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POPULATION GENETIC STRUCTURE OF COASTAL BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN THE NORTHERN BAHAMAS

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ABSTRACT

Population substructure has important implications for a species' ecology and evolution. As such, knowledge of this structuring is critical for the conservation and management of natural populations. Among marine mammals, many examples exist of species that enjoy a broad geographical distribution, yet are characterized by fine-scale population subdivisions. Coastal bottlenose dolphins have been studied extensively in a few regions globally, and these studies have highlighted a great diversity in both social strategies and demographic isolation. Here we use molecular genetic markers to examine the degree of population subdivision among three study sites separated by less than 250 km on Little Bahama Bank in the northern Bahamas. Mitochondrial DNA (mtDNA) sequence variation and microsatellite

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genotypes were used to assess partitioning of genetic variance among 56 individually recognized coastal ecotype bottlenose dolphins. Although resolved levels of genetic differentiation suggest gene flow among the three study sites, both nuclear and mitochondrial data indicate a significant degree of subdivision within the Little Bahama Bank population, and sex-based analyses suggest that patterns of dispersal may not be strictly biased toward males. These results corroborate the site fidelity documented through long-term photo-identification studies in the NE Bahamas, and highlight the need to consider independent subpopulation units for the conservation and management of coastal bottlenose dolphins in the Bahamas.

Key words: bottlenose dolphin, *Tursiops truncatus*, population structure, mammal, mtDNA, microsatellites, sex-biased dispersal.

The structuring of species into smaller constituent population units is an important factor that shapes many key demographic and evolutionary processes. As such, characterizing intraspecific population structuring not only provides fundamental information on the ecology and evolution of a species, but also yields information critical to the identification of biologically relevant management or conservation units.

Studies of population genetic structure among marine species have revealed a surprising degree of differentiation despite the lack of obvious physical population boundaries (*e.g.*, Encalada *et al*. 1996, Tolley *et al*. 2001, Ovenden *et al*. 2004, McMillen-Jackson *et al*. 2005). Many cetacean species are particularly inaccessible to field-based, direct examination of geographic structuring, owing to the nature and scale of the marine environment. These logistical constraints are further confounded by long generation times and a lack of understanding of the barriers to gene flow encountered by these highly vagile animals in a seemingly continuous marine habitat (Rosel *et al*. 1995, Avise 1998). Indirect assessment of population structure, according to the partitioning of genetic variation, offers an alternative approach to examining intraspecific population subdivision and patterns of dispersal in marine systems. Consequently, molecular genetic analysis has become an important tool for assessing the levels of population structuring among both coastal and oceanic cetacean species (Rosel *et al*. 1995, Brown-Gladden *et al*. 1997, Walton 1997, Berube *et al*. 1998, Pichler *et al*. 1998, Rosel *et al*. 1999, Escorza-Trevino and Dizon 2000).

Bottlenose dolphins (*Tursiops* sp.) are distributed across a wide range of habitats throughout temperate and tropical waters. Despite their potential for long-distance movements within this broad distribution, significant genetic differentiation has been detected both within ocean basins (Dowling and Brown 1993, Curry and Smith 1997, Natoli *et al*. 2004) and on a microgeographic scale within localized study sites (Krützen *et al*. 2004*b*, Möller and Beheregaray 2004). The structuring detected by molecular genetic surveys supports the site fidelity and stable social organization that has been described for *Tursiops* species through long-term direct observations of recognized individual dolphins (Wells *et al*. 1987, Connor *et al*. 1992, Connor *et al*. 2000). Independent studies in diverse geographic regions have highlighted not only the similarities in population structure among regions, but also the behavioral and ecological plasticity exhibited by *Tursiops* sp.

The northeastern Bahamas presents a unique location for examining the population structuring and dispersal patterns of *Tursiops truncatus.* Here, the coastal form of bottlenose dolphin inhabits the nearshore waters of Little Bahama Bank; a shallow

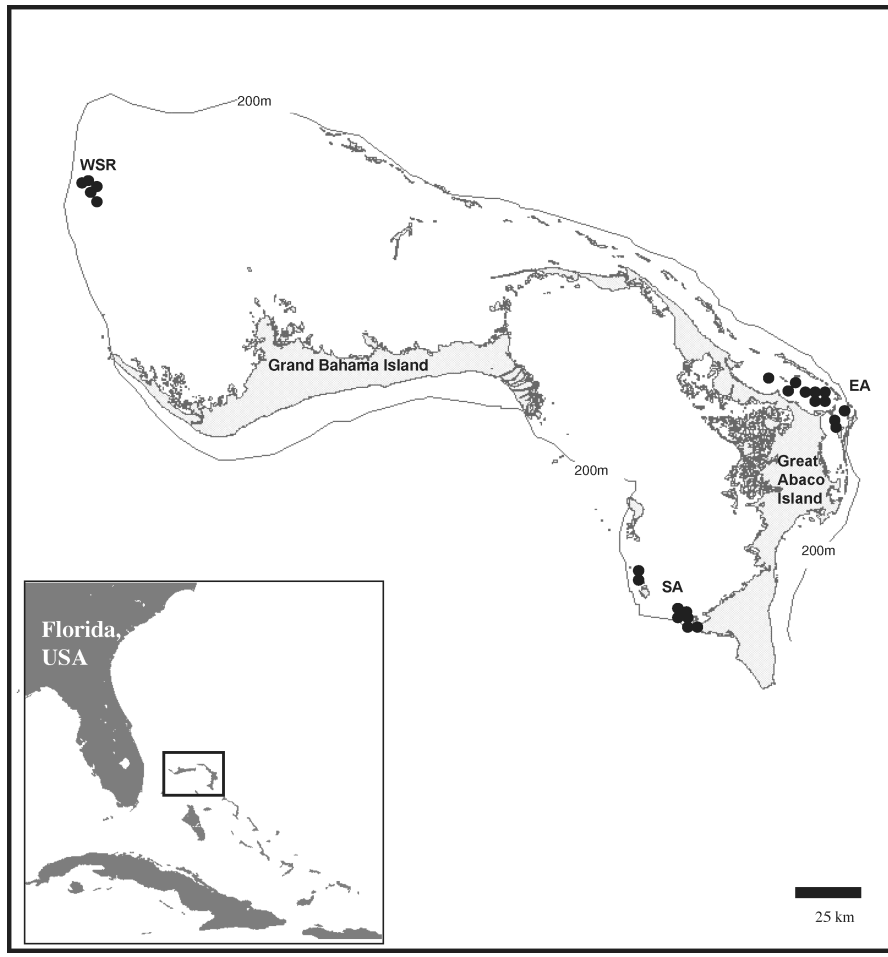

Figure 1. Map of the three sampling sites on Little Bahama Bank within the bounds of the 200-m-depth contour. Closed circles indicate sampling locations of ≥1 sample. EA = east Abaco; SA = south Abaco; WSR = White Sand Ridge.

sand bank system (Fig. 1) that is physically isolated from similar coastal habitats by the deep oceanic waters of Providence Channel to the south, and the Gulf Stream to the west. Photographic mark-recapture data sets in both east and south Abaco indicate relatively high degrees of residency among bottlenose dolphins in each site, punctuated by occasional movement of a few individuals between sites (Durban *et al*. 2000; Bahamas Marine Mammal Survey [BMMS], unpublished data). This observed pattern of residency also occurs among animals in a third site, White Sand Ridge off Grand Bahama Island (Rossbach 1997, Rogers *et al*. 2004), suggesting site fidelity and subdivision among the three Little Bahama Bank regions. Nonetheless, undocumented movements and genetic exchange likely occur outside the observations of these studies, requiring additional approaches to assess the degree of biologically

significant subdivision and the direction and extent of gene flow. In this study we examine the population structuring of bottlenose dolphins inhabiting the coastal waters on Little Bahama Bank, by incorporating direct information on the rate of intersite movement and individual identifications into an analysis of the geographic distribution of genetic variance, using both mitochondrial DNA (mtDNA) control region and nuclear microsatellite markers.

METHODS

Study Sites and Sample Collection

Little Bahama Bank is a relatively shallow sand bank with an average water depth less than 7 m, encompassing the islands of Grand Bahama and Great Abaco (approximately 17,149 km^2), in the northern Bahamas (Fig. 1). Boat-based surveys were conducted over four years (1997–2000) between June and October in two primary study sites centered in east (26°33.85′N, 77°04.25′W) and south Abaco (26°00′N, 77°25′W). During each Abaco encounter, individual bottlenose dolphins were identified photographically by the unique pattern of long-lasting, naturally occurring nicks in their dorsal fins (Würsig and Würsig 1977, Scott *et al*. 1990). These natural markings allow documentation of the composition of dolphin groups through photo-identification of individual animals. All photographs were graded for quality (based on lighting, focus, distance, and angle), and dolphins in high-quality images were assigned identification numbers with reference to existing photo-ID catalogs (BMMS). All identifications were confirmed by at least two of the authors (JD, DC, and KP). Dolphins identified during each encounter were compared to catalogs of all bottlenose dolphins previously photographed in the NE Bahamas, providing a record of the resighting of individuals within and between sites.

Samples of skin or feces were collected from photographically identified individual animals for genetic analyses during the 1998–2000 field seasons. Skin samples were obtained from free-swimming dolphins using a lightweight remote biopsy technique (Barrett-Lennard *et al*. 1996, Parsons *et al*. 2003*a*), and samples of sinking fecal matter were retrieved from the water column using the method described by Parsons (2001). Genetic sampling efforts concentrated in the two Abaco sites, were supplemented by opportunistic fecal sampling at a third site, White Sand Ridge, northwest of Grand Bahama Island during June–July 1999 and July–August 2000 (Fig. 1). In addition, blood samples from nine captive bottlenose dolphins were collected by veterinary personnel during routine husbandry practices at a captive dolphin facility in the Bahamas. Six of the captive dolphins were wild-caught in the east Abaco study area and were included in the population structure analyses.

DNA Extraction and mtDNA Control Region Sequencing

Total genomic DNA was extracted from blood and tissue samples using standard phenol/chloroform extraction protocols (Sambrook *et al*. 1989). DNA was extracted from fecal samples using the guanidine thiocyanate (GITC)/diatomaceous earth/vectaspin method described by Parsons *et al*. (1999). Particular caution was exercised in the extraction of DNA from fecal samples to guard against sample contamination. Both laboratory working surfaces and all equipment were sterilized prior to extractions, all metal instruments were wiped with ethanol and flamed, and disposable filter-tipped pipettes were used during all stages of fecal DNA extractions.

Primers L15926* (Eggert *et al*. 1998) and H16498 (Rosel *et al*. 1995) were used to amplify a 483-bp mitochondrial fragment encompassing the highly polymorphic, 5′ section of the control region (Hoelzel *et al*. 1998) using the polymerase chain reaction (PCR) conditions in Parsons *et al*. (1999). PCR products were purified using QIAquick PCR purification columns (Qiagen) and sequenced on an ABI 377 automated DNA sequencer using the BigDye sequencing kits (PE Biosystems) according to the manufacturer's instructions. PCR products from a random selection of samples, as well as those that revealed any sequence ambiguity, were sequenced in both directions to ensure accurate sequence determination.

To avoid potential biases due to the presence of first-order relatives, samples from one calf whose mother was also in the sample set was excluded from all population genetic analyses. One mother-offspring pair was included in the data set because they were sampled in two different study sites and represented a potential dispersal event.

Microsatellite Typing and Molecular Sex Determination

Skin, fecal, and blood samples were genotyped at 17 polymorphic di and tetranucleotide repeat cetacean microsatellite loci (EV14a, EV37a [Valsecchi and Amos 1996]; GATA098 [Palsbøll *et al*. 1997]; D08, D14, D22, D28, [Shinohara *et al*. 1997]; TexVet5, TexVet7 [Rooney *et al*. 1999]; DlrFCB1, DlrFCB2, DlrFCB4, DlrFCB5 [Buchanan *et al*. 1996]; KWM1b, KWM2a, KWM9b, KWM12a [Hoelzel *et al*. 1998]) following the PCR conditions specified in Parsons *et al*. (2003*b*). Alleles were amplified using a PCR containing [γ-^{32}P]-dATP labeled forward primers, separated by electrophoresis on 6% denaturing polyacrylamide gels, and visualized by autoradiography. Allele size was determined with reference to a simultaneously run M13mp8 DNA sequence ladder in every gel. Negative control reactions containing no genomic DNA were included in every set of amplifications to enable detection of contamination, and some samples were genotyped and electrophoresed multiple times to serve as positive controls and facilitate consistent allele sizing across gels.

The multiple-tubes genotyping method was employed to obtain accurate genotypes from fecal-derived DNA (Taberlet *et al*. 1996, Parsons 2001). Every fecal sample was screened for an average of four independent PCRs per locus, providing greater than 98% probability of deriving an accurate genotype (Parsons 2001), and all PCRs were performed using the same DNA extract. Multiple, conflicting heterozygous genotypes were never obtained from fecal samples, nor were multiple homozygous genotypes, suggesting that false alleles and allelic dropout were not prevalent in our data set. Genotyping problems encountered with fecal samples were limited to alleles that were difficult to score due to lack of sufficient amplified product, or samples that failed to amplify at a particular locus. Both of these potential errors were addressed by repeating the PCR in question. If repeated PCR still failed to produce a clear genotype, the individual in question was recorded as having an unknown genotype for that particular locus.

Sex was determined by co-amplification of a 147-bp fragment of the male-specific SRY gene (Richard *et al*. 1994) and a 211-bp microsatellite locus (EV37; Valsecchi and Amos 1996). This multiplexed PCR protocol guarded against erroneous female assignment due to general PCR failure by generating an internal positive control. Amplification reactions (10 μL) contained 0.5 μM of the SRY primers and 0.25 μM of the EV37 (Parsons *et al*. 2003*b*). PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide, and visualized under ultraviolet light.

Assessment of mtDNA Population Genetic Structure

CLUSTAL V (Higgins *et al*. 1992) was used to align multiple sequences, identify polymorphic nucleotide sites, and assign haplotypes. Nucleotide and haplotypic diversities were estimated from the mtDNA control region data for each of the three sampled regions according to Nei (1987) using the program DNASP version 3.0 (Rozas and Rozas 1999). Minimum-spanning networks were generated to infer the relationships among the haplotypes using both ARLEQUIN v.2, and the median-joining algorithm (Bandelt *et al*. 1999), implemented in NETWORK (http://www.fluxus-engineering.com).

Geographic structuring of mtDNA variation was assessed using an analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) performed for all individuals, and for each sex separately, using ARLEQUIN v.2 (Schneider *et al*. 2000). Pairwise F_{ST} values for haplotype frequencies alone, and ϕ_{ST} values incorporating Tamura-Nei's model of sequence mutation ($\alpha = 0.50$), were estimated. The significance of fixation indices was determined by comparing observed values to the null distribution of the test statistic generated by randomly permuting mtDNA haplotypes among the populations 10,000 times. White Sand Ridge samples were excluded from both the estimation of pairwise genetic differentiation, and the sex-specific analyses because of the limited sample size.

Assessment of Nuclear Population Genetic Structure

The program GENEPOP 3.1c (Raymond and Rousset 1995) was used to test for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium between all pairs of loci. Sequential Bonferroni correction (Rice 1989) was applied to adjust the significance levels for multiple tests.

The degree of genetic differentiation among the three sampling sites was quantified using standard fixation indices. Wright's F_{ST} was estimated from the microsatellite data using Weir and Cockerham's (1984) θ estimator, based on the infinite alleles model (IAM), using the program ARLEQUIN v.2. However, microsatellites are thought to evolve according to a stepwise model of mutation (SMM; Slatkin 1995, Goodman 1998), and therefore Slatkin's R_{ST}, accounting for differences in both sample size and allele size variance between loci, was estimated by ρ using the program RSTCALC (Goodman 1997). Both θ and ρ were calculated for the total data set, and for each sex separately, and significance was evaluated by randomly permuting genotypes among samples ($n = 10{,}000$). As with the analyses of mtDNA data, White Sand Ridge data was excluded, from the pairwise nuclear genetic differentiation, from the sex-specific analyses because of limited sample size, and from the estimation of ρ.

Multilocus microsatellite data were used to calculate pairwise relatedness for each site separately, using Lynch and Ritland's (1999) regression-based estimator (as in Parsons *et al*. 2003*b*). The validity of this metric was assessed by estimating relatedness for six pairs of known first-order relatives using genotyping data from both wild Abaco dolphins and captive dolphins. Differences in intrasexual relatedness between sexes, and within and among the Abaco sites were examined using the randomization resampling technique (10,000 permutations) implemented in RESAMPLING PROCEDURES v1.3 (http://www.uvm.edu/~dhowell/StatPages/Resampling/Resampling.html).

We also used a Bayesian model-based clustering approach (Luikart and England 1999, Pritchard *et al*. 2000, Eldridge *et al*. 2001) to assess structuring and identify potential migrants within the sampled Little Bahama Bank population. Given

the microsatellite data, this model attempts to assign individuals to subpopulations on the basis of their genotypes, while simultaneously estimating the allele frequencies that define each of these population units. Moreover, this method allows us to incorporate population information derived from direct photo-identification data, and test the validity of our subjective definitions of subpopulations. The Bayesian clustering method was implemented using the program STRUCTURE (Pritchard *et al*. 2000), which applies Markov Chain Monte Carlo (MCMC) methods to estimate these probabilities from the model.

To provide estimates in the form of direct probability distributions, Bayesian methods require prior probability distributions to be assigned to each of the unknown parameters being estimated (Gelman *et al*. 1995). The number of subpopulations (K) represented by the samples is a user-specified prior, here we tested the fit of models for each value of K (1–5) through a series of three to five independent MCMC runs during which data was collected for 10^6 iterations following a burn-in period of 50,000 iterations. Estimated values of $\Pr(X|K)$, where X is the observed genotype data and K is the number of clusters, or subpopulations, were generated for all values of K. From these data, the corresponding values of $\Pr(K|X)$ were calculated assuming a uniform prior on K (Pritchard *et al*. 2000) using the following equation:

$$\Pr(K_i|X) = \exp(\mathrm{Ln}\ \mathrm{P}(X|K_i)) \Big/ \sum_{K=1-5} (\exp[\mathrm{Ln}\ \mathrm{P}(X|K_i)]).$$

In addition to providing an overall probability of model fit for each K, STRUCTURE also estimates the probabilities of cluster membership for each individual dolphin, and facilitates the incorporation of prior information on the sampling location of individual dolphins. Incorporating geographic information in the model improves the accuracy of assigning individuals to clusters and improves the overall estimate of model fit (Pritchard *et al*. 2000). As suggested by Pritchard *et al*. (2000), the first set of models (Model I) fit to the data did not include geographic information (*i.e.*, the study site identity for each sample), to examine the clustering of individuals without the subjective definition of a population unit. The subsequent STRUCTURE models incorporating geographic information (Model II and III) were parameterized by two different values of v, the probability that an individual is an immigrant to the population being considered. In one model set, v was empirically estimated as the number of dolphins known to have moved between the two Abaco sampling locations ($n = 6$) as a proportion of the total number of different animals photo-documented in both of the sites between 1997 and 2000 ($v = 0.02$). To account for undocumented movements that may result in an underestimate of v, and to examine the sensitivity of the model to the choice of v, model fit was examined for $v = 0.05$ in the third model set. All of the above models were fit to the complete data set, as well as data for the two Abaco sites only, to explore the effect of the presence of sparse data from White Sand Ridge on model fit.

RESULTS

Dolphin Encounters and Sample Collection

All dolphin encounters were documented through high-quality identification photographs of every dolphin present in each group. Between 1997 and 2000, 148 individual dolphins were encountered in east Abaco, and 99 different dolphins were

Table 1. Summary statistics of photo-identification sighting data for bottlenose dolphins in Abaco, Bahamas, for data collected during 1997–2000. Standard deviations are presented in parentheses.

Bottlenose dolphins	East Abaco	South Abaco
Number of individuals identified	148	99
Number identified in first survey year	31	52
Number encountered in both 1997 and 2000	13 (41.94%)	26 (50%)
Mean number of years encountered	1.80 (±0.94)	1.89 (±1.25)
Average number seen in one survey year	66.25 (±41.32)	46.75 (±10.78)

encountered in south Abaco (Table 1). Of these 247 dolphins, six individuals (three females, one male, two sex unknown) that were originally encountered in east Abaco were subsequently photo-documented in south Abaco. These rare movements appeared to be transitory, as the animals did not remain in the south Abaco study area for prolonged periods, and three of the six have been subsequently resighted in east Abaco. In general, dolphins displayed considerable site fidelity. Most individuals were encountered in multiple years, and almost half of the individuals encountered during the first year of the study (1997) were also encountered in 2000 (Table 1).

During the study, 25 skin samples, 52 fecal samples, and 9 blood samples were collected. Samples could be assigned to individually identified dolphins for every biopsy sample, and 84% of fecal samples collected in Abaco. Duplicate fecal samples for which dolphin identity could not be ascertained at the time of collection were identified through direct comparison of mtDNA control region sequences, multilocus nuclear genotypes and molecular sex markers. After removing duplicate ($n = 17$) and unamplifiable ($n = 8$) fecal samples, tissue samples from 58 different bottlenose dolphins (47% of the average number of animals seen in a survey year; Table 1) were represented.

Mitochondrial Control Region Sequences

Multiple sequence alignment revealed 31 variable sites over 445 bp, defining 11 mtDNA control region haplotypes (Table 2). Three haplotypes (HAP-A, HAP-B and HAP-D) occurred in both east and south Abaco. However, none of the White Sand Ridge haplotypes was identified among the samples from either east Abaco or south Abaco, such that no haplotype occurred in all three geographic sites. A BLAST search of the 11 Bahamas *Tursiops truncatus* control region sequences on Genbank revealed a potential match of HapD with a published coastal *T. truncatus* sequence from the Gulf of Mexico (accession AY962620; Natoli *et al*. 2004). However, this match is equivocal because the published sequence is only 296 bases long, and 10 of the 31 variable sites identified among our 11 bottlenose dolphin haplotypes lie outside the span of this published sequence. We also identified potential sequence matches between our HapG and the western North Atlantic coastal haplotype WNACc, and between BAHk and three of our haplotypes (HapA, HapB, and HapF) when aligning our sequences with published sequences (Hoelzel 1998, Natoli *et al*. 2004). However, all of these matches are based on comparison of only 296 out of our 445 bp. Therefore, while these potential matches clearly indicate a high degree of sequence similarity, we cannot unequivocally confirm sequence identity with the published sequences.

Table 2. Mitochondrial control region sequences for bottlenose dolphins in the NE Bahamas. Polymorphic nucleotide sites defining the 11 mtDNA control region haplotypes and their occurrence in each of the three sampling locations (EA = east Abaco; WSR = White Sand Ridge; SA = south Abaco) are shown. Dots indicate nucleotide bases identical to the first sequence. Position 1 in the alignment below corresponds to the first guanine base in the sequence 5′-GAAAAAG-3′ at the start of the cetacean control region (Hoelzel et al. 1991).

	Variable site number				Genbank accession no.	EA	WSR	SA
	111111	1222222222	2333333333	3				
	1267011124	9046778889	9022225779	9				
	5634405849	3555480230	4345697042	3				
HapA_tt72	TTG–AACATC	ATCCTTCTCA	CAGATCCTCT	C	AF155162	5		1
HapB_Tt48	. . . –	. . . . .	. . AG.	.	AF378176	17		6
HapD_Tt65	. C . C . . . G . T	. . . . . CT .	. . A . . . T . . .	.	AF378177	5		1
HapE_Tt157	. . . –	G.	. . . . .	.	AF378178	2		
HapF_Fc50	. . . –	. . . . .	. . A	.	DQ118180		1	
HapG_Fc52	. CA– . . T . . .	. . T . C . . . . G	. GA . C	.	DQ118181		1	
HapH_Tt514	. . . –	. . . . . C	. GA.	.	AF155161			9
HapI_Tt520	. . . –	. . . . . T . . .	. . AG.	.	AF155160			4
HapK_Fc82	. C . –GG . . – .	. CTA–CTC . .	. GA. . . TC . C	T	DQ118182		1	
HapL_Fc83	C . . –	. . . . . C	. . A . . . . . T .	.	DQ118183		1	
HapM_Fc85	. C . –GG . . – .	. CTA–CTCT .	TGA. . TT. . C	T	DQ118184		1	

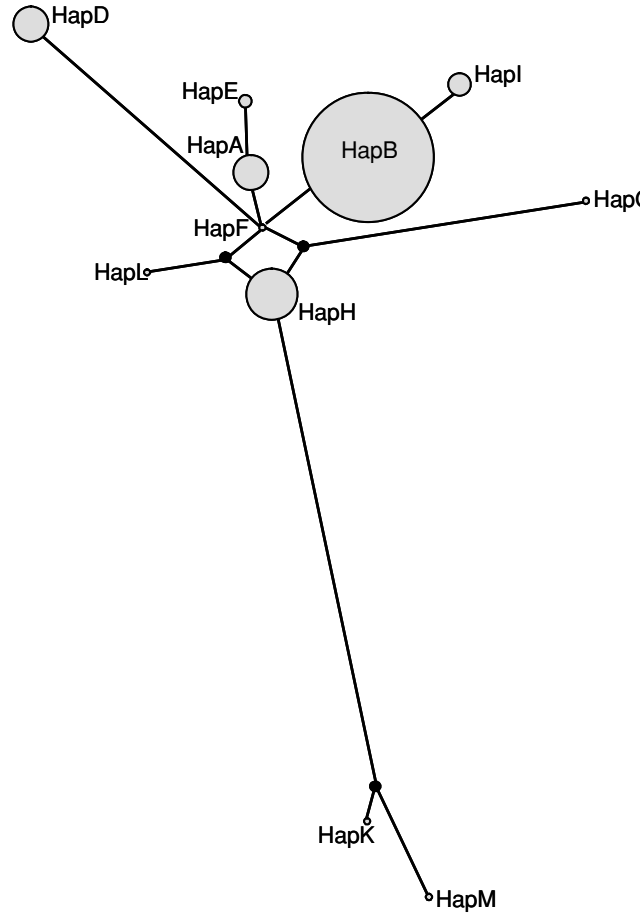

Figure 2. Minimum spanning network of the 11 *Tursiops truncatus* mitochondrial DNA haplotypes found in the NE Bahamas. Solid black circles indicate intermediate ancestral haplotypes, node size is approximately proportional to the number of individuals identified with that haplotype.

Two insertion-deletions (indels) located at positions 124 and 274 differentiated the White Sand Ridge haplotypes K and M from all others. The minimum spanning networks generated by both ARLEQUIN and NETWORK were identical in their topology, and therefore, only the latter is presented (Fig. 2). This network suggests the presence of two different clusters, and supports the division indicated by the indels necessary for alignment of the 11 mitochondrial control region sequences. In fact, alignment of HapK and HapM sequences with those from over 800 *T. truncatus* dolphins of known ecotype (data not shown) indicates that these two dolphins likely belong to the "offshore" or pelagic bottlenose dolphin ecotype.[2] Species designation

[2] Personal communication from P. Rosel, NOAA Fisheries, Southeast Fisheries Science Center, March 2005.

and taxonomic status within the genus *Tursiops* remains somewhat uncertain, however, a clear separation has been demonstrated between the "coastal" and "offshore" ecotypes (Hoelzel *et al*. 1998, Natoli *et al*. 2004). As such, all genetic data from the two likely "offshore" dolphins were excluded from the population genetic structure analyses. The results presented hereafter are based on the analysis of genetic data from 55 dolphins that were known to belong to the "coastal" ecotype, after the removal of one calf from a mother-offspring pair.

Across all samples, nucleotide diversity was 0.0066 (SD = 0.004) and haplotype diversity was 0.763 (SD = 0.046). Both measures of mitochondrial genetic diversity were greatest in the White Sand Ridge sample, where each of the three inshore ecotype dolphins possessed a unique mtDNA haplotype (Table 3). Analysis of molecular variance indicated a significant amount of structure among the three sampled regions on Little Bahama Bank, based on both haplotype frequency alone ($F_{ST} = 0.192$, $P < 0.0001$), and with genetic distance between mtDNA haplotypes incorporated ($\phi_{ST} = 0.162$, $P < 0.003$). All pairwise comparisons among the three sites also indicated a significant degree of structuring for F_{ST}, however, population differentiation between the Abaco sites and White Sand Ridge was not statistically significant for ϕ_{ST} (Table 4).

Microsatellite Diversity and Geographic Structuring

Unambiguous genotypes were obtained for all samples at 12–17 (mean = 16) microsatellite loci. The number of alleles resolved at each locus ranged from two to nine with a mean of 5.12 (Table 3), and microsatellite variation within sample regions was moderately high, with H_O values ranging from 0.563 to 0.638 (Table 3). Examination of microsatellite genotypic data across loci and across populations did not reveal any significant deviations from Hardy-Weinberg expectations (HWE) after Bonferroni correction for multiple tests was applied. Furthermore, no loci were consistently in linkage disequilibrium across all sites, and no populations were consistently in linkage disequilibrium across all loci.

Evidence of geographic structuring was assessed given the *a priori* assignment of individuals to the three regionally defined subpopulations in which they were sampled. Overall estimates of F_{ST} ($\theta = 0.040$, $P < 0.005$; $\rho = 0.022$, $P = 0.016$) from nuclear genotypes indicated significant differentiation among the three sampled regions, but levels of dispersal that are high enough to limit differentiation by genetic drift. Evidence of significant geographic structuring was also apparent in the pairwise population comparison between east Abaco and south Abaco ($\theta = 0.048$, $P = 0.0001$). Pairwise comparisons between Abaco sites and White Sand Ridge were precluded due to the small number of samples and loci genotyped at White Sand Ridge.

Bayesian clustering analysis indicated the presence of distinct genetic groups. Without prior information on sampling locations (Model I), the model best fit the data for $K = 1$ ($P = 0.99$; Table 5), reflecting the presence of some gene flow as indicated by the AMOVA analyses. However, when geographic information was included (Models II and III), the models with $K = 3$ ($P = 0.95$) fit the data better than the above model, and were not sensitive to the prior values of estimated movement rates (v; Table 5). When the White Sand Ridge data were excluded, identical patterns of individual assignment and model fit (without geographic information, $P = 0.99$ for $K = 1$; with geographic information, $P = 0.98$ for $K = 2$) were obtained, therefore, further consideration of the results refer to models that were fit to the data comprising individuals from all three locales.

Table 3. Geographic distribution of mitochondrial and microsatellite genetic diversity. Sample sizes and measures of genetic diversity are provided for "coastal" ecotype bottlenose dolphins in each of the three sampled regions. The number of males and females combined are less than the total number of individual dolphins sampled at each site due to the constraints of accurately determining sex from some fecal DNA extractions. If the sex-determining PCR was ambiguous, or failed, the sample remained of "unknown sex." Standard deviations are provided in parentheses.

	No. of individuals sampled	No. of males	No. females	Mean no. of alleles per locus	Mean H_E	Mean H_0	Gene diversity	Nucleotide diversity
East Abaco	31	19	11	4.530 (±1.630)	0.612 (±0.154)	0.638 (±0.157)	0.613 (±0.082)	0.0058 (±0.0023)
White Sand Ridge	3	0	1	2.000 (±0.655)	0.745 (±0.251)	0.611 (±0.391)	1.000 (±0.272)	0.0152 (±0.0113)
South Abaco	22	7	12	4.235 (±1.480)	0.609 (±0.126)	0.563 (±0.147)	0.700 (±0.062)	0.0054 (±0.0032)
Overall	56	–	–	5.118 (±1.965)	0.655 (±0.177)	0.604 (±0.232)	0.763 (±0.046)	0.0066 (±0.0044)

Table 4. Pairwise measures of genetic differentiation among the three subpopulations based on mtDNA control region sequence data. Significance values, in parentheses, were estimated by permuting the original data set 10,000 times. Distances between pairs of sites are approximated shortest distances by inshore waters.

	F_{ST}	ϕ_{ST}	Distance
East Abaco–South Abaco	0.181 (0.0002)	0.144 (0.003)	116 km
East Abaco–White Sand Ridge	0.284 (0.026)	0.283 (0.056)	226 km
South Abaco–White Sand Ridge	0.190 (0.033)	0.174 (0.111)	242 km
Overall	0.192 (<0.0001)	0.162 (<0.003)	

According to the probabilistic assignment of individuals to the inferred STRUCTURE clusters estimated from Model II with the empirically derived migration parameter (v), all of the east Abaco dolphins were "correctly" assigned to the east Abaco subpopulation (Fig. 3, Table 6). Of the three samples collected at White Sand Ridge, one was assigned a marginally higher probability of belonging to the south Abaco population ($P = 0.62$) than to its sampling locale, and this likely reflects the unrepresentative allele frequencies obtained for this site due to the small number of dolphins sampled. Two of the dolphins sampled in south Abaco were also assigned a greater probability ($P > 0.50$) of belonging to an alternate cluster. These two dolphins (Tt13 and Tt539, both adult females) were identified as potential immigrants to south Abaco, and assigned to the east Abaco cluster. The model-estimated probability of east Abaco membership for individual Tt13 of 0.810 concurs with our direct mark-recapture data in which this animal was repeatedly photo-documented in east Abaco between 1992 and 2000, temporarily migrated to south Abaco in late 2000 (where it was biopsy sampled), and has since returned to east Abaco (BMMS, unpublished data).

Table 5. Estimated posterior probabilities of K (number of subpopulations) for the Little Bahama Bank microsatellite genotyping data from all three study sites. The K with the greatest probability for each model is indicated by bold typeface.

	Without sampling location		With sampling location information			
			$v = 0.02$		$v = 0.05$	
	Model I		Model II		Model III	
K	Ln $P(X\|K)$	Pr$(K\|X)$[a]	Ln $P(X\|K)$	Pr$(K\|X)$[a]	Ln $P(X\|K)$	Pr$(K\|X)$[a]
1	−1,903	**0.999**	−1,942	~0	−1,903	~0
2	−2,320	~0	−1,959	~0	−1,927	~0
3	−2,427	~0	−1,902	**0.946**	−1,867	**0.950**
4	−2,125	~0	−1,905	0.047	−1,870	0.047
5	−1,916	2.26×10^{-6}	−1,907	0.006	−1,873	0.002

[a] Pr$(K|X)$ provides relative probabilities of models consistent with the data for each v, the probability that a sampled dolphin is a migrant.

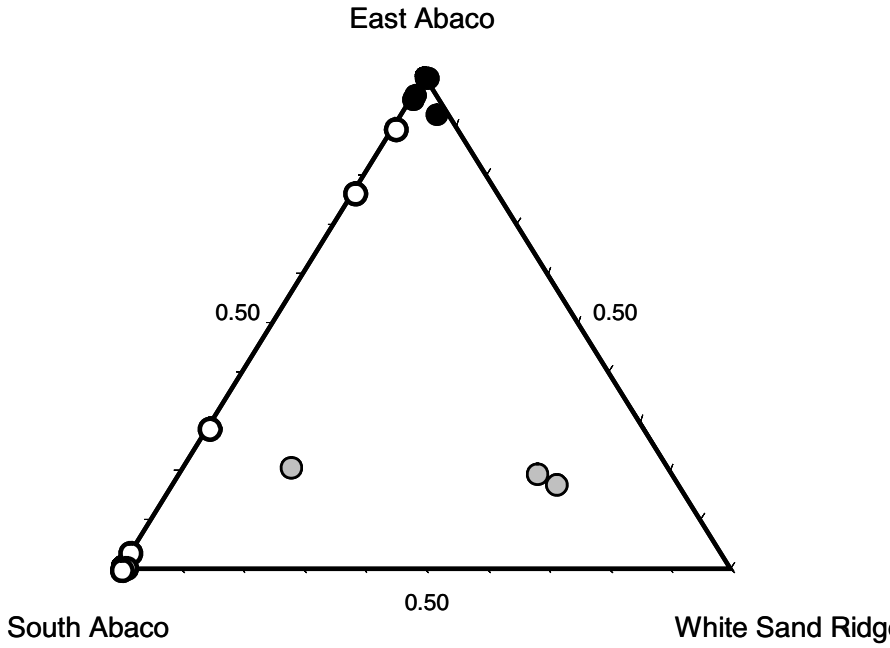

Figure 3. Ternary plot of the probabilistic assignment of individual bottlenose dolphins to the three study sites based on Bayesian cluster analysis performed using STRUCTURE. Assignment was based on the inclusion of the sampling location prior and the migration prior estimated from direct data ($v = 0.02$), for the best-fit model ($K = 3$). Dolphins sampled in east Abaco are represented by filled circles, those sampled in south Abaco and White Sand Ridge are indicated by open circles and gray circles, respectively.

Sex-Based Assessment of Population Structure

Sex-based analysis of the distribution of mtDNA genetic diversity between east and south Abaco suggested that the geographic apportioning of mitochondrial variation among the two sample sites accounted for more of the observed variation among males than among females. The AMOVA indicated that more than 26% ($P = 0.002$) of the observed mtDNA variation could be attributed to differentiation among males between the two sites (Table 7). In contrast, analysis of mtDNA sequences for Abaco

Table 6. Proportion of genotyped dolphins assigned to each of the three inferred population clusters for each sample region on Little Bahama Bank from the Bayesian clustering analysis, Model II (Table 5).

	Inferred cluster		
Sample site	1	2	3
East Abaco	0.994	0.002	0.004
White Sand Ridge	0.191	0.457	0.352
South Abaco	0.092	0.001	0.907

Table 7. Sex-specific measures of genetic differentiation between east Abaco and south Abaco inferred from mtDNA control region and microsatellite data. Statistical significance, in parentheses, was assessed by comparison with 10,000 random permutations of the data set, $^*P \leq 0.05$.

	mtDNA data		Microsatellite data	
	F_{ST}	ϕ_{ST}	θ	ρ
Females	0.095 (0.100)	0.093 (0.095)	0.029* (0.036)	0.012 (0.30)
Males	0.291* (0.002)	0.265* (0.002)	0.045* (0.003)	0.041* (0.041)

females did not reveal significant structuring (Table 7). Direct comparison of the AMOVA null distributions generated by random permutation of haplotypes among the populations for each sex indicated that the mtDNA population differentiation among males was significantly greater than that for females (Wilcoxon's signed rank test; F_{ST}, $Z = 17.37$, $P < 0.001$; ϕ_{ST}, $Z = 25.78$, $P < 0.001$).

Differentiation of nuclear variation also suggested high levels of genetic differentiation among Abaco males, however, unlike the mtDNA data, significant but low levels of differentiation among the Abaco sites were also resolved for females using the θ estimator (Table 7). Despite the significant difference found for females, the AMOVA null distributions still suggested greater genetic differentiation among male bottlenose dolphins (Wilcoxon's signed rank test; $Z = 87.04$, $P < 0.001$). Moreover, the STRUCTURE (Model II) posterior probabilities of a dolphin being assigned to the site in which they were sampled were greater, although not significantly so, for males than for females (males, mean $= 0.997 \pm 0.003$ SD; females, mean $= 0.907 \pm 0.246$ SD; t-test: $t = -1.69$, $P = 0.054$).

Pairwise relatedness estimated using Lynch and Ritland's (1999) metric yielded values that closely approximated that expected for first-order relatives for the five genotyped mother-offspring pairs, with a mean of 0.482 (SD = 0.068). The mean intrasexual pairwise relatedness derived from the microsatellite genotypes did not differ between sexes in either location (east Abaco, $t = -0.082$, $P = 0.933$; south Abaco, $t = -1.063$, $P = 0.300$), suggesting that within a study site, the average degree of genetic relatedness is the same among females as among males. Moreover, both males and females exhibited significantly higher intrasexual relatedness within, compared to between, Abaco subpopulations (females, $t = 5.726$, $P < 0.0001$; males, $t = 4.083$, $P = 0.0001$; Fig. 4).

Discussion

The spatial genetic structuring of both microsatellite and mtDNA genotypes revealed significant levels of genetic differentiation and restricted gene flow among the three study sites in the northern Bahamas. Moreover, analysis of pairwise relatedness indicated significantly higher relatedness within than between Abaco sampling locations, for both sexes. While it must be acknowledged that these results are based on a limited sample size, they support the site fidelity suggested by direct individual-based photographic sightings data and indicate significant population subdivision and philopatry among bottlenose dolphins on Little Bahama Bank.

Among the "coastal" ecotype bottlenose dolphins sampled in the NE Bahamas, nine mitochondrial control region haplotypes (four to five haplotypes per sampling

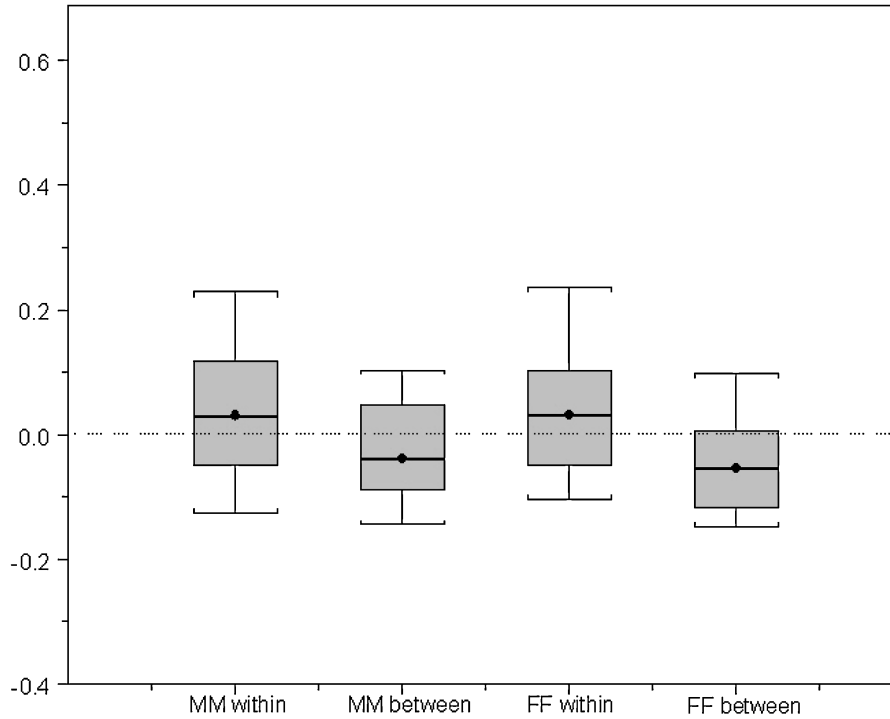

Figure 4. Intrasexual pairwise relatedness within and between Abaco bottlenose dolphin subpopulations. Whiskers indicate the median, 10th, and 90th percentiles. Pairwise relatedness between males denoted as MM (within subpopulation, mean = 0.043 ± 0.143 SD; between subpopulations, mean = −0.024 ± 0.101 SD), relatedness between females denoted as FF (within subpopulation, mean = 0.050 ± 0.141 SD; between subpopulations, mean = −0.038 ± 0.107 SD).

region) were found. The amount of genetic diversity found in mitochondrial surveys of *Tursiops* sp. varies considerably from population to population. In the coastal waters of Australia, where Möller and Beheregaray (2001) resolved five different mtDNA haplotypes in a sample of 57 bottlenose dolphins in southeastern Australia, and Krützen *et al*. (2004*b*) found only eight different mtDNA haplotypes in a sample of 220 different dolphins from Shark Bay waters, genetic diversity appears to be considerably lower than in the NE Bahamas. Bottlenose dolphins from a small population in NE Scotland exhibited exceptionally low levels of mitochondrial genetic diversity where only two different haplotypes were found in a sample of 15 dolphins (Parsons *et al*. 2002). In contrast, surveys of *Tursiops* sp. in Chinese waters (Wang *et al*. 1999), and those representing *Tursiops truncatus* in the Mediterranean Sea (Natoli *et al*. 2004) and western North Atlantic populations (Hoelzel *et al*. 1998, Natoli *et al*. 2004) reported levels of genetic diversity similar to, or greater than, those resolved in this study. However, comparison across studies is confounded by methods of sample collection and sample type, where samples collected across a geographically defined population could encompass several population subdivisions that would be undetected without

reference to individual dolphin identifications and sighting histories. Likewise, sampling regimes such as that applied in this study and that employed by Möller and Beheregaray (2001) which was based only on "resident" dolphins, could result in sampling that is biased towards genetic relatives.

Contrary to the relatively high levels of mtDNA genetic diversity, values of H_O from the nuclear microsatellite data, ranging from 56% to 64% for the three sites, were lower than those reported in a study of population structure of *Tursiops* sp. in Shark Bay, Australia (Krützen *et al*. 2004*b*). These differences may be due to the particular microsatellite loci used in each study, or may be attributed to differences in the number of genotyped dolphins. Both studies employed some loci isolated from *Tursiops* sp., as well as heterologous PCR primers isolated from other cetacean species. The regions flanking microsatellites tend to be highly conserved across cetaceans and loci isolated from the genome of one species can often be successfully cross-amplified in other species (*e.g.*, Schlotterer *et al*. 1991, Buchanan *et al*. 1996, Valsecchi and Amos 1996, Shinohara *et al*. 1997). However, caution has been recommended when amplifying microsatellite loci in species other than those from which they were isolated due to the possibility of non-amplifying alleles (Pemberton *et al*. 1995). The lack of deviation from Hardy-Weinberg equilibrium and lack of evidence for heterozygote deficiencies in our data suggest that the difference in heterozygosity is most likely due to sample size effects.

Estimates of genetic differentiation resolved for both mtDNA and nuclear markers clearly indicate a significant degree of structuring within this population, and limited intersite dispersal of both males and females. Moreover, pairwise relatedness between sites was significantly lower than that within sites for both sexes, supporting the population subdivision indicated by both behavioral observations and analyses of molecular variance. The higher F_{ST} estimates resolved from mitochondrial data suggest a greater degree of genetic differentiation compared to the nuclear microsatellite markers. This trend is expected given the uniparental inheritance of the mitochondrial genome (Whitlock and McCauley 1999), and may also be indicative of female philopatry and male-mediated gene flow (Burg *et al*. 1999, Lyrholm *et al*. 1999, Eizirik *et al*. 2001, Girman *et al*. 2001). However, the AMOVA based on nuclear markers suggests that while dispersal is likely high enough to limit absolute differentiation by genetic drift, those levels are sufficiently low that homogenization of the dolphin population on Little Bahama Bank is prevented. While we acknowledge that the small sample sizes available for this study (particularly from the White Sand Ridge location) limit the power of an AMOVA, the sample size effects would likely result in a failure to detect population structure. Furthermore, the strong pairwise differentiation between dolphins from the two Abaco locations (which are geographically closer to one another than to White Sand Ridge), and the assignment of Abaco dolphins to two distinct clusters in the STRUCTURE analysis supports the population subdivision detected, and suggests demographic independence of the two Abaco locales.

Significant structuring over relatively short geographical distances has also been documented for coastal *Tursiops truncatus* in other geographical locations. Populations of bottlenose dolphins along the Gulf of Mexico and southeastern coasts of the United States were found to have significant differentiation of mtDNA haplotypes (Dowling and Brown 1993, Natoli *et al*. 2004). In Sarasota Bay, Florida, long-term behavioral observations indicated considerable site fidelity (Wells *et al*. 1987, Wells 1991), and population structuring along the central west coast of Florida was supported by molecular data (Duffield and Wells 1991, 2002). Genetic structuring in

aduncus-type bottlenose dolphin populations has also been documented on both the east and west coasts of Australia. On the east coast, Möller and Beheregaray (2004) have documented population differentiation between two sites separated by approximately 400 km. Significant population structuring was also described using both nuclear and mitochondrial markers for Shark Bay dolphins on the west coast over much shorter distances (Krützen *et al*. 2004*b*). The degree of subdivision described for these socially structured bottlenose dolphin populations in such diverse locales is concordant with the genetic consequences of the fission-fusion social systems and site fidelity they express.

Many cetacean species exhibit social structures that are characterized by highly stable social or maternal fidelity. Bottlenose dolphins are often described as having a fission-fusion social system (Wells *et al*. 1987), where larger fluid groups generally comprise some smaller long-term stable associations that are sometimes based on kinship (*e.g.*, male alliances; Krützen *et al*. 2003, Parsons *et al*. 2003*b*). Fidelity to these social groups is likely to have a measurable impact on the degree and direction of dispersal and gene flow among local subpopulations (Stortz 1999).

Recent Australian studies have suggested that, as with most mammals, the dispersal of males is greater than that of females in bottlenose dolphins (Krützen *et al*. 2004*b*, Möller and Beheregaray 2004). Krützen *et al*. (2004*b*) describe a process by which male-mediated gene flow occurs through the expansion of their home range to an adult range that is larger, but incorporates the area of their natal range. In contrast, sex-based analysis of both microsatellite and mtDNA data for the Bahamas indicate greater genetic differentiation among Abaco males than females. If dispersal were female biased as suggested by the sex-based fixation indices, we would expect greater male-male pairwise relatedness compared to female-female pairwise relatedness within each study site. However, analysis of pairwise relatedness found that the within-site pairwise relatedness for females did not differ from that estimated for males, and relatedness for both sexes is greater within than between the Abaco study sites. While a general lack of effective dispersal between sites is evident for both sexes, the data suggest that if there is a sex bias in dispersal it is in the direction of females rather than males. Alternatively, the observed patterns may reflect the effect of related male-male allied pairs in our data set. Allied male-male pairs have been previously identified in both Abaco study sites on the basis of analyses of long-term association patterns and behavioral observations (see Parsons *et al*. 2003*b*). Although 12 male alliances (each comprising two dolphins) have been identified, only six are comprised of males that have both been genotyped. Removal of one male from each of those six alliances from the genetic data did not significantly affect the average degree of relatedness among males, nor did it have an effect on the AMOVA results (data not shown). Furthermore, although kinship has been demonstrated to be a factor in the formation of male-male alliances in the Bahamas, the estimated relatedness within allied pairs is markedly lower than that expected for first-order relatives (mean $= 0.13 \pm 0.07$, Parsons *et al*. 2003*b*), and as such, their inclusion in population structure analyses is unlikely to bias results.

Little Bahama Bank is characterized by relatively continuous habitat suitable for inshore bottlenose dolphins. Although field-based studies have documented a relatively low rate of movement between sites for both males ($n = 1$) and females ($n = 3$), it is likely that some dispersal events are undetected considering the mobility and ranging patterns of this species. However, high dispersal potential does not necessarily translate into high levels of realized gene flow, even among highly vagile large vertebrates (Paetkau *et al*. 1995, Avise 1998). The complex social organization exhibited

by bottlenose dolphins may prevent large-scale mixing of individuals, thereby maintaining population substructuring. Moreover, kinship among males has previously been shown to play an important role in establishing male alliances in the Bahamas (Parsons *et al*. 2003*b*), and a certain degree of natal philopatry among males would be beneficial for establishing and maintaining alliances among kin. The inclusive fitness benefit derived from limited dispersal of adult males has also been invoked to explain the patterns of genetic differentiation found among *Tursiops* sp. around Shark Bay, Australia (Krützen *et al*. 2004*b*).

Observational studies in several different geographic regions have described "resident" female bottlenose dolphins that are occasionally absent from a core study area for prolonged periods (BMMS, unpublished data; Duffield and Wells 2002), and populations that comprise both resident and non-resident animals (Möller and Beheregaray 2004). Paternity assessments for two long-term studies have also resulted in an appreciable number of calves sired by non-resident males (Duffield and Wells 1991, 2002; Krützen *et al*. 2004*a*). These data seem to suggest that despite the high degree of site fidelity documented through behavioral observations and supported by genetic differentiation, some gene flow is occurring between subpopulations or communities, and this may be mediated by both males and females. While both the geographic structuring and long-term observations suggest limited natal dispersal of both sexes, even short-term transient movements provide the opportunity for mating outside the natal unit while still enjoying the fitness benefits of long-lasting social bonds.

Despite a high potential for long distance movements, significant population structuring has been detected for several species of small cetaceans. This is particularly true for species that exhibit social structures characterized by stable, long-lasting affiliations. As with comparable studies in other geographic regions, we resolved significant differentiation among study sites separated by less than 200 km using both mitochondrial and nuclear genetic markers. While this study highlights the apparent similarities of bottlenose dolphin population structure among diverse locations, it also suggests that, in the Bahamas, dispersal may not be strictly male-biased. These data not only provide important information for the conservation and management of this nearshore species, but also provide insight into the extreme behavioral and ecological plasticity of *Tursiops* species.

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