Genetic diversity of the green turtle nesting populations in Yaeyama Archipelago, Japan

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ABSTRACT
Patterns of mitochondrial DNA (mtDNA) variation were used to analyze the genetic diversity and population genetic structure of green turtle (*Chelonia mydas*) populations in Yaeyama Archipelago, southwestern Japan. Six mtDNA haplotypes were observed among 42 nesting green turtles. Nucleotide diversities at Yaeyama populations are higher than other sea turtle populations, reflecting all rookeries are comprised of a mixture of divergent haplotypes. Because genetic diversity is believed to represent the ability of populations to adapt or respond to environmental changes, it is important to protect Yaeyama nesting colonies for conserving this endangered species.

KEYWORDS: *Chelonia mydas*, mitochondrial DNA, genetic analysis, conservation genetics

INTRODUCTION

Information on the genetic diversity and population structure of female green turtle, *Chelonia mydas*, is necessary to define conservation priorities for this highly endangered species. In recent reports, mitochondrial DNA (mtDNA) assays have proven useful for resolving divergence within a gene phylogeny and migratory behavior in female sea turtles (Bass et al., 1996; Bourjea et al., 2007; Encalada et al., 1996). However, the geographical specificity of genetic diversity and population structure apparently varies widely (Bass et al., 1996; Chassin-Noria et al., 2004). Therefore, it is necessary to study the local nesting populations for proper management strategies.

In this study, mtDNA control region sequences were analyzed to resolve the genetic diversity and population genetic structure of Yaeyama Archipelago nesting populations of green turtle. Yaeyama Archipelago, located in southwestern Japan, has major nesting sites for green turtle which is a northern limit of green turtle nesting in the Pacific Ocean.

MATERIALS AND METHODS

A total of 42 nesting females were sampled from nesting sites in Ishigaki and Iriomote Islands, Yaeyama Archipelago, Japan. Nesting females were identified by tagging during nightly patrol. Minute pieces of tissue, which were secondarily recovered while punching hind flippers for tagging, were collected and stored in 99% EtOH. Samples were collected in 1998, 2003, 2006, and 2007.

DNA was extracted from a small amount of tissue and prepared for polymerase chain reaction (PCR) by using Blood & Tissue Genomic DNA Extraction Miniprep System (VIOGENE). About 520-bp segment of the mtDNA control region was amplified using primers CMMTF1 (5'-CTT AAA CCT TCA TCC CCG GTC-3') and TCR6 (5'-CAA GTA AAA CTA CCG TAT GCC AGG-3'; Norman et al., 1994). Typically, 3 μL of template was used in 20-μL PCR reactions with Ex Taq polymerase (Takara) using standardized conditions of denaturing 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 60 s for 35 cycles. Amplification was verified by electrophoresis of 10 μL of each reaction in 1 % agarose gel. Low-density products (two of the 42 samples) were x1000 diluted and prepared for seminested PCR. Seminested PCR were performed with primers LTCM2 (5'-GGT CCC CAA AAC CGG AAT CC-3'; Allard et al., 1994) and TCR6.

The sequencing reactions (forward and reverse) were performed with dye terminators (Big Dye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems). Products were purified with the CleanSEQ (Agencourt) and ran through a 3130xl sequencing analyzer (Applied Biosystems).

Sequencing alignments were performed with the software CLUSTAL W version 2.0 (Larkin et al., 2007). Population genetic parameters estimated in ARLEQUIN version 3.1 (Excoffier et al., 2005). Estimates of nucleotide diversity and haplotype

diversity were used to quantify genetic diversity. Neighbor-joining tree (Saitou and Nei, 1987) was drawn and bootstrap analysis (1000 replicates) was computed with the software MEGA4 (Tamura et al., 2007). 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 and Nei, 1993).

RESULTS

Six mtDNA haplotypes were observed among 42 green turtle. Of 32 polymorphic sites, 28 were transitions, one site contained both a transition and a transversion, and three sites were characterized by indels. The six haplotypes differed by between 1 and 26 observed mutations. The neighbor-joining tree of the six haplotypes (Fig. 1) identified three distinct clades: clade 1 (including A, B, and C), clade 2 (including D and E), and clade 3 (F alone). Haplotype diversity was calculated as 0.5401 and nucleotide diversity was calculated as 0.021.

DISCUSSION

While haplotype diversity of Yaeyama nesting population is in the range of estimates from other sea turtle populations (0.25 – 0.60 in Chassin-Noria et al., 2004; 0.20 – 0.62 in Lahanas et al., 1994), nucleotide diversity is higher than these populations (0.0018 – 0.0052 in Chassin-Noria et al., 2004; 0.004 – 0.005 in Lahanas et al., 1994), reflecting that the Yaeyama nesting population comprised a mixture of divergent haplotypes (Fig. 1). Genetic diversity represents the ability of populations to adapt or respond to environmental changes, and hence it is important to conserve Yaeyama nesting colonies for protecting this endangered species.

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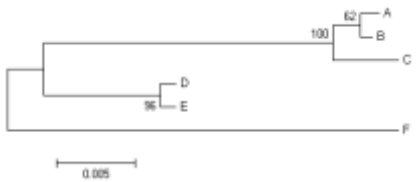

Fig. 1 Neighbor-joining tree based on the mtDNA control region sequences. Bootstrap values (1000 replicates) are indicated on the branches.

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