Molecular Ecology (2005) **14**, 2389–2402 doi: 10.1111/j.1365-294X.2005.02598.x

Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*)

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Abstract

Complex population structure can result from either sex-biased gene flow or population overlap during migrations. Loggerhead turtles (*Caretta caretta*) have both traits, providing an instructive case history for wildlife management. Based on surveys of maternally inherited mtDNA, pelagic post-hatchlings show no population structure across the northern Atlantic ($\phi_{ST} < 0.001$, $P = 0.919$), subadults in coastal habitat show low structure among locations ($\phi_{ST} = 0.01$, $P < 0.005$), and nesting colonies along the southeastern coast of the United States have strong structure ($\phi_{ST} = 0.42$, $P < 0.001$). Thus the level of population structure increases through progressive life history stages. In contrast, a survey of biparentally inherited microsatellite DNA shows no significant population structure: $R_{ST} < 0.001$; $F_{ST} = 0.002$ ($P > 0.05$) across the same nesting colonies. These results indicate that loggerhead females home faithfully to their natal nesting colony, but males provide an avenue of gene flow between regional nesting colonies, probably via opportunistic mating in migratory corridors. As a result, all breeding populations in the southeastern United States have similar levels of microsatellite diversity ($H_E = 0.70–0.89$), whereas mtDNA haplotype diversity varies dramatically ($h = 0.00–0.66$). Under a conventional interpretation of the nuclear DNA data, the entire southeastern United States would be regarded as a single management unit, yet the mtDNA data indicate multiple isolated populations. This complex population structure mandates a different management strategy at each life stage. Perturbations to pelagic juveniles will have a diffuse impact on Atlantic nesting colonies, mortality of subadults will have a more focused impact on nearby breeding populations, and disturbances to adults will have pinpoint impact on corresponding breeding populations. These findings demonstrate that surveys of multiple life stages are desirable to resolve management units in migratory marine species.

Keywords: dispersal, homing behaviour, marine turtles, microsatellite DNA, migratory behaviour, mitochondrial DNA

Received 9 November 2004; revision accepted 1 April 2005

Introduction

Isolated populations or management units (MUs: Moritz 1994) are typically characterized by genotype frequency shifts, as well as differences in key demographic features, including age structure, survivorship, fecundity, and sex ratio. These populations will prosper or perish without significant input from other populations, providing a compelling mandate for an independent management regime. Such populations are also the potential wellsprings of future biodiversity (geminate evolutionary units: Bowen 1998).

Prior to the availability of genetic assays, population resolution was accomplished primarily with mark–recapture studies, direct observation, or geographical inference. Allozyme and mtDNA assays provided more efficient means to resolve populations, but always with a gap between population structure in the genetic sense (which requires

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fewer than 10 effective migrants per generation: Wright 1931; Mills & Allendorf 1996) and population structure in the management sense (which can allow dozens of migrants without compromising demographic independence). Microsatellite surveys are beginning to close this gap, as multilocus assignment tests can resolve population members even under conditions of moderate gene flow (Rannala & Mountain 1997; Cornuet *et al*. 1999; Goudet *et al*. 2002).

In migratory animals, the resolution of populations can be confounded by two factors:

1 Geographic overlap, in which demographically independent populations mingle at feeding habitats or during migratory phases. Examples of population overlap can be found in birds (Wenink & Baker 1996; Wennerberg 2001), fishes (Grant *et al*. 1980; Wirgin *et al*. 1997), mammals (Baker *et al*. 1994, 1998), and reptiles (Bowen *et al*. 1996; Bolten *et al*. 1998). A critical question about population integrity is whether gametic exchange occurs during intervals of overlap.
2 Sex-biased dispersal, in which gene flow between populations is accomplished primarily by one gender. For many mammals and birds, males disperse prior to reproduction, while females are philopatric to natal area (Greenwood 1980; Blundell *et al*. 2002). In other species, the role of sex-biased dispersal may depend on scale. For example, spatially dependent sex-biased dispersal has a major impact on population structuring in the lake-dwelling brook charr, *Salvelinus fontinalis* (Fraser *et al*. 2004). For the purposes of this study, it is important to note that genetic exchange does not require dispersal of individuals between populations, but can occur when migratory populations overlap.

Cases of population overlap and sex-biased dispersal abound, and collectively may encompass a majority of migratory species. From a wildlife management perspective, stock integrity can ebb and flow on a seasonal basis, or at different life stages. The philopatry of females can be countered by opportunistic mating by males, so that each gender yields a different measure of genetic isolation. This is known as complex population structure (Bowen 1997; Kassahn *et al*. 2003), and the corresponding management implications have seldom been addressed.

In the last two decades, gender-specific genetic markers have been profitably applied to resolve sex-biased dispersal (Mossman & Wasser 1999; Scribner *et al*. 2001; Goudet *et al*. 2002; Wirgin *et al*. 2002; Arnaud-Haond *et al*. 2003). In pine trees, for example, the mitochondrial genome is maternally inherited (through seeds), the chloroplast genome is paternally inherited (through pollen), and the nuclear genome has the usual biparental (diploid) inheritance (Mogensen 1996). In bivalves of the genus *Mytilus*, males and females maintain distinct mtDNA lineages that are highly divergent (Stewart *et al*. 1995). In most mammals, male dispersal will be recorded in surveys of the sex (Y) chromosome, but will have no impact on the genetic architecture registered in maternally inherited mtDNA. For this reason, the Y chromosome has proven useful for tracking male-mediated dispersals of *Homo sapiens* (Zegura *et al*. 2004), while mtDNA has illuminated female dispersal (Bonatto & Salzano 1997). Genomes or chromosomes with different inheritance pathways will have different responses to dispersal, selection, lineage sorting, population crashes, and isolation events; therefore each class of genetic markers can provide unique insights (e.g. Allendorf & Seeb 2000; Buonaccorsi *et al*. 2001; Lu *et al*. 2001).

Loggerhead turtles (*Caretta caretta*) have both population overlap and sex-biased dispersal, and therefore may be especially valuable for dissecting the conservation implications of complex population structure. This migratory marine reptile has two distinct juvenile stages, the first being an oceanic stage after hatching (Carr 1987; Bolten 2003a). For post-hatchling turtles departing the nesting beaches of the northwestern Atlantic, this oceanic habitat extends from the Grand Banks (Newfoundland, Canada) to the Azores and Madeira, as well as the Mediterranean Sea (Bolten *et al*. 1998; Laurent *et al*. 1998; LaCasella *et al*. 2005). Older juveniles return to the shallow coastal waters of the northwest Atlantic, where they switch to benthic feeding and consume hard-shelled invertebrates (Bolten 2003b; Hopkins-Murphy *et al*. 2003). Upon reaching sexual maturity, female loggerheads make cyclic reproductive migrations to breed and nest in the vicinity of their natal beach (Bowen *et al*. 1993). Male loggerheads may make a similar migration to breeding areas near their natal beach (see FitzSimmons *et al*. 1997a, b).

In order to resolve the conservation implications of complex population structure, here we assemble loggerhead genetic data from three previously published surveys of North Atlantic populations, and an unpublished thesis. These studies include mtDNA data for oceanic juveniles (N = 455: Bolten *et al*. 1998; LaCasella *et al*. 2005), coastal subadults (N = 1437: Bowen *et al*. 2004), nesting females (N = 514: Encalada *et al*. 1998; Bowen *et al*. 2004), and nuclear DNA data (nDNA; microsatellite loci) for nesting females (N = 463: Pearce 2001). The primary lessons learned from mtDNA and microsatellite surveys of loggerhead turtles are (i) either genetic assay, taken in isolation, could lead to disastrously incorrect conclusions about the management of migratory species, and (ii) surveys of distinct life stages are desirable, and surveys of breeding populations are of paramount importance.

Methods

Sample collections were made in the interval 1989–2003. The nesting populations that are the primary focus of this

study are located in the southeast United States from North Carolina to the northern Gulf of Mexico (Fig. 1) plus an 'outgroup population' in Bahia, Brazil. Prior to the advent of polymerase chain reaction (PCR) methodology, samples from the nesting beaches consisted of whole eggs and moribund hatchlings. Subsequently, specimens from nesting beaches and feeding areas were collected as small blood aliquots (usually less than 1 mL) or tissue biopsy plugs. Rookery sample sizes range from $N = 11$ for the mtDNA survey of Bahia, Brazil, to $N = 123$ for the microsatellite survey of southeastern Florida (Table 1). Details of the sample collections and mtDNA analyses are available in Bowen *et al*. (1993, 1994, 2004) and Encalada *et al*. (1998). Corresponding information for feeding populations is available in Bolten *et al*. (1998), Bowen *et al*. (2004), and LaCasella *et al*. (2005).

In brief, a 391-base-pair (bp) fragment located in the control region of the mitochondrial genome was amplified with PCR methodology using primers in Table 2, as described by Allard *et al*. (1994) and Norman *et al*. (1994). Resulting sequences were assigned haplotype numbers based on the website maintained by the Archie Carr Center for Sea Turtle Research (http://accstr.ufl.edu/ccmtdna.html).

The rookery locations (Fig. 1) include the same groupings as in the mtDNA survey by Bowen *et al*. (2004) with two modifications: (i) the Georgia nesting colony is grouped with Jacksonville County in the northeastern corner of

Fig. 1 Map of surveyed nesting locations for the loggerhead turtle indicated as Florida northern Gulf (FL-NG), Florida southern Gulf (FL-SG), Dry Tortugas (FL-DT), Florida southern Atlantic (FL-SA), Florida Volusia County (Vo-FL), Florida northern Atlantic (FL-NA), Georgia (GA), South Carolina (SC) and North Carolina (NC). Subadult feeding populations range from Texas to the northeastern states (NE US). In the analyses of nesting populations, the FL-NA sample is combined with the adjacent GA sample, based on geographical proximity and extensive sharing of a single mtDNA haplotype.

Table 1 Sample sizes for nesting populations in the southeastern United States and Bahia, Brazil, as described in Bowen *et al*. (2004) for the mtDNA control region survey, and Pearce (2001) for the five microsatellite loci

Locus	FL-NG	FL-SG	FL-DT	FL-SA	Vo-FL	GA	SC	NC	BA
mtDNA	49	45	58	64	49	43	20	28	11
Dc107	42	46	23	123	42	51	24	26	80
Ccm2	42	46	23	123	45	51	24	27	81
Ccar176	42	46	23	123	43	51	24	26	81
Cc141	41	46	23	123	45	51	24	27	81
Cc7	41	46	23	122	45	51	24	26	81

Abbreviations: FL-NG, Florida Peninsula, northern Gulf of Mexico; FL-SG, Florida Peninsula, southern Gulf of Mexico; FL-DT, Dry Tortugas at the southern end of the Florida Keys; FL-SA, Florida Peninsula, southern Atlantic coast; Vo-FL, Volusia County, Florida (north of Cape Canaveral); GA, Georgia and adjacent Jacksonville County, FL (FL-NA in Fig. 1); SC, South Carolina; NC, North Carolina; BA, Bahia, Brazil.

Table 2 Primer sequences and annealing temperatures (T_a °C) for the mtDNA control region and the five microsatellite loci used to survey loggerhead nesting colonies (Norman *et al*. 1994; FitzSimmons 1998; Pearce 2001 and unpublished data from N. FitzSimmons and P. Dutton)

Locus	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)	T_a (°C)
mtDNA	TTGTACATCTACTTATTTACCAC	GTACGTACAAGTAAAACTACCGTATGCC	52
DC107	GTCACGGAAAGAGTGCCTGC	CAATTTGAGGTTATAGACC	55
CCM2	TGGCACTGGTGGATT	TGACTCCCAAATACTGCT	58
Ccar176	GGCTGGGTGTCCATAAAAGA	TTGATGCAGGAGTCACCAAG	60
CC141	CAGCAGGCTGTCAGTTCTCCAC	TAGTACGTCTGGCCTGACTTT	56
CC7	TGCATTGCTTGACCAATT AGTGAG	ACATGTATAGTTGAGGAGCAAGTG	56

Florida. With the exception of one individual, these adjacent locations have the same haplotype at 100% frequency. (ii) The nesting population in Volusia County, Florida (Vo-FL in Fig. 1) is added as a distinct category. Previously there has been some question about whether this area comprises a distinct management unit, or whether it represents an area of overlap between nesting colonies to the north and south (Encalada *et al*. 1998). These two modifications make the groupings for mtDNA analysis concordant with the groupings for microsatellite analysis.

The mtDNA diversity among populations was measured with an analysis of molecular variance (AMOVA) as implemented in ARLEQUIN version 2.000 (Schneider *et al*. 2000). The same software package was used to estimate haplotype diversity and nucleotide diversity (Nei 1987; Excoffier & Slatkin 1995). In all tests that required estimates of sequence divergence, we used the Tamura–Nei model of nucleotide substitutions, which was designed for control region sequences (Tamura & Nei 1993).

The microsatellite data include two loci (CC7 and CC141) developed for loggerhead turtles by FitzSimmons (1998; personal communication), one locus (Ccar176) developed for loggerhead turtles by Moore & Ball (2002), and one locus (DC107) developed for leatherback turtles (P. Dutton, personal communication). One additional locus (CCM2) was developed specifically for this study (Pearce 2001). In brief, genomic DNA was digested with *Sau*3AI and fragments in the size range of 400–1500 bp were ligated to *Sau*3AI linkers and purified with a QIAquick Gel Extraction Kit (Qiagen). Fragments containing microsatellite loci were identified by hybridization with biotinylated probes containing a CA_5 repeat. Enriched DNA fragments were amplified with *Sau3AI* primer and cloned with the TOPO-TA Cloning Kit (Invitrogen). Clones were screened again by hybridization with a CA probe, and 25 plasmids that tested positive were sequenced. Primers were designed for the flanking regions of 10 candidate loci using the software package OLIGO Primer Analysis (Molecular Biology Insights). However, 9 of these 10 loci were either monomorphic in initial screening, or would not amplify consistently, leaving only one additional locus to add to the four previously characterized loci (see Table 2).

Polymerase chain reactions (PCR) for the microsatellites included an initial denaturation step at 93 °C for 3 min, followed by six cycles of denaturation at 92 °C for 30 s, annealing at 55–60 °C for 55 s (see Table 2 for primer-specific annealing temperatures), and extension at 72 °C for 1 min 25 s, followed by 30 cycles of denaturation at 89 °C for 30 s, annealing at 2 °C lower than previous cycle for that primer pair, and extension at 72 °C for 1 min 25 s, finishing with extension at 72 °C for 10 min.

Estimates of gene flow calculated from Wright's hierarchical *F*-statistics, or Weir & Cockerham's (1984) comparable estimators, should be robust to unmeasured selection if allele frequencies do not deviate significantly from those expected under a neutral model (Slatkin & Barton 1989). Therefore, we tested each microsatellite locus for significant deviations from neutral expectations across all individuals sampled, using Slatkin's exact test program, ENUMERATE (Slatkin 1997). For mtDNA sequence data, we tested for significant deviations from neutral expectations using both the infinite-alleles and infinite-sites approaches as implemented in ARLEQUIN 2.000 (Schneider *et al*. 2000).

Allelic data was converted to input format for data analysis programs using the MS toolkit (Park 2001) macro for Excel. We tested conformity to Hardy–Weinberg (HW) expectations via Markov chain permutation (Guo & Thompson 1992) as implemented in ARLEQUIN. Independent assortment of microsatellite loci was also tested in ARLEQUIN, using a likelihood-ratio test (Slatkin & Excoffier 1996) against an empirical distribution obtained by permutation. Finally, we used ARLEQUIN to perform exact tests of population differentiation (Raymond & Rousset 1995; Goudet *et al*. 1996). We used a chain length of 500 000 steps, with a 10 000 step dememorization, for all permutation tests.

Tests for population subdivision based on microsatellites were made using both the infinite allele model (IAM) and the stepwise-mutation model (SMM). We calculated Weir & Cockerham's (1984) unbiased estimator of Wright's F_{ST} (θ) on a locus-by-locus basis using the Tools for Population Genetic Analyses (TFPGA, version 1.3) software (Miller 1997). We used RSTCALC (version 2.2; Goodman 1997) to calculate Goodman's ρ, an unbiased estimator of Slatkin's R_{ST} (Slatkin 1995). Throughout the manuscript where we refer to either F_{ST} or R_{ST} we are reporting these unbiased estimators (θ and ρ).

Estimates of gene flow based on F_{ST} and its analogues rely on the island model assumptions of equal population sizes and symmetric migration rates; however, these assumptions rarely hold in natural populations (Whitlock & McCauley 1999). Therefore we estimated gene flow using a maximum-likelihood method based on a coalescent approach (Beerli & Felsenstein 1999) implemented in MIGRATE version 1.7.6.1. (Beerli 2002). For all analyses, the default settings of MIGRATE were used except that the number of short and long Markov chains and the number of trees sampled were increased (20 short chains sampling 10 000 trees and five long chains sampling 100 000 trees, following an initial 'burn-in' period of 10 000 trees). Analyses involving microsatellite loci were performed under the 'allele model' rather than the 'microsatellite model' because allele frequency distributions of these loci did not fit strict expectations of the SMM. Because convergence problems are common with Markov chain estimations, we performed each analysis three times, and the values presented herein are the mean of three replicate runs.

Indirect estimates of migration rate (*Nm*) based on F_{ST} are less prone to bias and more conservative than R_{ST}-based

Table 3 Genetic diversity indices for nesting populations in the southeastern United States and Bahia, Brazil, as described in Bowen *et al*. (2004) for the mtDNA control region survey, and Pearce (2001) for five microsatellite loci. Location abbreviations are defined in Table 1

Nesting beach	mtDNA *h*	DC107 H_E/H_O	CCM2 H_E/H_O	Ccar176 H_E/H_O	CC141 H_E/H_O	CC7 H_E/H_O
FL-NG	0.383	0.796	0.700	0.868	0.874	0.782
		0.857	0.691	0.881	0.756	0.902
FL-SG	0.664	0.800	0.728	0.775	0.879	0.788
		0.804	0.804	0.739	0.891	0.696
FL-DT	0.254	0.777	0.783	0.752	0.886	0.820
		0.696	0.696	0.609	0.739*	0.739
FL-SA	0.567	0.778	0.768	0.816	0.872	0.820
		0.797	0.756	0.764	0.878	0.781
Vo-FL	0.511	0.812	0.787	0.814	0.852	0.787
		0.762	0.800	0.767	0.778	0.667
GA	0.035	0.784	0.745	0.846	0.867	0.844
		0.804	0.745	0.902	0.784	0.863
SC	0.000	0.790	0.719	0.803	0.858	0.804
		0.750	0.750	0.750	0.833	0.875
NC	0.000	0.796	0.775	0.814	0.861	0.773
		0.769	0.704	0.923	0.741	0.769
BA	0.000	0.744	0.625	0.782	0.680	0.582
		0.761	0.587	0.804	0.674	0.478

*Indicates the only significant departure from Hardy–Weinberg equilibrium, based on exact tests ($P < 0.05$) prior to Bonferroni correction.

estimates for moderate sample sizes (50 or less) and numbers of loci (fewer than 20) (Gaggiotti *et al*. 1999). Furthermore, the microsatellites used herein do not provide a good fit for the SMM. Therefore, we have only used estimates of *Nm* derived from F_{ST} for subsequent analyses. Mantel tests comparing gene flow estimates based on coalescent and F_{ST}-based approaches were run using GENALEX version 5.1 (Peakall & Smouse 2001).

Results

The mtDNA results are previously published, and details are available in Bowen *et al*. (1993, 1994, 2004), Bolten *et al*. (1998), Encalada *et al*. (1998) and LaCasella *et al*. (2005). Haplotype and nuclear DNA diversities are detailed in Tables 3 and 4.

The distribution of mtDNA haplotypes indicates three levels of population structure, corresponding to three life stages (Fig. 2). First, based on our re-analysis of the data in Bolten *et al*. (1998) and LaCasella *et al*. (2005), the pelagic juvenile populations that inhabit the eastern Atlantic (Azores and Madeira, $N = 131$) and western-central Atlantic (Grand Banks to Azores, $N = 324$) are not significantly different ($\phi_{ST} < 0.001$; $P = 0.919$). Second, the subadults that feed along the coast of North America ($N = 1437$) have low but significant population structure ($\phi_{ST} = 0.0088$, $P = 0.016$). Third, the nesting colonies of the west Atlantic are highly structured, with $\phi_{ST} = 0.428$ ($P < 0.001$) for the nine sample locations in Table 1, or $\phi_{ST} = 0.420$ ($P < 0.001$) for the eight locations in the southeastern US. Volusia County (Vo-FL), the area of possible overlap between nesting colonies to the north and south, is significantly different from Georgia and adjacent Jacksonville County (GA; $\phi_{ST} = 0.306$; $P < 0.001$), but is not significantly different from the rookery to the south (FL-SA; $\phi_{ST} = 0.014$; $P = 0.178$).

Table 4 Summary statistics for the five microsatellite loci used to survey loggerhead turtle nesting colonies

Locus	Size	*K*	H_E	H_O	*P*
DC107	158–186	11	0.779	0.787	0.33
CCM2	169–195	10	0.750	0.733	0.08
Ccar176	117–181	29	0.812	0.795	0.84
CC141	186–220	16	0.868	0.805	0.18
CC7	209–247	18	0.793	0.751	0.11
Mean		16.8	0.800	0.774	0.31

Size, allele size in bp; *K*, number of alleles; H_E, mean expected heterozygosity per locus; and H_O, mean observed heterozygosity per locus. *P*, overall fit to Hardy–Weinberg expectations, based on an exact test.

All five microsatellite loci showed high levels of polymorphism with 10–29 alleles (Tables 3 and 4), and mean expected heterozygosities per location ranged from $H_E = 0.582$ (Bahia) to $H_E = 0.886$ (FL-Dry Tortugas). Observed heterozygosities per locus were also high, ranging from $H_O = 0.478$ (CC7 in Bahia) to $H_O = 0.923$ (Ccar176 in North Carolina) (Table 3). All alleles were distinguished by increments of 2 bp, in keeping with the CA motif of the five loci. With a single exception, all five loci fit expectations for

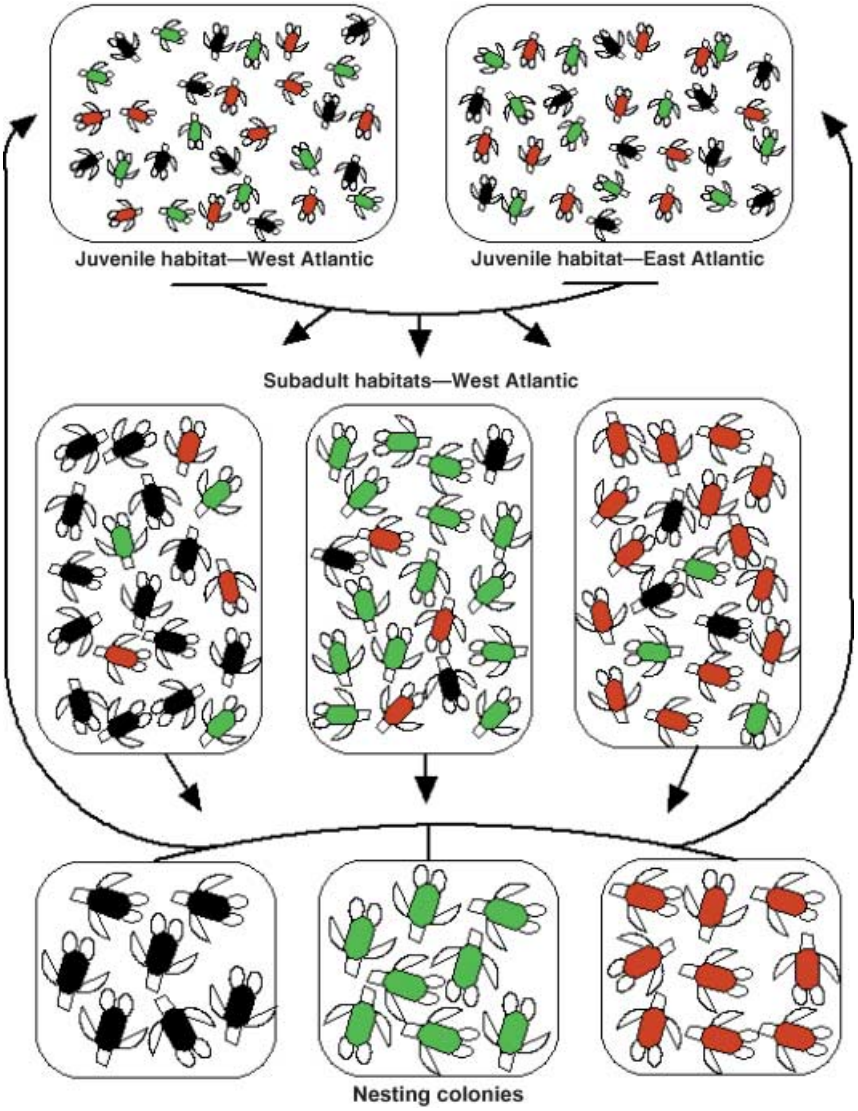

Fig. 2 A model of loggerhead population structure in the North Atlantic, using three hypothetical rookeries designated by red, green and black icons. The mtDNA data indicate a stepwise increase in population structure through juvenile, subadult, and adult stages. In the juvenile stage, turtles from all three rookeries intermingle, and no population structure is apparent between eastern and western edges of the North Atlantic Gyre. In the subadult stage, turtles tend to recruit to neritic feeding habitat in the vicinity of their natal rookery, inducing low but significant population structure. In the adult turtles, females (and possibly males) have high site fidelity to breeding/nesting habitat, inducing strong population structure.

Hardy–Weinberg equilibrium at all locations (Table 3), and none of the loci had significant departures after Bonferroni correction for multiple tests. There was only one significant case of linkage disequilibrium among any of the loci, and again that comparison is not significant after Bonferroni correction for multiple tests. Likewise, none of the five microsatellite loci showed any significant deviations from the Ewen's sampling distribution ($0.1 > P < 0.9$).

With five exceptions involving the Georgia (GA) nesting colony, all pairwise comparisons based on microsatellites across the eight nesting populations in the southeast United States are nonsignificant; after Bonferroni correction for multiple tests, only northwest Florida (FL-NG) and Georgia populations are significantly different (Table 5). All eight comparisons to Brazil remain significant after Bonferroni correction, for a total of 9 of 36 significant pairwise comparisons (Table 5). This contrasts sharply with the mtDNA surveys across the same nine locations in which 29 of 36 pairwise comparisons are significant after Bonferroni correction (Table 5). For the entire data set, $R_{ST} = 0.009$ ($P < 0.01$) and $F_{ST} = 0.01$ ($P < 0.001$) compared to mtDNA $\phi_{ST} = 0.428$ ($P < 0.001$). For the southeastern United States, $R_{ST} < 0.001$ ($P > 0.5$) and $F_{ST} = 0.002$ ($P = 0.07$) compared to mtDNA $\phi_{ST} = 0.420$ ($P < 0.001$).

Migration rates (Nm) based on microsatellites were significantly higher than those based on mtDNA sequence data (paired t-test, d.f. = 107, $P < 0.05$; Table 6). Furthermore, estimates of gene flow derived from an F_{ST}-based approach showed no correlation with those derived from a coalescent-based approach for either microsatellite (Mantel $r^2 = 0.003$; $P = 0.46$), or mtDNA sequence data (Mantel $r^2 = 0.007$; $P = 0.25$).

Table 5 Genetic partitions among eight nesting populations in the southeast United States plus Bahia, Brazil. Above the diagonal are partitions based on five microsatellite loci (F_{ST} values), below the diagonal are partitions based on mtDNA sequence comparisons (ϕ_{ST} values) from Bowen *et al*. (2004) with additional unpublished data. On the diagonal are nucleotide diversity values (π values, in bold) for each nesting population. Significant values ($P < 0.05$) based on permutation tests are indicated with asterisks. Abbreviations are defined Table 1

	FL-NG	FL-SG	FL-DT	FL-SA	Vo-FL	GA	SC	NC	BA
FL-NG	**0.0192**	0.0052	0.0057	0.0024	0.0015	0.0167*	0.0008	−0.0022	0.0398*
FL-SG	0.1645*	**0.0276**	0.0007	−0.0001	−0.0001	0.0049	−0.0032	−0.0044	0.0521*
FL-DT	0.6682*	0.3252*	**0.0073**	−0.0011	0.0068	0.0072	0.0010	−0.0049	0.0455*
FL-SA	0.1050*	−0.0100	0.3787*	**0.0268**	−0.0009	0.0052*	0.0004	−0.0039	0.0514*
Vo-FL	0.0151	0.0491	0.5338*	0.0135	**0.0247**	0.0064*	0.0001	−0.0001	0.0622*
GA	0.1747*	0.5123*	0.9039*	0.4123*	0.3060*	**0.0018**	0.0100*	0.0072*	0.0827*
SC	0.1372*	0.4272*	0.8910*	0.3479*	0.2436*	−0.0225	**0.0000**	−0.0017	0.0385*
NC	0.1592*	0.4601*	0.9004*	0.3734*	0.2711*	−0.0138	0.0000	**0.0000**	0.0461*
BA	0.2171*	0.3542*	0.8612*	0.2974*	0.2356*	0.7667*	1.0000*	1.0000*	**0.0000**

Table 6 Migration estimates based on either microsatellite alleles or mtDNA haplotypes among each of the nine sampling sites defined in Table 1. Estimates of migration from columns (source) to rows (recipient) are based on a coalescent approach using the computer program MIGRATE. Values are the mean of three replicates (see text)

Microsatellite-based migration estimates

	FL-NG	FL-SG	FL-DT	FL-SA	Vo-FL	GA	SC	NC	BA
FL-NG	—	2.206	1.839	5.337	2.366	2.430	2.606	2.374	4.643
FL-SG	2.279	—	1.425	7.115	2.907	2.060	1.542	2.321	2.261
FL-DT	1.578	1.674	—	3.628	1.844	2.787	0.455	1.972	2.657
FL-SA	3.004	6.629	1.582	—	5.315	3.489	3.095	3.456	7.808
Vo-FL	1.192	2.047	1.992	4.889	—	1.412	2.202	1.286	3.142
GA	1.274	1.486	0.962	4.321	1.657	—	2.646	2.303	4.095
SC	1.665	1.496	0.696	2.682	2.451	1.518	—	2.268	1.150
NC	4.289	1.023	0.855	8.312	1.697	3.132	3.998	—	6.396
BA	3.211	0.922	1.979	5.701	3.139	4.006	1.787	1.650	—

mtDNA-based migration estimates

	FL-NG	FL-SG	FL-DT	FL-SA	Vo-FL	GA	SC	NC	BA
FL-NG	—	0.767	0.548	< 0.001	< 0.001	0.110	< 0.001	< 0.001	< 0.001
FL-SG	0.526	—	< 0.001	1.579	9.976	< 0.001	< 0.001	< 0.001	< 0.001
FL-DT	< 0.001	< 0.001	—	< 0.001	1.790	< 0.001	< 0.001	< 0.001	0.217
FL-SA	4.583	< 0.001	< 0.001	—	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Vo-FL	< 0.001	< 0.001	2.648	< 0.001	—	0.681	3.606	< 0.001	< 0.001
GA	< 0.001	< 0.001	< 0.001	0.610	0.081	—	2.511	1.090	< 0.001
SC	< 0.001	< 0.001	< 0.001	0.039	< 0.001	1.975	—	< 0.001	< 0.001
NC	0.006	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.029	—	< 0.001
BA	< 0.001	< 0.001	0.037	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	—

Discussion

Migratory marine animals often have complex population structure, which can result from overlap during migrations or sex-biased dispersal. In global surveys of the sperm whale (*Physeter macrocephalus*), significant population structure is recorded in mtDNA sequence comparisons ($G_{ST} = 0.03$, $P < 0.001$: Lyrholm & Gyllensten 1998), but not in microsatellite comparisons ($G_{ST} = 0.001$, $P = 0.232$: Lyrholm *et al*. 1999). A similar pattern is apparent in humpback whales (*Megaptera novaengliae*: Baker *et al*. 1994, 1998; Palumbi & Baker 1994), bottlenose dolphins (*Terciops aduncus*: Möller & Beheregaray 2004), Dall's porpoises (*Phocoenoides dalli*: Escorza-Trevino & Dizon 2000), shortfin mako shark (*Isurus oxyrinchus*: Schrey & Heist 2003) and Patagonian toothfish (*Dissostichus eleginoides*: Shaw *et al*. 2004). In a

survey of white sharks (*Carcharodon carcharias*), the mtDNA data indicated strong population structure (F_{ST} = 0.81 between South Africa and Australia) while a microsatellite survey reveals no significant structure (Pardini *et al*. 2001). For these cases, male-mediated gene flow can readily explain the lower population structure registered in nuclear markers relative to mitochondrial markers.

In the first global genetic survey of a sea turtle (green turtle; *Chelonia mydas*), Karl *et al*. (1992) reported low population structure in single copy nuclear DNA (Atlantic F_{ST} = 0.130, Indo-Pacific F_{ST} = 0.126), relative to a parallel survey of mtDNA (Atlantic G_{ST} = 0.63, Indo-Pacific G_{ST} = 0.71; Bowen *et al*. 1992). This finding is confirmed with microsatellite surveys across the same range (Atlantic F_{ST} = 0.038, Indo-Pacific F_{ST} = 0.024: for a detailed analysis of each class of markers see Roberts *et al*. 2004). The conclusion of male-mediated gene flow in *Chelonia mydas* is supported by comparative surveys of mtDNA and microsatellites in the West Pacific and the Indian Ocean (FitzSimmons *et al*. 1997b). Notably, male-mediated gene flow occurs even though males are homing to breeding areas adjacent to the nesting habitat (FitzSimmons *et al*. 1997a).

In every case that has been published to date, sea turtles register lower population genetic structure in nDNA assays relative to mtDNA (FitzSimmons *et al*. 1996; Schroth *et al*. 1996). Part of this pattern can be explained by the haploid inheritance of mtDNA, imparting a fourfold lower effective population size relative to diploid nuclear loci (Birky *et al*. 1983). Under these circumstances, population differentiation by genetic drift will be more rapid in mtDNA than in nDNA loci. However, other factors must be invoked when measures of population divergence differ by an order of magnitude (Roberts *et al*. 2004). This trend reaches an extreme in the comparison of loggerhead nesting colonies of the northwestern Atlantic: population structure is high in mtDNA surveys (ϕ_{ST} = 0.420, $P < 0.001$) but is not significant with microsatellites (F_{ST} = 0.002, P = 0.07; $R_{ST} < 0.001$, $P > 0.5$).

Loggerhead life history and population genetics

Hatchling loggerhead turtles leave the nesting beaches of the northwest Atlantic and subsequently occupy oceanic (pelagic) habitats across the North Atlantic and the Mediterranean Sea. Casale *et al*. (2002) reported evidence of male-biased dispersal of Atlantic juveniles into the Mediterranean, and Chaloupka & Limpus (2002) also reported greater dispersal of juvenile males in the southwest Pacific. However, the North Atlantic juveniles appear to be a homogenous mixture of turtles from source populations, with no significant differences in haplotype composition between the western-central and eastern Atlantic ($\phi_{ST} < 0.001$, P = 0.919, based on re-analysis of the data in Bolten *et al*. 1998 and LaCasella *et al*. 2005). Contributions to this pool of juveniles are roughly proportional to the size of source (nesting) populations (Bolten *et al*. 1998; LaCasella *et al*. 2005).

After an oceanic phase that can last a decade or more (Bjorndal *et al*. 2000), pelagic juvenile turtles switch to shallow (neritic) habitats along the continental coastline of North America (although this switch is not immutable, as subadults and adults can switch back to pelagic feeding: Hatase *et al*. 2002b; Witzell 2002). Therefore the transition from juvenile to subadult phase can involve a trans-oceanic migration (Bowen *et al*. 1995; Bolten *et al*. 1998), and recruitment to the same coastline that hosts the familial nesting beaches (Bolten 2003b). At this stage, subadult turtles are not uniformly distributed along the eastern coast of North America (ϕ_{ST} = 0.0088, $P < 0.005$), and haplotype frequency differences are significantly correlated between coastal feeding populations and adjacent nesting populations (Mantel test R^2 = 0.52, P = 0.001; Bowen *et al*. 2004). Hence genetic data indicate that the subadult populations are not a random mix, but are homing to their region of origin, a conclusion supported indirectly by tag recapture data (Avens *et al*. 2003). Homing of subadults (ϕ_{ST} = 0.0088, $P <$ 0.005) is less precise than that of breeding adults (ϕ_{ST} = 0.420, $P < 0.001$), as indicated by the occurrence of subadult turtles far outside the range of nesting habitat (Ehrhart *et al*. 2003; Hopkins-Murphy *et al*. 2003). Nonetheless, this behaviour places subadult turtles at elevated frequencies in the vicinity of their natal nesting colonies.

Additional mtDNA studies indicate that contributions to subadult habitats are influenced by the size of regional source (nesting) populations (Norrgard & Graves 1996; Rankin-Baransky *et al*. 2001; Engstrom *et al*. 2002; Witzell *et al*. 2002; Bass *et al*. 2004). The large rookery in southern Florida contributes most of the subadult turtles feeding along this coast, with additional contributions from the rookeries at the Yucatan Peninsula, Dry Tortugas, Gulf of Mexico, and the Atlantic states of Georgia, South Carolina, and North Carolina. While the composition of juvenile pelagic aggregates in the North Atlantic can be explained by the size of source (nesting) populations, the composition of subadult feeding aggregates is guided by at least two influences: the size of source populations and proximity to these source populations.

After a decade (or more) in subadult habitat, the turtles switch to adult habitats, which are largely unknown but suspected to include the Caribbean Basin. As a consequence of natal homing behaviour of loggerhead females, most nesting populations are distinguished by differences in the frequency of mtDNA haplotypes (Encalada *et al*. 1998; Hatase *et al*. 2002a; Bowen 2003).

Loggerhead turtles have an elaborate life history, reviewed in detail by Bolten & Witherington (2003). For the purposes of this treatment, two additional points bear consideration. First, marine turtles do not have differentiated sex chromosomes, and the sex of emerging hatchlings is

under hormonal control (Owens 1997; Carthy *et al*. 2003). Temperature-dependent sex determination is widespread in turtles, and in this case warmer incubation temperatures produce females, and lower temperatures produce males (Mrosovsky 1994). Second, loggerhead turtles exhibit polyandry, with 31% of nests in Florida having multiple sires (Moore & Ball 2002).

Population overlap in loggerhead turtles

The mtDNA surveys indicate no genetic structure among juvenile (oceanic) populations, low genetic structure among subadult (coastal) populations, and high genetic structure among adult (breeding) populations (Fig. 2).

Three lessons are apparent:

1 Genetic surveys of migratory species on feeding grounds or migratory corridors may be misleading. Loggerhead sea turtles on feeding grounds are mixed aggregates including cohorts from several breeding populations. The same conditions may apply to widely distributed fishes, including migratory sharks, billfishes, and tunas. Surveys of migratory stages are valuable in the context of global phylogeography and mixed-stock analysis, but may miss the fine-scale population structure that is crucial for defining management units (Carlsson *et al*. 2004; Hueter *et al*. 2004).

2 Different management regimes are appropriate at different life stages. For loggerhead turtles, disturbances to the juvenile populations will have a diffuse impact on nesting colonies across the North Atlantic (and possibly the Mediterranean). The large FL-SA population, with perhaps 70% of the nesting effort in this region, will absorb 70% of the disturbances to juvenile populations. The primary threat at this stage may be longline fisheries, which result in thousands of mortalities every year (Witzell 1999; Lewison *et al*. 2004). The primary regulatory bodies are international agencies including the International Commission for the Conservation of Atlantic Tunas (ICCAT) and the United Nations.

In contrast, disturbance to the subadult populations will have a more direct impact on nearby nesting colonies. The primary threats here are shrimp fisheries, dredging, and pollution (Henwood & Stuntz 1987; Studt 1987; Bjorndal *et al*. 1994; Slay 1995; Sakai *et al*. 2000; Storelli & Marcotrigiano 2003), and the primary regulatory bodies are those agencies responsible for coastal resources, such as the US National Marine Fisheries Service. Perhaps half of the subadults feed near their natal rookery, so that disturbances here will have a strong impact on local nesting populations. This must elevate the conservation priorities for habitat in the vicinity of small and dwindling nesting colonies.

Finally, disturbance to the nesting females will yield specific damage to the corresponding reproductive population. The primary threats are shrimp trawling, beach disturbance, erosion, and construction. The primary regulatory bodies are municipal, state and federal agencies. Clearly at each life history stage there are different threats, different responsibilities, and different prospects.

3 Ecosystem-based protection is not sufficient to manage migratory marine species. One of the most promising advances in marine conservation is the development of marine protected areas (MPAs) on an ecosystem scale (Norse & Crowder 2004; Sobel & Dahlgren 2004). Recent field studies have confirmed the efficacy of MPAs for these ecological goals (Halpern & Warner 2002; Friedlander *et al*. 2003; Halpern 2003), but do not fully address the needs of migratory species. The genetic surveys of juvenile loggerhead turtles confirm suspected links between nesting colonies in the northwest Atlantic and distant feeding populations in the northeast Atlantic and Mediterranean Sea (Carr 1987; Bolten *et al*. 1998; Laurent *et al*. 1998; LaCasella *et al*. 2004). These ocean-wide connections raise doubts about protecting specific ecosystems as a comprehensive management option for loggerhead turtles and other migratory species. For an animal that begins life on a Florida beach, feeds in the east Atlantic and Mediterranean as a juvenile, forages in Florida as a subadult, and occupies the Caribbean Sea as an adult, what ecosystem protection will suffice?

In the case of migratory species, the solution is not ecosystem protection alone, but taxon-specific protection of vulnerable life stages (Bowen & Roman 2005). The specific management strategy will depend on the idiosyncratic life histories of the target species. In sea turtles this clearly includes nesting beaches and juvenile-feeding habitat, for whales it must include the calving grounds in sheltered coastal areas, and in marine fishes it will include spawning aggregates and coastal nurseries.

Sex-biased gene flow in loggerhead turtles

The microsatellite survey indicates no population structure among nesting colonies of the southeastern United States. Taken alone, these data would mandate that regional nesting colonies are a single management unit. Yet the surveys of mtDNA indicate strong population structure among nesting colonies. Two lessons can be drawn from these data:

1 Concerns about inbreeding and corresponding loss of genetic diversity are alleviated for the smaller nesting colonies in the southeastern United States. A key feature of loggerhead population structure is the differences in diversity indices for nesting populations. The mtDNA diversity varies tremendously among nesting colonies of the southeastern United States (h = 0.000–0.664) while the corresponding measures for microsatellites are uniformly high (H_E = 0.700–0.886; Table 3). There is considerable debate about what measures of genetic diversity are

relevant for 'healthy' populations (Lande & Shannon 1996; DeWoody & DeWoody 2005), but it is clear from the microsatellite survey that the smallest and the largest nesting colonies in the southeastern United States have comparable levels of nuclear DNA diversity.

In organisms with chromosomal sex determination, the reduced diversity in mtDNA could carry corresponding expectations for heterogametic (sex-specific) chromosomes. This concern does not apply to sea turtles, which have hormonal (temperature-dependant) sex determination. Furthermore, multiple paternity in egg clutches should retard the loss of diversity by genetic drift. Collectively these factors reduce management concerns about the genetic health of small nesting populations in the southeast United States.

2 Male-mediated gene flow does not detract from the classification of breeding areas as independent populations. For this point, it is helpful to consider the extremes of gender-specific extirpation. What would happen if all the males were eliminated from the breeding habitat adjacent to the nesting beach? The nesting population would continue, because some of the females were inseminated on feeding grounds or migratory corridors, before arriving at the breeding/nesting habitat. In contrast, what would happen if the females were eliminated? The nesting population would be extinct. Females are the essential vessels that transmit the threads of life from generation to generation (Avise 1995). Their nesting site fidelity defines reproductive populations, regardless of male behaviour.

Perhaps the most valuable lesson from this and similar studies is that either nDNA surveys (allozymes, microsatellites, etc.) or mtDNA surveys, taken in isolation, can be positively misleading for migratory species with complex population structure (e.g. Castella *et al*. 2001; Scribner *et al*. 2001; Gay *et al*. 2004). A management plan based on nDNA data alone would indicate a single management unit for nesting colonies of loggerhead turtles of the southeastern United States, a disastrous premise. The mtDNA surveys, if applied at the junctions where populations overlap on feeding or migratory areas, would likewise yield a misleading picture. Genetic surveys of juvenile turtles, taken alone, would indicate a single panmictic population, obscuring the true structure of subadults and nesting adults. Therefore it is desirable to survey multiple life stages to uncover cryptic aspects of population structure (Toonen 2001). Many of the genetic surveys of tunas, billfishes, and sharks are based on adults sampled on feeding habitat, and many of these surveys indicate very low population structure. These surveys are valuable for resolving range-wide population structure and for illuminating cryptic evolutionary partitions. However, when the same species are surveyed in nursery habitats, fine-scale population structure can emerge (Carlsson *et al*. 2004; Hueter *et al*. 2004). To define populations and management units of migratory marine animals, an optimal strategy would be to survey all life stages. It is especially important to survey at the source: the breeding/nesting habitat in the case of sea turtles, the calving grounds in the case of whales, or the spawning/nursery habitat in the case of fishes. Only at these locations will the essential population structure be revealed.

Acknowledgements

This study was made possible by outstanding contributions from K.A. Bjorndal, A.B. Bolten, S.-M. Chow, P. Dutton, S. Epperly, S.A. Karl, R. Klinger, E. LaCasella, G. Marcovaldi and M. Marcovaldi. The microsatellite data were produced by A. Pearce. For advice and assistance we thank E. Almira, D. Amorocho, J. Avise, D. Bagley, S. Baker, G. Balazs, A. Ball, J. Becker, M. Bostrom, M. Camhi, R. Carthy, P. Castaneda, M. Chaloupka, R. Chapman, C. Coogan, M. Courtney, R. Cowie, K. Diemer, A. Dizon, M. Dodd, K. Dodge, E. Dunlavey, L. Ehrhart, S. Encalada, L. Fisher, N. FitzSimmons, A. Foley, J. Foote, J. Frazier, M. Godfrey, S. Grant, M. Harris, S. Hopkins-Murphy, A. Huff, L. Iocco, S. Johnson, N. Kamezaki, M. Lamont, B. Liebert, A. Meylan, R. Mezich, J. Musick, L. Ogren, T. Okuyama, L. Parris, F. Percival, E. Possardt, N. Price, R. Reardon, S. Reynolds, J. Richardson, N. Richardson, J. Roman, S. Shanker, P. Taylor, W. Teas, J. Thome, W. Witzell, S. Woodley-Miller, M. Zacks, M. Zatcoff, and J. Zurita. For logistic support we thank the Archie Carr Center for Sea Turtle Research, Broward County Environmental Quality Control Board, Florida Department of Natural Resources, Florida Cooperative Fish and Wildlife Research Unit, Georgia Department of Natural Resources, NOAA-Fisheries Molecular Genetics Laboratory, Marine Mammal Stranding Center (New Jersey, USA), Maryland Department of Natural Resources, Massachusetts Audubon Wellfleet Bay Wildlife Sanctuary, the Marine Education, Research, and Rehabilitation Institute (Delaware, USA), North Carolina Department of Environment and Natural Resources, Projeto Tartaruga Marinha (TAMAR; Brazil), Riverhead Foundation for Marine Research and Preservation (New York, USA), South Carolina Department of Natural Resources, the Volusia County Turtle Patrol (Florida, USA), the DNA Sequencing Core at the University of Florida, and the US National Park Service. We thank S. White for drafting Fig. 1. For valuable critiques we thank Louis Bernatchez, two anonymous reviewers, and the Zoology Department Journal Club at UH Manoa. Research was funded by the US National Marine Fisheries Service, US National Science Foundation, US Fish and Wildlife Service, National Geographic Society, Turner Foundation, and private contributions. This is contribution #1192 from the Hawaii Institute of Marine Biology, and contribution #6536 from SOEST.

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