is shown for $P < .05$ (*) and $P < .005$ (**).

no significant differentiation between Elato, Ngulu, and Ulithi Atolls, suggesting some genetic exchange between regional rookeries (Dethmers et al. 2006) but strong genetic partitioning from other green turtle rookeries in Southeast Asia and Australia. However, that study was limited to low sample sizes ($n = 9$, 15, and 25) for the FSM rookeries and shorter 384 bp fragment of the mtDNA control region. Here we have provided a more detailed view of the geographic distribution of genetic variation that builds on previous work and reveals significant structuring among the green turtle populations across the western and central Pacific using new sample sites, larger sample sizes, and longer mtDNA fragments.

Stock Structure across the Western and Central Pacific

The analysis of 805 samples from the 25 rookeries identified at least seven MUs across the study area. These sampled rookeries represent either localized areas with high nesting

density (e.g., Ulithi Atoll, FSM) or clusters of islands and atolls covering a large area with scattered low-density nesting (e.g., the Marshall Islands). Data from the Marshall Islands were affected by various sample sizes due to the scattered nature of the nesting habitat (samples were collected across nine nesting beaches). However, our results indicate that groups of nesting beaches that are isolated from more distant rookeries by >500 km can be considered genetically distinct MUs. Although not ideal, combining the samples from nearby nesting beaches can be justified when rookeries are within 500 km and the sample size is too low for a statistical test. Tagging studies from the Great Barrier Reef showed some inter- and intranesting movement within scales of 300 km (Limpus et al. 2009). In our study all rookeries separated by more than 1,000 km were significantly differentiated from each other, whereas neighboring rookeries within 500 km showed no genetic differentiation. We found moderate (but significant) genetic structure within Micronesian MUs ($F_{ST} < 0.2$), but differentiation was much higher between rookeries in Micronesia and those in Melanesia and Polynesia. When looking at the overall distribution of genetic variation, the AMOVA showed strong partitioning of genetic variation among the three different regions (Micronesia, Melanesia, and Polynesia) when using sequence-based Φ_{ST}, with 77.24% of the genetic variation distributed among the three regions. This pattern is likely driven by strong isolation of the New Caledonia rookery in Melanesia, which shares no haplotypes with any other rookery in this study. However, a comparison (using the shorter 384 bp) with samples from the Great Barrier Reef and the Coral Sea (Dethmers et al. 2006) showed that the New Caledonia stock shares four of eight haplotypes with turtles nesting along the Great Barrier Reef and the Coral Sea, although New Caledonia was distinct (Dethmers et al. 2006). These patterns of genetic connectivity and isolation are undoubtedly shaped by regional oceanographic currents that act as barriers to dispersal and have led to isolation of Coral Sea populations (see Dethmers et al. 2006). There are several nesting populations that were absent from our study, including those in the Cook Islands, Vanuatu, Solomon Islands, and Papua New Guinea. Additional sampling from French Polynesia nesting sites may also reveal further fine-scale structure, and longer 770 bp sequence data are needed for the northeastern Australian rookeries so that a comprehensive regional analysis can be completed.

On a broader scale, there is high genetic differentiation between rookeries in the western Pacific and those in the eastern Pacific (including the Hawaiian Islands), indicating a strong biogeographic barrier across the Pacific Ocean when it comes to dispersal of nesting females, because no haplotypes are shared between the eastern and the western Pacific (Dutton et al. 2008; P.H.D. et al., unpubl. data). Similarly, there is a strong barrier to dispersal of female lineage between the Pacific island rookeries (including those nesting on the Great Barrier Reef) and those nesting in Southeast Asia (Dethmers et al. 2006). The extent to which biogeography over evolutionary timescales has shaped the current observed patterns of genetic variation is the subject of a broader phylogeographic analysis currently under way (M.P.J., N. Fitzsimmons, J. Bourjea, T. Hamabata, J. Reece, P.H.D., unpubl. data).

Implications for Management and Conservation

Studies using both satellite telemetry and flipper tagging have shown that green turtles nesting in these regions may migrate to several distant foraging areas. For example, green turtles satellite tagged while nesting at Gielop and Ulithi Atoll (FSM) migrated to areas such as Malaysia, the Philippines, and Japan (Kolinski 1995). Most turtles tagged at Rose Atoll in American Samoa migrated to foraging areas in Fiji (Craig et al. 2004). Two females tagged in Guam went to the Philippines and Japan (see Maison et al. 2010). From RMI turtles went to Bikini Atoll, the Philippines, and Kiribati (Kabua et al. 2012), and turtles tagged at Scilly Atoll in French Polynesia have been recorded in Tonga, New Caledonia, Vanuatu, the Cook Islands, and Fiji (Balazs et al. 1995). Also, a small proportion

of adult female turtles foraging along the Great Barrier Reef nest in New Caledonia (Limpus et al. 2009). These studies highlight the long distance traveled by some turtles between nesting and foraging areas and shows that turtles from one MU will migrate to a number of different foraging areas, resulting in foraging populations of mixed stock origin. This overlap of MUs at foraging areas presents a challenge to delineating the geographic boundaries of MUs. Furthermore, although adequate for characterizing female reproductive boundaries linked to nesting stocks, exclusive use of mtDNA markers may overlook the role that male-mediated gene flow may have in demographic structuring. Results from a study using a comprehensive set of nuclear Single Nucleotide Polymorphism (SNP) and microsatellite markers found significant structure among Pacific green turtle rookeries that was consistent with mtDNA results and suggest that male-mediated gene flow is not as extensive as previously believed (Roden et al. 2013). A similar analysis with these nuclear markers is needed to determine whether the fine-scale structuring exists among the maternally distinct western and central Pacific MUs we identified.

The extent of connectivity between nesting populations and different feeding habitats determines the geographical scale of conservation efforts directed at individual MUs. Knowing the proportion of turtles belonging to different MUs at foraging areas is important to conducting meaningful threat assessments of marine turtle populations. The data presented here provide a much-needed baseline that can be used as a reference of potential source nesting stocks for determining stock origin of turtles at foraging areas or caught in fisheries (Bolker et al. 2007). Many of the Pacific island green turtle nesting populations are thought to be depleted, and in some places, such as Fiji, nesting is rare, yet harvest of animals captured in local waters, presumably from other MUs, is widespread (Jit 2007). Other local management applications include impact assessment required for certain development projects, such as harbor construction in Pago Pago, American Samoa, where green turtles forage. In addition our results can enable assignment of stock origin of animals caught in pelagic fisheries, such as the American Samoa–based longline fishery, which interacts with juvenile green turtles.

Within the rookeries studied here sea turtles are managed by a variety of national and international laws and therefore protected to various degrees. Some territories fall under the jurisdiction of the United States (e.g., American Samoa, Guam, and the Northern Mariana Islands) and protect turtles under the ESA. Others have local regulations in place to manage green turtle populations such as the FSM, Marshall Islands, Palau, New Caledonia, and French Polynesia. Overall the amount of nesting data for the majority of the region is scarce. Although some countries have established monitoring of nesting beaches (e.g., Yap State, FSM), most have only limited and fragmented information. In many of the Pacific islands indigenous and subsistence harvest is permitted, but harvest levels and the impact on local and regional populations are unknown. Our results will now enable stock-specific threats assessments to be carried out for the western and central Pacific using mtDNA. Additional work using a comprehensive array of informative nuclear markers will be required to further improve understanding of the demographic connectivity among nesting populations (Lowe and Allendorf 2010, Roden et al. 2013). Genetic stock assessment of sea turtle populations at both nesting and foraging habitats is important for a better understanding of the long-term implications of management or mismanagement of those populations, and it may have broader conservation implications if appropriately used.

ACKNOWLEDGMENTS

We thank SPREP, the late Lui Bell, Nancy Fitzsimmons, Irene Kelly, and Kyle Van Houtan for their help with coordinating sample collections. We also thank R. LeRoux, S. Roden, and C. Allen for valuable comments on the manuscript. A. Lanci, V. Pease, and A. Konopacki helped with laboratory analysis. The following people, communities, and organizations helped with field collec-

tions: Ruth Utzurrum, Katerine Schletz-Saili (American Samoa), Tammy Summers, the Falalop, Ulithi Community, the FSM Office of Marine Resources, Department of Resources and Development, the Oceanic Society, Yap Cap and Yap State Marine Resources Management Division; the field crew in Ulithi including X. Maigul, M. Lingelmar, A. Sau, J. Mangirechog, J. P. Malisou, L. Mal, B. Malpuluw, V. Thawalmara, A. Suwel, A. Yangolug, E. Yatch, E. Rusumal, J. Mangirechog, M. Fasong, N. Malifang, J. Waithog, J. Maremog, J. J. Hasog, G. Hacheglyeg, M. Dohwel, Q. Yefalgoi, H. Remaatiul, R. J. Lutwog, K. Dothwel, R. Yurus, M. Tabungmai; the data collectors of Women United Together in the Marshall Islands (WUTMI) including K. Boktok, M. Langidrik, H. Jacob, E. Senight, R. Jidok, A. Bunglick, R. Jelke, R. Jidok, R. and A. deBrum, L. Mejbon; C. Bigler, H. Heine (Project Coordinators); N. Vander Velde (Biologist), the Marshall Islands Marine Resources Authority (MIMRA), and E. Kabua; the CNMI Department of Lands and Natural Resources/Division of Fish and Wildlife Sea Turtle Program; the Palau Bureau of Marine Resources; for American Samoa: U. R. Tulafono (former DMWR director), R. Matagi-Tofiga (current DMWR director); for New Caledonia: Christophe Fonfreyde du Service de la marine marchande et des pêches maritimes, Fred Avril, province Nord, and Natacha Agudo; Sophie and Kalami Taputu and family for access to nesting sites at Mopelia; for Guam: Shawn Wusstig, Guam Division of Aquatic and Wildlife Resources. Thanks to Patrick Opay, Irene Kelly, and Ray Clarke (NMFS-PIRO) for partial funding support.

Literature Cited

Abreu-Grobois, F. Alberto, J. A. Horrocks, A. Formia, P. H. Dutton, R. A. LeRoux, X. Velez-Zuazo, L. S. Soares, and A. B. Meylan. 2006. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. Page 179 *in* M. Frick, A. Panagopoulous, A. F. Rees, and K. Williams, eds. Proceedings of the 26th Annual Symposium on Sea Turtle Biology, Island of Crete, Greece.

Balazs, G. H., P. Siu, and J. P. Landret. 1995. Ecological aspects of green turtles nesting at Scilly Atoll in French Polynesia. Pages 7–10 *in* J. I. Richardson and T. H. Richardson, eds. Proceedings of the 12th Annual Workshop on Sea Turtle Biology and Conservation. NOAA Tech. Memo. NMFS-SEFSC-361.

Bolker, B., T. Okayama, K. A. Bjorndal, and A. B. Bolten. 2007. Incorporating multiple mixed stocks in mixed stock analysis: "Many-to-many" analyses. Mol. Ecol. 16:685–695.

Bolten, A. B. 2003. Variation in sea turtle life history patterns: Neritic vs. oceanic developmental stages. Page 455 *in* Peter L. Lutz, John A. Musick, and Jeanette Wyneken, eds. The biology of sea turtles. Vol. 2. CRC Press, Boca Raton, Florida.

Bourjea, J., S. Lapègue, L. Gagnevin, D. Broderick, J. A. Mortimer, S. Ciccione, D. Roos, C. Taquet, H. Grisel, and H. Grizel. 2007. Phylogeography of the green turtle, *Chelonia mydas*, in the Southwest Indian Ocean. Mol. Ecol. 16:175–186.

Bowen, B. W., A. L. Bass, L. S. Soares, and R. J. Toonen. 2005. Conservation implications of complex population structure: Lessons from the loggerhead turtle (*Caretta caretta*). Mol. Ecol. 14:2389–2402.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs, and J. C. Avise. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46:865–881.

Chaloupka, M., K. A. Bjorndal, G. H. Balazs, A. B. Bolten, L. M. Ehrhart, C. J. Limpus, H. Suganuma, S. Troëng, and M. Yamaguchi. 2008. Encouraging outlook for recovery of a once severely exploited marine megaherbivore. Glob. Ecol. Biogeogr. 17:297–304.

Craig, P., D. M. Parker, R. E. Brainard, M. Rice, and G. H. Balazs. 2004. Migrations of green turtles in the central South Pacific. Biol. Conserv. 116:433–438.

Dethmers, K. E. M., D. Broderick, C. Moritz, N. N. FitzSimmons, C. J. Limpus, S.

Lavery, Scott D. Whiting, M. Guinea, R. I. T. Prince, and R Kennett. 2006. The genetic structure of Australasian green turtles (*Chelonia mydas*): Exploring the geographical scale of genetic exchange. Mol. Ecol. 15:3931–3946.

Drummond, A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, C. Duran, M. Field, J. Heled, M. Kearse, S. Markowitz, R. Moir, S. Stones-Havas, S. Sturrock, T. Thierer, and A. Wilson. 2011. Geneious V5.4. http://www.geneious.com/.

Dutton, P. H. 1996. Methods for collection and preservation of samples for sea turtle genetic studies. Pages 17–24 *in* Brian W. Bowen and W. N. Witzell, eds. Proceedings of the International Symposium on Sea Turtle Conservation Genetics, Miami, Florida. NOAA Tech. Memo. NMFS-SEFSC-396.

Dutton, P. H., G. H. Balazs, R. A. LeRoux, Shawn K. K. Murakawa, P. Zárate, and L. Sarti Martínez. 2008. Composition of Hawaiian green turtle foraging aggregations: mtDNA evidence for a distinct regional population. Endem. Spec. Res. 5:37–44.

Dutton, P. H., C. Hitipeuw, M. Zein, S. R. Benson, and S. M. Al-Ghais. 2007. Status and genetic structure of nesting populations of leatherback turtles (*Dermochelys coriacea*) in the western Pacific. Chelon. Conserv. Biol. 6:47.

Dutton, P. H., S. E. Roden, K. R. Stewart, E. L. LaCasella, M. Tiwari, A. Formia, J. C. Thomé, S. R. Livingstone, S. Eckert, D. Chacon-Chaverri, P. Rivalan, and P. Allman. 2013. Population stock structure of leatherback turtles (*Dermochelys coriacea*) in the Atlantic revealed using mtDNA and microsatellite markers. Conserv. Genet. 14:625–636.

Encalada, S. E., P. N. Lahanas, K. A. Bjorndal, B. Bolker, M. M. Miyamoto, and B. W. Bowen. 1996. Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: A mitochondrial DNA control region sequence assessment. Mol. Ecol. 5:473–483.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin Suite Ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10:564–567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479–491.

FitzSimmons, N. N., C. J. Limpus, J. A. Norman, A. R. Goldizen, J. D. Miller, and C. Moritz. 1997. Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proc. Natl. Acad. Sci. U.S.A. 94:8912–8917.

Formia, A., B. J. Godley, J. F. Dontaine, and M. W. Bruford. 2006. Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. Conserv. Genet. 7:353–369.

Hirth, H. F. 1997. Synopsis of the biological data on the green turtle, *Chelonia mydas* (Linnaeus 1758). U.S. Fish Wildl. Serv. Biol. Rep. 97 (1).

Jensen, M. P., N. N. FitzSimmons, and P. H. Dutton. 2013. Molecular genetics of sea turtles. Pages 135–154 *in* J. Wyneken, K. J. Lohmann, and J. A. Musick, eds. The biology of sea turtles. Vol. 3. CRC Press, Boca Raton, Florida.

Jit, J. N. 2007. Status of sea turtle conservation in Fiji: An assessment of the international, regional and national focus. M.A. thesis, University of the South Pacific, Suva. www.sprep.org/att/IRC/eCOPIES/Countries/Fiji/6.pdf.

Kabua, E., M. Langidrik, K. Boktok, D. M. Parker, and K. P. Frutchey. 2012. Marine turtle–fisheries interaction mitigation training, data collection activities and satellite telemetry in the Republic of the Marshall Islands. Page 120 *in* L. Belskis, M. Frick, A. Panagopoulou, A. F. Rees, and K. Williams, eds. Proceedings of the 29th Annual Symposium on Sea Turtle Biology and Conservation, Brisbane, Australia. NOAA Tech. Memo. NMFS-SEFSC-630.

Kolinski, S. 1995. Migrations of the green turtle, *Chelonia mydas*, breeding in Yap State, Federated States of Micronesia. Micronesica 28 (1): 1–8.

Lahanas, P. N., M. M. Miyamoto, K. A. Bjorndal, and A. B. Bolten. 1994. Molecular evolution and population genetics of greater Caribbean green turtles (*Chelonia mydas*) as inferred from mitochondrial DNA control region sequences. Genetica 94:57–66.

LeRoux, R. A., P. H. Dutton, F. A. Abreu-Grobois, C. J. Lagueux, C. L. Campbell, E. Delcroix, J. Chevalier, J. A. Horrocks, Z. Hillis-Starr, S. Troëng, E. Harrison, and S. Stapleton. 2012. Re-examination of population structure and phylogeography of hawksbill turtles in the Wider Caribbean using longer mtDNA sequences. J. Hered. 103:806–820.

Limpus, C. J., I. Bell, and J. D. Miller. 2009. Mixed stocks of green turtles foraging on Clack Reef, Northern Great Barrier Reef, identified from long term tagging studies. Mar. Turtle Newsl. 123:3–5.

Limpus, C. J., P. J. Couper, and M. A. Read. 1994. The green turtle, *Chelonia mydas*, in Queensland: Population structure in a warm temperate feeding area. Mem. Queensl. Mus. 35:139–154.

Limpus, C. J., and D. G. Walter. 1980. The growth of immature green turtles (*Chelonia mydas*) under natural conditions. Herpetologica 36:162–165.

López-Castro, M. C., V. Koch, A. Mariscal-Loza, and W. J. Nichols. 2010. Long-term monitoring of black turtles *Chelonia mydas* at coastal foraging areas off the Baja California Peninsula. Endang. Spec. Res. 11:35–45.

Lowe, W. H., and F. W. Allendorf. 2010. What can genetics tell us about population connectivity? Mol. Ecol. 19:3038–3051.

Maison, K. A., I. Kinan-Kelly, and K. P. Frutchey. 2010. Green turtle nesting sites and sea turtle legislation throughout Oceania. NOAA Tech. Memo. NMFS-F/SPO-110.

Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16:1215.

Monzón-Argüello, C., C. Rico, A. Marco, P. Lopez, and L. F. López-Jurado. 2010. Genetic characterization of eastern Atlantic hawksbill turtles at a foraging group indicates major undiscovered nesting populations in the region. J. Exp. Mar. Biol. Ecol. 387:9–14.

Moritz, C. 1994. Defining 'Evolutionary Significant Units' for conservation. Trends Ecol. Evol. 9:373–375.

Musick, J. A., and C. J. Limpus. 1997. Habitat utilization and migration in juvenile sea turtles. Pages 137–163 *in* The biology of sea turtles. Vol. 1. CRC Press, Boca Raton, Florida.

National Marine Fisheries Service and U.S. Fish and Wildlife Service. 2007. Green sea turtle (*Chelonia mydas*) 5-year review: Summary and evaluation. NMFS Office of Protected Resources, Silver Spring, Maryland, and USFWS Southeast Region, Jacksonville, Florida.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Pilcher, N. J., M. Chaloupka, and E. Woods. 2012. Hawaiian green sea turtle: Regional assessment. Page 13 *in* IUCN Red List of Endangered Species.

Raymond, M., and F. Rousset. 1995. An exact test for population differentiation. Evolution 49:1280–1283.

Roden, S. E., P. A. Morin, A. Frey, G. H. Balazs, P. Zarate, I.-J. Cheng, and P. H. Dutton. 2013. Green turtle population structure in the Pacific: New insights from SNPs and microsatellites. Endem. Spec. Res. 20:227–234.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbour Laboratory Press, New York.

Senko, J., V. Koch, W. M. Megill, R. R. Carthy, R. P. Templeton, and W. J. Nichols. 2010*a*. Fine scale daily movements and habitat use of East Pacific green turtles at a shallow coastal lagoon in Baja California Sur, Mexico. J. Exp. Mar. Biol. Ecol. 391:92–100.

Senko, J., M. C. López-Castro, V. Koch, and W. J. Nichols. 2010*b*. Immature East Pacific green turtles (*Chelonia mydas*) use multiple foraging areas off the Pacific coast of Baja California Sur, Mexico: First

evidence from mark-recapture data. Pac. Sci. 64:125–130.

Shamblin, B. M., A. B. Bolten, K. A. Bjorndal, P. H. Dutton, J. T. Nielsen, F. A. Abreu-Grobois, K. J. Reich, B. E. Witherington, D. A. Bagley, L. M. Ehrhart, A. D. Tucker, D. S. Addison, A. Arenas, C. Johnson, R. R. Carthy, M. M. Lamont, M. G. Dodd, M. S. Gaines, E. LaCasella, and C. J. Nairn. 2012. Expanded mitochondrial control region sequences increase resolution of stock structure among North Atlantic loggerhead turtle rookeries. Mar. Ecol. Prog. Ser. 469:145–160.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512–526.

Taquet, C. 2007. Diversité et différenciation génétiques des populations de tortues vertes (*Chelonia mydas*) dans les sites de ponte et d'alimentation du sud-ouest de l'océan Indien: Application aux stratégies de conservation de l'espèce. PhD. diss, Université de la Réunion, Biologie Marine.

Trevor, A. P. 2010. Turtle research and monitoring database system (TREDS): Annual report 2009. Apia, Samoa, SPREP. http://www.sprep.org/att/publication/000769_Final_TREDS_Annual_Report_2010.pdf.

Tuato'o-Bartley, N., T. E. Morrell, and P. Craig. 1993. Status of sea turtles in American Samoa in 1991. Pac. Sci. 47:215–221.

Wallace, B. P., A. D. DiMatteo, A. B. Bolten, M. Y. Chaloupka, B. J. Hutchinson, F. A. Abreu-Grobois, J. A. Mortimer, J. A. Seminoff, D. Amorocho, K. A. Bjorndal, J. Bourjea, B. W. Bowen, R. Briseno-Duenas, P. Casale, B. C. Choudhury, A. Costa, P. H. Dutton, A. Fallabrino, E. M. Finkbeiner, A. Girard, M. Girondot, M. Hamann, B. J. Hurley, M. Lopez-Mendilaharsu, M. A. Marcovaldi, J. A. Musick, R. Nel, N. J. Pilcher, S. Troëng, B. Witherington, and R. B. Mast. 2011. Global conservation priorities for marine turtles. PLoS ONE 6 (9): e24510. doi:10.1371/journal.pone.0024510.

Supplemental TABLE S1. Sequences from 770 bp of the mtDNA Control Region Haplotypes Detected among Western Pacific Populations of Green Turtles (Chelonia mydas). Shaded Area Marks the Previously Sequenced "Short" 384 bp Region. (•) Have the Same Base as CmP20.1, and (-) Are Insertions or Deletions. Genbank Accession Numbers Are Indicated.

Base Pair Position

Haplotype	Genbank ID	20	47	48	49	70	71	72	73	76	135	146	147	199	209	211	213	214	215	222	223	227	231	232
CmP20.1	AB819806	T	T	A	A	-	-	-	-	T	A	T	T	G	G	G	T	T	G	C	A	T	A	A
CmP20.2	KF311744	•	•	•	•	-	-	-	-	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•
CmP20.3	KF311745	•	•	•	•	-	-	-	-	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP20.4	KF311746	•	•	•	•	-	-	-	-	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•
CmP22.1	KF311747	•	•	•	•	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP31.1	KF311748	•	•	•	•	A	C	T	A	•	•	•	•	•	A	A	•	•	•	T	•	•	•	•
CmP32.1	KF311749	•	•	•	•	-	-	-	-	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•
CmP40.1	KF311750	C	-	-	-	A	C	T	A	•	•	•	•	•	•	A	•	C	A	T	•	•	•	•
Cmp44.1	KF311751	•	•	•	•	A	C	T	A	•	•	•	•	•	A	A	•	•	•	T	G	•	•	•
CmP44.2	KF311752	•	•	•	•	A	C	T	A	•	G	•	•	•	A	A	•	•	•	T	G	•	•	•
CmP47.1	KF311753	•	•	•	•	-	-	-	-	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•
CmP49.1	AB819808	C	-	-	-	A	C	T	A	•	•	•	•	•	•	A	C	C	A	T	•	•	•	•
CmP56.1	KF311754	•	-	-	-	A	C	T	A	•	•	•	C	A	•	A	•	C	A	T	•	C	G	G
CmP61.1	KF311755	•	•	•	•	-	-	-	-	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•
CmP65.1	KF311756	•	-	-	-	A	C	T	A	•	•	•	C	A	•	A	•	C	A	T	•	C	G	G
CmP65.2	KF311757	•	-	-	-	A	C	T	A	•	•	•	C	A	•	A	•	C	A	T	•	C	G	G
CmP67.1	KF311758	•	•	•	•	-	-	-	-	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•
CmP77.1	KF311759	•	•	•	•	-	-	-	-	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•
CmP80.1	KF311760	•	•	•	•	A	C	T	A	•	•	•	•	•	A	A	•	•	•	T	G	•	•	•
CmP85.1	KF311761	•	-	-	-	A	T	T	A	•	•	•	C	A	•	A	•	C	A	T	•	•	G	G
CmP91.1	KF311762	C	-	-	-	A	C	T	A	•	•	•	•	•	•	A	C	C	A	T	•	•	•	•
CmP117.1	KF311763	•	•	•	•	-	-	-	-	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•
CmP123.1	KF311764	•	•	•	•	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP160.1	KF311765	•	•	•	•	A	C	T	A	•	•	•	•	•	A	A	•	•	•	T	G	•	•	•
CmP173.1	KF311766	•	-	-	-	A	T	T	A	•	•	•	C	A	•	A	•	C	A	T	•	•	G	•

Haplotype	Genbank ID	310	313	316	336	339	361	369	395	403	405	409	411	412	422	423	448	449	450	463	469	470	471	476	487
CmP20.1	AB819806	T	C	A	A	G	C	A	A	C	A	G	G	G	T	T	T	C	T	T	A	C	G	T	T
CmP20.2	KF311744	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP20.3	KF311745	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP20.4	KF311746	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP22.1	KF311747	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP31.1	KF311748	•	•	G	•	A	•	G	•	T	G	A	A	•	•	•	C	T	•	C	G	T	A	•	•
CmP32.1	KF311749	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•
CmP40.1	KF311750	C	•	•	G	•	•	•	G	T	G	A	A	•	C	C	C	T	•	C	G	T	A	C	•
Cmp44.1	KF311751	•	T	G	•	A	•	G	•	T	G	A	A	•	•	•	C	T	•	C	G	T	A	•	•
CmP44.2	KF311752	•	T	G	•	A	•	G	•	T	G	A	A	•	•	•	C	T	•	C	G	T	A	•	•
CmP47.1	KF311753	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	C	•	•	•	•	•	•
CmP49.1	AB819808	C	•	•	G	•	•	•	G	T	G	A	A	•	C	C	C	T	•	C	G	T	A	C	•
CmP56.1	KF311754	•	•	•	•	•	T	•	G	•	•	•	A	•	•	•	•	T	•	C	G	T	A	•	C
CmP61.1	KF311755	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP65.1	KF311756	•	•	•	•	•	T	•	G	•	•	•	•	•	•	•	•	T	•	C	G	T	A	•	C
CmP65.2	KF311757	•	•	•	•	•	T	•	G	•	•	•	•	•	•	•	•	T	•	C	G	T	A	•	C
CmP67.1	KF311758	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CmP77.1	KF311759	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	C	•	•	•	•	•	•
CmP80.1	KF311760	•	•	G	•	A	•	G	•	T	G	A	A	•	•	•	C	T	•	C	G	T	A	•	•
CmP85.1	KF311761	•	•	•	•	•	•	•	G	•	G	•	•	•	•	•	•	T	•	C	G	T	•	•	C
CmP91.1	KF311762	C	•	•	G	•	•	•	•	T	G	A	A	•	C	C	C	T	•	C	G	T	A	C	•
CmP117.1	KF311763	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	C	•	•	•	•	•	•
CmP123.1	KF311764	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•
CmP160.1	KF311765	•	T	G	•	A	•	G	•	T	G	A	A	•	C	•	C	T	•	C	G	T	A	•	•
CmP173.1	KF311766	•	•	•	•	•	•	•	G	•	G	•	•	•	•	•	•	T	•	C	G	T	•	•	C

Haplotype	Genbank ID	508	509	510	511	512	513	514	515	516	517	536	591	627	643	653	654	662	663	667	697	724	730	748	749	752
CmP20.1	AB819806	-	-	-	-	-	-	-	-	-	-	G	T	C	C	A	G	G	C	G	T	T	T	T	-	C
CmP20.2	KF311744	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•
CmP20.3	KF311745	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•
CmP20.4	KF311746	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•
CmP22.1	KF311747	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•
CmP31.1	KF311748	-	-	-	-	-	-	-	-	-	-	A	•	T	T	G	•	A	T	A	•	•	C	•	-	•
CmP32.1	KF311749	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•
CmP40.1	KF311750	-	-	-	-	-	-	-	-	-	-	A	C	•	•	G	A	•	T	A	C	•	C	C	-	T
Cmp44.1	KF311751	-	-	-	-	-	-	-	-	-	-	A	•	T	T	G	•	A	T	A	•	•	C	•	-	•
CmP44.2	KF311752	-	-	-	-	-	-	-	-	-	-	A	•	T	T	G	•	A	T	A	•	•	C	•	-	•
CmP47.1	KF311753	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	T	•	•	•	•	•	-	•
CmP49.1	AB819808	-	-	-	-	-	-	-	-	-	-	A	C	•	•	G	A	•	T	A	C	•	C	C	-	T
CmP56.1	KF311754	-	-	-	-	-	-	-	-	-	-	A	C	•	T	G	•	•	T	A	C	•	C	•	T	•
CmP61.1	KF311755	C	C	T	T	T	G	G	T	T	G	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•
CmP65.1	KF311756	-	-	-	-	-	-	-	-	-	-	A	C	•	T	G	•	•	T	A	C	•	C	•	T	•
CmP65.2	KF311757	-	-	-	-	-	-	-	-	-	-	A	C	•	T	G	•	•	T	A	C	C	C	•	T	•
CmP67.1	KF311758	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•
CmP77.1	KF311759	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	T	•	•	•	•	•	-	•
CmP80.1	KF311760	-	-	-	-	-	-	-	-	-	-	A	•	T	T	G	•	A	T	A	•	•	C	•	-	•
CmP85.1	KF311761	-	-	-	-	-	-	-	-	-	-	A	C	•	T	G	•	•	T	A	C	•	C	•	T	•
CmP91.1	KF311762	-	-	-	-	-	-	-	-	-	-	A	C	•	•	G	A	•	T	A	C	•	C	C	-	T
CmP117.1	KF311763	C	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	T	•	•	•	•	•	-	•
CmP123.1	KF311764	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•
CmP160.1	KF311765	-	-	-	-	-	-	-	-	-	-	A	•	T	T	G	•	A	T	A	•	•	C	•	-	T
CmP173.1	KF311766	-	-	-	-	-	-	-	-	-	-	A	C	•	T	G	•	•	T	A	C	•	C	•	T	•