OPEN ACCESS

The Journal of Threatened Taxa is dedicated to building evidence for conservation globally by publishing peer-reviewed articles online every month at a reasonably rapid rate at www.threatenedtaxa.org. All articles published in JoTT are registered under Creative Commons Attribution 4.0 International License unless otherwise mentioned. JoTT allows unrestricted use of articles in any medium, reproduction, and distribution by providing adequate credit to the authors and the source of publication.

Journal of Threatened Taxa

Building evidence for conservation globally

www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

Article

Genetic diversity of the Green Turtle (Testudines: Cheloniidae: *Chelonia mydas* (Linnaeus, 1758)) population nesting at Kosgoda Rookery, Sri Lanka

E.M.L. Ekanayake, T. Kapurusinghe, M.M. Saman,
D.S. Rathnakumara, P. Samaraweera & R.S. Rajakaruna

26 June 2017 | Vol. 9| No. 6 | Pp. 10261–10268
10.11609/jott.2797.9.6.10261-10268

For Focus, Scope, Aims, Policies and Guidelines visit http://threatenedtaxa.org/About_JoTT
For Article Submission Guidelines visit http://threatenedtaxa.org/Submission_Guidelines
For Policies against Scientific Misconduct visit http://threatenedtaxa.org/JoTT_Policy_against_Scientific_Misconduct
For reprints contact <info@threatenedtaxa.org>

Publisher/Host

Partner

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 June 2017 | 9(6): 10261–10268

Genetic diversity of the Green Turtle (Testudines: Cheloniidae: *Chelonia mydas* (Linnaeus, 1758)) population nesting at Kosgoda Rookery, Sri Lanka

ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

OPEN ACCESS

E.M.L. Ekanayake [1], T. Kapurusinghe [2], M.M. Saman [3], D.S. Rathnakumara [4], P. Samaraweera [5] & R.S. Rajakaruna [6]

[1,6] Department of Zoology, Faculty of Science, University of Peradeniya, Galaha Road, Peradeniya 20400, Sri Lanka
Current address: [1] Bio Conservation Society (BCSL), 19/1/2, Heerassagala Road, Kandy, Sri Lanka
[1,6] Postgraduate Institute of Science, University of Peradeniya, Galaha Road, Peradeniya 20400, Sri Lanka
[1,2,3,4] Turtle Conservation Project (TCP), 72/4 Old Galle Road, Walana, Panadura, Sri Lanka
[5] Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka
[1] lalitheml@yahoo.com, [2] kjthushan@yahoo.co.uk, [3] samantcp@yahoo.com, [4] rathnakumaratcp@yahoo.com, [5] psam@pdn.ac.lk, [6] rupikar@pdn.ac.lk (corresponding author)

Abstract: We determined the genetic diversity of the Green Turtle *Chelonia mydas* (Linneaus, 1758) nesting at Kosgoda rookery, the second largest sea turtle aggregation on the southwestern coast of Sri Lanka. Skin tissue samples were collected from 68 nesting females and genetic diversity was estimated using six microsatellite loci. High genetic diversity was observed within the population as all loci analyzed were highly polymorphic with a total of 149 alleles observed. The mean number of alleles per locus was 24.7 and the mean observed and expected heterozygosity across all loci were 0.75 and 0.93, respectively. It appears that five out of six loci were not in Hardy-Weinberg equilibrium, while micro-checker analysis suggested that the Kosgoda Green Turtle population was possibly in equilibrium. The viability of a population is unlikely to be reduced if high genetic diversity is maintained within it. Although the Green Turtle population nesting at Kosgoda is small compared to other nesting rookeries in the world, the high genetic diversity observed suggests that the population may not be undergoing a bottleneck.

Keywords: Genetic diversity, heterozygosity, Indian Ocean, microsatellites, Sri Lanka

DOI: http://doi.org/10.11609/jott.2797.9.6.10261-10268 | **ZooBank:** urn:lsid:zoobank.org:pub:F2524EBF-A7D2-407F-A400-416A4B227D0C

Editor: Oguz Turkozan, Adnan Menderes University, Aydın, Turkey. **Date of publication:** 26 June 2017 (online & print)

Manuscript details: Ms # 2797 | Received 17 May 2016 | Final received 16 May 2017 | Finally accepted 31 May 2017

Citation: Ekanayake, E.M.L., T. Kapurusinghe, M.M. Saman, D.S. Rathnakumara, P. Samaraweera & R.S. Rajakaruna (2017). Genetic diversity of the Green Turtle (Testudines: Cheloniidae: *Chelonia mydas* (Linnaeus, 1758) population nesting at Kosgoda Rookery, Sri Lanka. *Journal of Threatened Taxa* 9(6): 10261–10268; http://doi.org/10.11609/jott.2797.9.6.10261-10268

Copyright: © Ekanayake et al. 2017. Creative Commons Attribution 4.0 International License. JoTT allows unrestricted use of this article in any medium, reproduction and distribution by providing adequate credit to the authors and the source of publication.

Funding: National Science Foundation, Sri Lanka (SIDA/2005/BT/01), UNDP-GEF/SGP small grant program, International Foundation for Science, Sweden (A/3863- 1).

Competing interests: The authors declare no competing interests.

Author Details: see end of this article.

Author Contribution: EMLE: Acquired and analyzed data and wrote the manuscript; TK: Supervised the data collection in the field; MMS and DSR: Collected data and samples from the field; PS: Contributed to study design and interpretation of data, supervised DNA work; RSR: Developed the concept, supervised the lab work and interpretation of data, edited and revised the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript.

Acknowledgements: We thank the Turtle Conservation Project (TCP) staff for their technical support and the Department of Wildlife Conservation (DWC), Sri Lanka for granting permission to carry out this study, Nancy FitzSimmons and M.P. Jensen for their valuable advice and guidance during the study, and Andrea Phillott for editing the manuscript. We also thank the National Science Foundation, Sri Lanka (SIDA/2005/BT/01), UNDP-GEF/SGP small grant program, and the International Foundation for Science, Sweden (A/3863- 1) for financial support.

INTRODUCTION

Conservation efforts are directed at preserving the existing genetic variation within endangered species (Lynch 1996). There are three basic measures of genetic variation: heterozygosity within individual genomes, allelic variation among individuals within a population, and allele frequency differences among populations. Genetic variation plays a critical role in the ability of populations to respond to changing environments, and thus it likely plays an important role in the survival and adaptability of species.

The Green Turtle *Chelonia mydas* is listed as Endangered in the IUCN Red List of Threatened Animals (Seminoff 2004). Knowledge of population structure is important in defining conservation priorities for this endangered species. Mitochondrial DNA (mtDNA) is often used to assess genetic diversity, population structure and migration patterns of sea turtles (Bjorndal et al. 2005; Formia et al. 2006; Naro-Maciel et al. 2007; Bowen & Karl 2007; LeRoux et al. 2012). Microsatellite loci have also been used to assess genetic variation within populations (FitzSimmons et al. 1995; Gullberg et al. 1997; Lee 2008) and among populations (DeWoody & Avise 2000). Microsatellite markers are ideal for studies of genetic diversity because of their high frequency in the genome, high polymorphism, co-dominance and the cost-effectiveness of analysis. Because microsatellites are bi-parentally inherited they can reveal patterns of sex-biased gene flow, especially when used in combination with mtDNA (FitzSimmons et al. 1997). Even DNA from degraded or poorly preserved specimens may contain a sufficient number of copies of a short microsatellite target sequence suitable for amplification (Queller et al. 1993). The earliest sea turtle microsatellite markers developed by FitzSimmons et al. (1995) and FitzSimmons (1998) are particularly well-used, with some loci having been applied to almost all of the sea turtle species studied using microsatellites (Lee 2008). Many new microsatellite markers have been developed for Green Turtles recently (Dutton & Frey 2009; Frey et al. 2013). Most of the studies use both mtDNA and microsatellite analysis to improve knowledge of the genetic diversity of sea turtles (Naro-Maciel et al. 2008; Yilmaz et al. 2011; Bagda et al. 2012).

Genetic studies have not been conducted for any of the five species of sea turtles nesting in Sri Lanka, although many studies have examined genetic structure of Green Turtles in the Indian Ocean (Karl et al. 1992; Dethmers et al. 2006; Bourjea et al. 2007; Dethmers & Baxter 2010). Dethmers et al. (2006) examined the mtDNA variation across 27 Green Turtle rookeries in Australasia and recorded 17 genetically distinct breeding stocks, regarded as management units, consisting either of individual rookeries or groups of rookeries separated by more than 500km. Bourjea et al. (2007) used mtDNA variation to analyze the genetic structure of southwestern Indian Ocean Green Turtle populations and detected gene flow from the Atlantic Ocean into the Indian Ocean via the Cape of Good Hope. The current study investigated the genetic diversity of the Green Turtle population nesting at Kosgoda Rookery using microsatellites. Although more than 90% of the nests recorded at Kosgoda Rookery are of Green Turtle (Ekanayake et al. 2008) its population is small compared to other rookeries in the world with a mean of 298.4 annual nests (Ekanayake et al. 2010), among which about half of the hatchlings show multiple paternity (Ekanayake et al. 2013a). Assessing the genetic diversity among and within individuals of this nesting population will provide important information for defining conservation priorities for this endangered species. This information is important in developing global, multi-scale regional management units as highlighted in Wallace et al. (2010) describing the complexities in sea turtle population structures and thus providing a flexible, dynamic framework for evaluating threats, identifying high diversity areas and most importantly bridging data gaps.

Kosgoda Turtle Rookery (6.550000 N & 80.033333 E) on the southwestern coast of Sri Lanka (Fig. 1) hosts the second largest Green Turtle rookery in Sri Lanka, with year-round nesting. We collected tissue samples from nesting females after they had completed the nesting process. A small area in one of the front flippers was cleaned with 95% ethanol and a skin scraping (~0.5cm^2) was taken using a sterile surgical blade. Samples were stored in 1.5ml centrifuge tubes containing 95% ethanol. The tag number was noted if the female had already been tagged, or a new tag was placed to ensure the same female was not sampled multiple times. Permission to collect tissue samples was obtained from the Department of Wildlife Conservation (DWC), Sri Lanka.

Extraction of genomic DNA and amplification of microsatellites

Approximately 0.1g of skin tissue from each sample was placed on a glass plate and cut into small pieces using a sterile surgical blade. The tissue was placed in 500µl of a 5% aqueous suspension of Chelex® (BioRad, Richmond, U.S.A.) in 1.5ml Eppendorf tube and incubated for three hours at 55^{0}C with continuous shaking. Samples were vortexed for 30s and placed in a heating block at 95^{0}C for

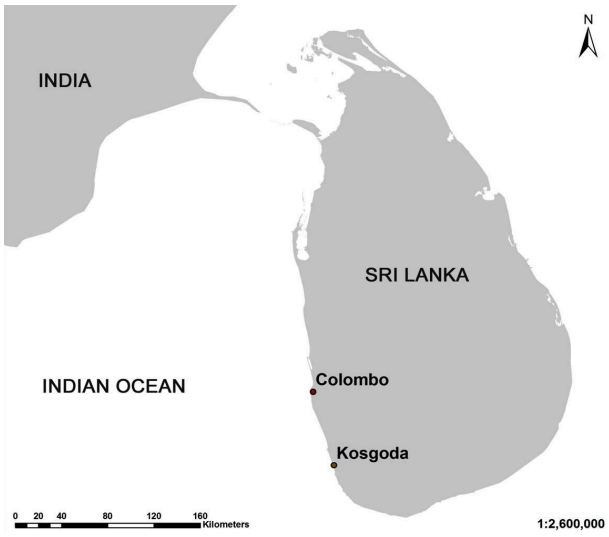

Figure 1. The Kosgoda nesting beach on the southwestern coast of Sri Lanka.

five minutes. After centrifugation at 11,600g (RCF) for five minutes, the supernatant containing extracted DNA was removed and stored at -20^{0}C.

Six di-nucleotide microsatellite loci, Cm3, Cm58, Cm72, Cm84, Cc117 (FitzSimmons et al. 1995) and Cc7 (FitzSimmons 1998), were amplified by polymerase chain reaction (PCR). Four of these loci (Cm3, Cm58, Cm72, and Cm84) were developed from Green Turtle DNA (FitzSimmons et al. 1995) and the remaining two (Cc117, Cc7) were from Loggerhead Turtle DNA (FitzSimmons et al. 1995; FitzSimmons 1998). The PCR mixture contained 3µl of template DNA, 0.5 µM of each primer (forward and reverse), 0.2mM dNTPs, 0.5 unit of Taq DNA polymerase, 1.5 µl of 10 x PCR buffer with $MgCl_2$ in a final volume of 10 µl. The amplification procedure consisted of initial denaturation at 95^{0}C for 2 min followed by 35 cycles with primer annealing at 55^{0}C for Cc7, 62^{0}C for Cm3, Cm58, Cm72 and Cc117, or 64^{0}C for Cm84 for 1 min, strand synthesis at 72^{0}C for 1 min and denaturation at 95^{0}C for 45 sec, followed by final extension at 72^{0}C for 7 min.

Polyacrylamide gel electrophoresis

Amplified products were separated by electrophoresis on a 6% denaturing polyacrylamide gel. To denature the samples, 1.5µl of loading buffer was mixed with 10µl of PCR product and incubated at 95^{0}C for five minutes. Tubes were then immediately placed on ice for rapid cooling. The samples were loaded and the gel (48cm) was run at 1,400v for three hours at 45–50 ^{0}C using a 20bp DNA ladder as a size standard (Bangalore Genei, Pvt Ltd. India). Gels were fixed with 100ml of 5% acetic acid for 20 minutes with gentle shaking, washed twice with distilled water and stained with 0.4% silver nitrate for 20 minutes, then washed in distilled water. DNA bands were visualized after developing the gels in 3% NaOH. A solution of 5% acetic acid was added to prevent further development. The size of the DNA bands was determined using a gel documentation system (Bio-Vision, Germany, 3000-WL/26M) and Vision-Capt (Version 14.3) software. Alleles of approximately one bp difference in size were scored to the nearest even number.

Data analysis

The number of alleles for each locus was determined, and the mean number of alleles per locus and the allele frequencies for each locus were calculated. Deviations from Hardy-Weinberg equilibrium (HWE) and heterozygosity (observed and expected) for each locus were calculated by the Markov chain method, following the exact test of Guo & Thompson (1992) with 1,000,000 forecasted chain length and 100,000 dememorization steps using Arlequin software (Version 3.1, Excoffier, Laval, Schneider, 2005). Estimates of within population structure (fixation indices, F_{IS}), analysis of molecular variance (AMOVA, Excoffier et al. 1992), measures of genetic diversity, and tests of pairwise linkage disequilibrium (Slatkin & Excoffier 1996) were also calculated using Arlequin software. The microsatellite data were analysed using the software Micro-Checker (Version 2.2.3) to check for null alleles (van Oosterhout et al. 2004).

RESULTS

All microsatellite loci were highly polymorphic (Table 1). A total of 149 alleles were observed for the six microsatellites (Fig. 2). The most polymorphic locus was Cm72, with 40 alleles, and the least polymorphic was Cm58, with 15 alleles (Fig. 2). The mean number of alleles per locus was 24.7. Although there were predominant alleles observed in all loci, no allele had a frequency of greater than 20%. A high proportion of uncommon (< 5% occurrence) alleles were observed at each locus, ranging from 46.7% (at Cm58) to 85.0% (at Cm72) of alleles (Fig. 2). The mean observed and expected heterozygosity across all loci was 0.75 (range 0.66–0.86) and 0.93 (range 0.89–0.97), respectively (Table 1). The Arlequin analysis indicated all loci (exact test, $p<0.05$) displayed deviations from HWE due to a deficit of heterozygous individuals. The mean F_{IS} over all loci was 0.2; the F_{IS} for each locus is given in Table 1. The positive F_{IS} values indicate that there are fewer heterozygous individuals than would be

Table 1. Microsatellite data from six loci for the Green Turtle population nesting at Kosgoda, Sri Lanka, including observed and expected heterozygosity and results of tests for Hardy-Weinberg equilibrium and F_{IS} estimates

Locus	Sample size	No. of alleles	Allelic range	Observed het	Expected het	F_{IS}
Cm58	63	15	118-148	0.81*	0.89	0.09*
Cm3	60	26	136-208	0.75**	0.93	0.20***
Cm72	55	40	226-328	0.73**	0.97	0.25***
Cc117	55	22	206-256	0.75**	0.93	0.20***
Cm84	57	25	304-366	0.67***	0.94	0.30***
Cc7	61	20	148-198	0.82***	0.93	0.12**
Mean ±SD	57±7.2	24.7±8.57	-	0.75±0.06	0.93±0.03	0.20

*indicates p < 0.05, ** indicates p < 0.01, *** indicates p < 0.001. Het = heterozygosity; FIS = fixation indices

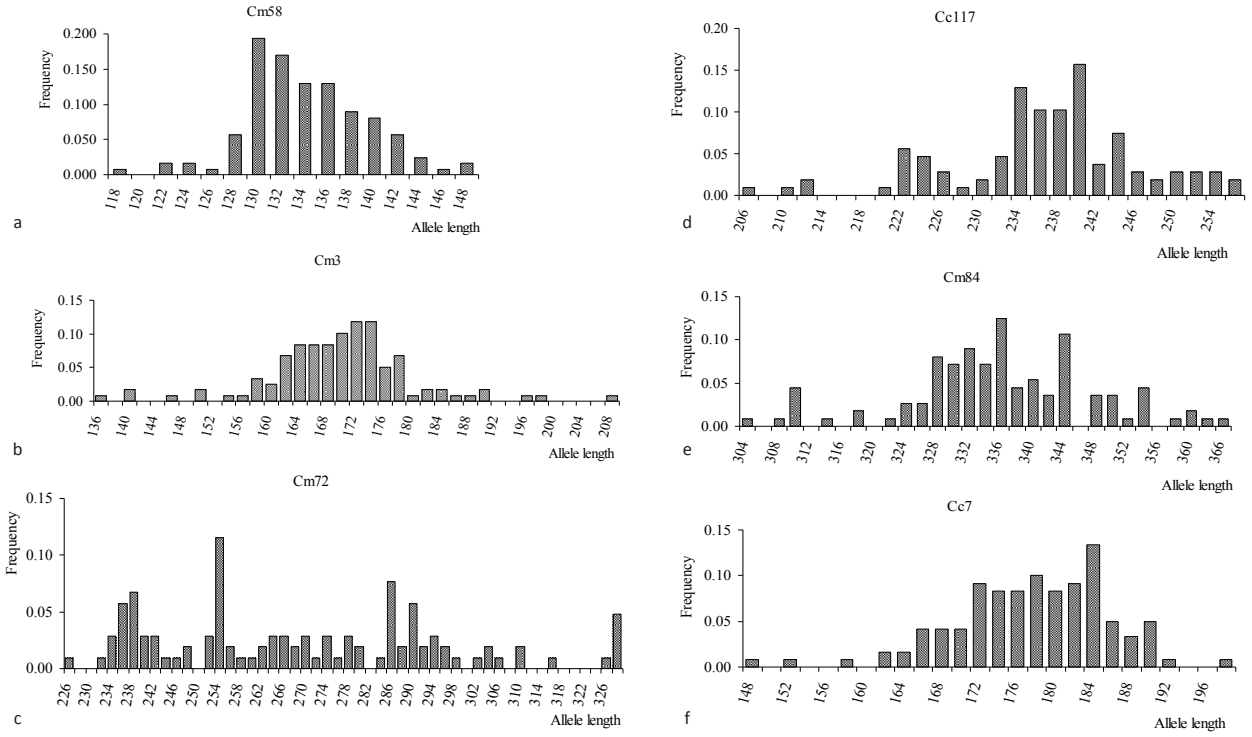

Figure 2. Allele frequencies for the six microsatellite loci (a = Cm58, b = Cm3, c = Cm72, d = Cc117, e = Cm84 and f = Cc7) of Green Turtles at the Kosgoda Turtle Rookery, Sri Lanka.

Table 2. Results from the Micro-Checker Version 2.2.3

Locus	Null alleles	Stuttering	Large allele dropout
Cm58	No	No	No
Cm3	Yes	No	No
Cm72	Yes	No	No
Cc117	Yes	Yes	No
Cm84	Yes	No	No
Cc7	Yes	No	No

expected for all the loci, suggesting possible inbreeding. Hence, about 20% of the population would be expected to have both alleles derived from inbreeding at one locus at least; however, the observed heterozygosity may be an underestimation due to the presence of null alleles. Therefore, data were analyzed using Micro-Checker software that detects the occurrence of null alleles. All loci, except Cm58, have null alleles (Table 2) therefore this population is possibly in HWE.

The mean gene diversity, as estimated by Arlequin, over all six loci was 0.65 (± 0.36, SD). The nested analysis of molecular variance (AMOVA) was calculated (average

Table 3. Calculated p values from HWE test results for the linkage disequilibrium of all pairs of loci (above diagonal) and presence of linkage disequilibrium (below diagonal)

Locus	Cm58	Cm3	Cm72	Cc117	Cm84	Cc7
Cm58	*	0.55545	0.00158	0.02158	0.00129	0.15812
Cm3	-	*	0.00000	0.00139	0.00564	0.02109
Cm72	+	+	*	0.00000	0.00248	0.00139
Cc117	+	+	+	*	0.00248	0.39040
Cm84	+	+	+	+	*	0.00941
Cc7	-	+	+	-	+	*

Significant linkage disequilibrium indicated by $P < 0.05$ and presence (+) or absence (-)

Table 4. Genetic variation at microsatellite loci in nesting Green Turtle populations around the world. Modified and updated from Robert et al. 2004

Location	Sample size	Microsatellite loci												Source**
		Cm3		Cm58		Cm72		Cm84		Cc117		Cc7		
		A	H	A	H	A	H	A	H	A	H	A	H	
Ascension	46	8	0.44	15	0.80	27	0.65	21	0.78	-	-	-	-	1
Brazil	21	9	0.52	10	0.71	19	0.76	21	0.81	-	-	-	-	1
Venezuela	44	9	0.37	9	0.59	26	0.86	26	0.55	-	-	-	-	1
Florida	21	9	0.71	8	0.48	16	0.86	22	0.86	-	-	-	-	1
Cyprus	25	5	0.40	7	0.76	18	0.80	14	0.68	-	-	-	-	1
Suriname	15	9	0.67	7	0.73	14	0.80	16	1.00	-	-	-	-	1
Africa	19	6	0.53	8	0.68	18	0.84	10	0.79	-	-	-	-	1
Quintana Roo	7	4	0.57	7	0.86	9	1.00	10	1.00	-	-	-	-	1
Costa Rica	49	11	0.69	11	0.67	31	0.94	26	0.86	-	-	-	-	1
Hawaii	22	13	0.82	8	0.41	18	0.64	19	0.73	-	-	-	-	1
Australia	16	15	0.75	8	0.81	19	0.88	16	0.88	-	-	-	-	1
Galapagos	8	9	0.88	5	0.88	12	0.75	14	0.88	-	-	-	-	1
Michoacan	7	7	0.57	6	0.71	11	0.86	12	0.86	-	-	-	-	1
Polynesia	3	4	1.00	4	0.67	4	0.33	5	1.00	-	-	-	-	1
Japan	19	10	0.74	8	0.74	20	1.00	18	1.00	-	-	-	-	1
Oman	15	13	0.53	9	0.80	15	0.87	17	0.87	-	-	-	-	1
Western Australia	69.9 ± 8.0	22	0.91*	12	0.85*	33	0.96*	-	-	16	0.88*	-	-	2
Gulf of Carpentaria	40.3 ± 7.3	18	0.87*	10	0.84*	28	0.94*	-	-	13	0.81*	-	-	2
Northern GBR	65.8 ± 3.3	23	0.92*	10	0.81*	35	0.96*	-	-	19	0.91*	-	-	2
Southern GBR	98.0 ±5.4	22	0.92*	10	0.96*	37	0.96*	-	-	19	0.88*	-	-	2
Ubatuba, Brazil	114	14	0.61	11	0.77	33	0.97	16	0.78	-	-	13	0.82	3
Almofala, Brazil	117	16	0.60	11	0.79	35	0.93	19	0.85	-	-	19	0.82	3
Kosgoda, Sri Lanka	57.0 ± 3.2	25	0.79	16	0.86	41	0.74	23	0.77	24	0.76	20	0.82	4

Number of alleles (A) and observed heterozygosity (H) are shown. * Expected heterozygosity; GBR = Great Barrier Reef
** 1 - Robert et al. 2004; 2 - FitzSimmons et al. 1997; 3 - Naro-Maciel et al. 2007; 4 - Present Study

over 6 loci) and genetic variation of 19.3% among individuals (variance components, 0.54) and 80.7% within individuals (variance components, 2.26) were observed.

Pairwise significance linkage disequilibrium ($p<0.05$) was observed for many pairs of loci (Table 3).

DISCUSSION

A high genetic diversity within and among individuals of the Green Turtle population nesting at Kosgoda Rookery was observed. All six microsatellite loci tested were highly polymorphic in the Kosgoda Green Turtle population. According to population genetics theory, a locus is considered polymorphic when the frequency of the most common allele is equal to, or less than, 0.99 (Ahmad et al. 1977). The mean number of alleles per locus, and the number of alleles per locus, was higher in Green Turtles nesting at Kosgoda when compared with other nesting Green Turtle populations (Table 4). A population with high genetic diversity has a greater chance of possessing at least some individuals with a genetic constitution that enables survival if new pressures, such as environmental disasters, occur. If genetic diversity is very low, the population is at risk as none of the individuals may possess characteristics that allow adaptation to the new environmental conditions. Such a population could suddenly disappear. Although the nesting Green Turtle population at Kosgoda is not very large, viability of the population in the next few generations is unlikely to be substantially reduced by genetic factors.

Genetic diversity in a population can also be estimated by the mean levels of heterozygosity in addition to the mean number of alleles per locus, or the percentage of polymorphic loci. Heterozygosity is an important measurement of population diversity at the genetic level, and has drawn much attention from ecologists and aquaculturists (Xu et al. 2001). Conservation of genetic variability is important to the overall viability of populations, because decreased genetic variability leads to increased levels of inbreeding and reduced fitness (Young et al. 1996; Frankham 2006). When analyzed using Arlequin software, heterozygosity deficiency was observed in the Kosgoda Green Turtle population; however, the mean H_o was within the range of many other Green Turtle populations in the world (Table 4). High levels of heterozygosity in Green Turtle populations has been reported in several studies using microsatellite markers (FitzSimmons et al. 1995; Rico et al. 1996; Norris et al. 1999). The H_o was higher than the H_e in most of the population studies by Roberts et al. (2004). The H_o of the Kosgoda population of nesting Green Turtles was less than H_e for all six loci based on tests for HWE. Hence, the Kosgoda Green Turtle population appears to deviate from HWE. Deviation from HWE at some microsatellite loci was reported by Roberts et al. (2004) in studies on Green Turtle populations. Reduced heterozygosity is one of the factors contributing to departure from HWE, and may result from one or more factors including the presence of null alleles (Callen et al. 1993; Pemberton et al. 1995; Roberts et al. 2004), small sample size and inbreeding (Kumar et al. 2006). Null alleles might occur at microsatellite loci, resulting in weak or absent bands after amplification (O'Connell & Wright 1997), a disadvantage of microsatellite markers. According to Shriver et al. (1995) the problem of small sample size can be overcome by using multiple independent loci to increase resolution and statistical confidence. Even with arguments for larger sample sizes, a sample size of 15 per location has been determined as sufficient for genetic analyses of sea turtles (including microsatellite surveys; Roberts et al. 2004). In the current study, 68 samples were analysed with six microsatellite loci; therefore, the sample size can be considered sufficient to resolve the genetic diversity of the nesting Green Turtle population at Kosgoda.

Although the mean F_{IS} value indicated the heterozygote deficiency, the results of analysis by Micro-Checker, which takes into account the presence of null alleles, suggested that the Kosgoda nesting Green Turtle population was possibly in HWE. Hence, heterozygosity deficiency could be due to the presence of null alleles. The stutter bands cause problems in differentiation between homozygous and heterozygous individuals (Luty et al. 1990; DeWoody et al. 2006) and can contribute to high heterozygosity. The Micro-Checker result, however, suggested stuttering might have resulted in scoring errors only in locus Cc117 (Table 2). Therefore, the contribution of stutters to heterozygosity observed in the present study may be negligible; however, gene flow from outside populations would be beneficial to avoid inbreeding and the future erosion of genetic diversity.

The nest paternity of the Green Turtle population at Kosgoda was analyzed using the same microsatellites for the six loci and reported evidence of multiple paternity with 47% of the clutches are being sired by two (62.5%) or three (37.5%) males (Ekanayake et al. 2013a). Further, the results of successive clutch analysis show that the dominant father sired 50.0% of the total offspring followed by 33.3% by the second male. Moreover, the study also reported the same paternal alleles at all six loci in successive clutches, suggesting that the male or males that sired the first clutch also sired the others for a given female. This provides evidence for multiple mating with the same male during a nesting season and/or sperm storage. In populations where multiple matings occur, knowledge of its prevalence and effects on paternity distribution within a natural assemblage is critical to

comprehend population structure. This information can therefore be of great importance to the management and conservation of threatened species such as sea turtles.

Rekawa and Kosgoda have been identified as major Green Turtle nesting rookeries in Sri Lanka, and many minor rookeries have also been identified (Amarasooriya 2000). Green Turtles from Rekawa and Kosgoda rookeries have migrated thousands of kilometers across the seas to the east and west of Sri Lanka (Ekanayake et al. 2013b; Richardson et al. 2013). Although the Green Turtles nesting at these rookeries may be different populations, there is a high possibility of mixing within these populations or with nesting populations from other countries while at the feeding grounds or along migratory pathways. The Green Turtle population that nests at the Kosgoda Rookery of Sri Lanka has high genetic diversity. Despite its small size, its high genetic diversity will be vital to carry all the healthy useful characters to the progeny. If the Kosgoda Green Turtle population is declining, it is not due to genetic factors and hence, necessary actions should be taken to reduce natural and anthropogenic threats to the population. Although it was initially planned to carry out the study using all the major Green Turtle nesting sites in Sri Lanka, the permits (from the Department of Wildlife Conservation, Sri Lanka) were issued to collect samples only from the Kosgoda Rookery. Further genetic sampling and analysis is needed from other Green Turtle rookeries in the country to further understand the genetic diversity of the Green Turtles in Sri Lanka and determine if they are of different genetic stock.

The sea turtle population nesting at Rekawa has declined by ~60% during the last decade (Ekanayake et al. 2012). Although a similar decline was not observed in the Green Turtle population nesting at the Kosgoda Rookery (Ekanayake et al. 2010), nesting population is small and they face many threats. Information about population structure is important in defining conservation priorities for this species, as the viability of a population is unlikely to be reduced if high genetic diversity is maintained.

REFERENCES

Ahmad, M., D.O.F. Skibinski & J.A. Beardmore (1977). An estimate of the amount of genetic variation in the common mussel *Mytilus edulis. Biochemical Genetics* 15(9/10): 833–846.

Amarasooriya, K.D. (2000). Classification of marine turtle nesting beaches of southern Sri Lanka, pp. 228–237. In: Pilchar, N. & G. Ismail (eds.). *Sea Turtles of The Indo Pacific, Research, Management and Conservation*. Proceedings of the second ASEAN Symposium and workshop on sea turtle biology and conservation.

Bagda, E., F. Bardakci & O. Turkozan (2012). Lower genetic structuring in mitochondrial DNA than nuclear DNA among the nesting colonies of Green Turtles (*Chelonia mydas*) in the Mediterranean. *Biochemical Systematics and Ecology* 43: 192–199.

Bjorndal, K.A., A.B. Bolten & S. Troeng (2005). Population structure and genetic diversity in green turtles nesting at Tortuguero, Costa Rica, based on mitochondrial DNA control region sequences. *Marine Biology* 147: 1449–1457.

Bourjea, J., S. Lapegue, L. Gagnevin, D. Broderick, J.A. Mortimer, S. Ciccione, D. Roos, C. Taquet & H. Grizel (2007). Phylogeography of the green turtle, *Chelonia mydas* in the southwest Indian Ocean. *Molecular Ecology* 16: 175–186.

Bowen, B.W. & S.A. Karl (2007). Population genetics and phylogeography of sea turtles. *Molecular Ecology* 16: 4886–4907.

Callen, D.F., A.D. Thompson, Y. Shen, H.A. Phillips, R.I. Richards, J.C. Mulley & G.R. Sutherland (1993). Incidence and origin of "null" alleles in the (AC)n microsatellite markers. *American Journal of Human Genetics* 52: 922–927.

Dethmers, K.E.M. & P.W.J. Baxter (2010). Extinction risk analysis of exploited Green Turtle stocks in the Indo-Pacific. *Animal Conservation* 14: 140–150.

Dethmers, K.E.M., D. Broderick, C. Moritz, N.N. FitzSimmons, C.J. Limpus, S. Lavery, S. Whiting, S., Guinea, R.I.T Prince & R. Kennett (2006). The genetic structure of Australasian Green Turtles (*Chelonia mydas*): exploring the geographical scale of genetic exchange. *Molecular Ecology* 15: 3931–3946.

DeWoody, J., J.D. Nason & V.D. Hipkins (2006). Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes* 6: 951–957.

DeWoody, J.A. & J.C. Avise (2000). Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology* 56: 461–473;
http://doi.org/10.1371/journal.pone.0015465

Dutton, P.H. & A. Frey (2009). Characterization of polymorphic microsatellite markers for the green turtle (*Chelonia mydas*). *Molecular Ecology Resources* 9: 354–356.

Ekanayake, E.M.L., R.S. Rajakaruna, T. Kapurusinghe, M.M. Saman, P. Samaraweera & K.B. Ranawana (2012). A declining trend of nesting frequency of sea turtles at the largest rookery in Sri Lanka. T.T. Jones and B.P. Wallace (Comp.). *Proceedings of the thirty-first Annual Symposium on Sea Turtle Biology and Conservation.* NOAA Technical Memorandum, NOAA NMFS-SEFSC-631, 201.

Ekanayake, E.M.L., T. Kapurusinghe, M.M. Saman, & A.M.D.S. Rathnakumara (2013b). Re-nesting movements and post-nesting migrations of green turtles tagged in two turtle rookeries in Sri Lanka. J. Blumenthal, A. Panagopoulou, and A.F. Rees, Compilers. *Proceedings of the Thirtieth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-640, 123.

Ekanayake, E.M.L., T. Kapurusinghe, M.M. Saman, P. Samaraweera, K.B. Ranawana, & R.S. Rajakaruna (2010). Nesting Behaviour of the Green Turtle at Kosgoda Rookery, Sri Lanka. *Ceylon Journal of Science (Biological Science*) 39(2): 109–120.

Ekanayake, E.M.L., T. Kapurusinghe, M.M. Saman, P. Samaraweera, K.B. Ranawana and R.S. Rajakaruna (2013a). Paternity of Green Turtles (*Chelonia mydas*) clutches laid at Kosgoda, Sri Lanka. *Herpetological Conservation and Biology* 8: 27–36.

Ekanayake, E.M.L., T. Kapurusinghe, M.M. Saman, R.S. Rajakaruna, P. Samaraweera & K.B. Ranawana (2008). Nesting Frequency of Marine Turtles Visiting on Kosgoda Beach, Southern Sri Lanka. Rees, A.F., M. Frich, A. Panagopoulou & K. Williams (Comp.). *Proceedings of the Twenty-Seventh Annual Symposium on Sea Turtle Biology and Conservation*, South Carolina, USA. NOAA Technical Memorandum NMFS-SEFSC-569: 232–233.

Excoffier, L., G. Laval & S. Schneider (2005). Arlequin, version 3.1: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.

Excoffier, L., P. Smouse & J. Quattro (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.

FitzSimmons, N.N. (1998). Single paternity of clutches and sperm storage in the promiscuous green turtle (*Chelonia mydas*). *Molecular Ecology* 7: 575–584.

FitzSimmons, N.N., C. Moritz & S.S. Moor (1995). Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Molecular Biology and Evolution* 12(3): 432–440.

FitzSimmons, N.N., C. Moritz, C.L. Limpus, L. Pope & R. Prince (1997). Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian Green Turtle populations and male-biased gene flow. *Genetics* 147: 1843–1854.

Formia, A., B.J. Godley, J.F. Dontaine & M.W. Bruford (2006). Mitochondrial DNA diversity and phylogeography of endangered Green Turtle (*Chelonia mydas*) populations in Africa. *Conservation Genetics* 7: 353–369.

Frankham, R. (2006). Genetics and Extinction. *Biological Conservation* 126: 131–140.

Frey, A., P.H. Dutton & G.H. Balazs (2013). Insights on the demography of cryptic nesting by green turtles (*Chelonia mydas*) in the main Hawaiian Islands from genetic relatedness analysis. *Journal of Experimental Marine Biology and Ecology* 442: 80–87.

Gullberg, A., H. Tegelstrom & M. Olsson (1997). Microsatellites in the Sand Lizard *(Lacerta agilis)*: description, variation, inheritance, and applicability. *Biochemical Genetics* 35: 281–295.

Guo, S.W. & E.A. Thompson (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48(2): 361–372.

Karl, S.A., B.W. Bowen & J.C. Avise (1992). Global population genetic structure and male-mediated gene flow in the green turtle *(Chelonia mydas)*: RFLP analyses of anonymous nuclear loci. *Genetics* 131: 163–173.

Kumar, S., J. Gupta, N. Kumar, K. Dikshit, N. Navani, P. Jain & M. Nagarajan (2006). Genetic variation and relationships among eight Indian Riverine Buffalo breeds. *Molecular Ecology* 15: 593–600.

Lee, P.L.M. (2008). Molecular ecology of marine turtles: New approaches and future directions. *Journal of Experimental Marine Biology and Ecology* 356: 25–42.

LeRoux, R.A., P.H. Dutton, F.A. Abreu-Grobois, C.J. Lagueux, C.L. Campbell, E. Delcroix, E. Chevalier, J.A. Horrocks, Z. Hillis0starr, S. Troeng, E. Harrison & S. Stapleton (2012). Re-examination of population structure and phylogeography of Hawksbill Turtles in the wider Caribbean using longer mtDNA sequences. *Journal of Heredity* 103(6): 806–820.

Luty, J.A., Z. Guo, H.F. Willard, D.H. Ledbetter, S. Ledbetter & M. Litt (1990). Five polymorphic microsatellite VNTRs on the human X chromosome. *American Journal of Human Genetics* 46(4): 776–783.

Lynch, M. (1996). A quantitative-genetic perspective on conservation issues, pp. 471–501. In: Avise, J.C. & J.L. Hamrick (eds.). *Conservation Genetics: Case Studies from Nature*. Chapman and Hall, New York.

Naro-Maciel, E., J.H. Becker, E.H.S.M. Lima, M.A. Marcovaldi & R. Desalle (2007). Testing dispersal hypotheses in foraging green Sea Turtles (*Chelonia mydas*) of Brazil. *Journal of Heredity* 98(1): 29–39.

Naro-Maciel, E., M. Le, N.N. FitzSimmons & G. Amato (2008). Evolutionary relationships of marine turtles: a molecular phylogeny based on nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution* 49: 659–662.

Norris, A.T., D.G. Bradley & E.P. Cunningham (1999). Microsatellite genetic variation between and within farmed and wild Atlantic Salmon (*Salmo salar*) populations. *Aquaculture* 180: 247–264.

O'Connell, M. & J.M. Wright (1997). Microsatellite DNA in fishes. *Biomedical and Life Sciences* 7(3): 331–363.

Pemberton, J.M., J. Slate, D.R. Bancroftt & J.A. Barrett (1995). Nonamplifying alleles at microsatellite a caution for parentage and population loci: studies. *Molecular Ecology* 4: 249–252.

Queller, D.C., J.E. Strassmann & C.R. Hughes (1993). Microsatellites and kinship. *Trends in Ecology and Evolution* 8: 285–288.

Richardson, P.B., A.C. Broderick, M.S. Coyne, L. Ekanayake, T. Kapurusinghe, C. Premakumara, S. Ranger, M.M. Saman, M.J. Witt & B.J. Godley (2013). Satellite telemetry reveals behavioural plasticity in a Green Turtle population nesting in Sri Lanka. *Marine Biology* 160(6): 1415–1426.

Rico, C., I. Rico & G. Hewitt (1996). 470 Million years of conservation of microsatellite loci among fish species. *Proceedings of the Royal Society Biological Sciences* 263(1370): 549–557; http://doi.org/10.1098/rspb.1996.0083

Roberts, M.A., T.S. Schwartz & S.A. Karl (2004). Global population genetic structure and male-mediated gene flow in the Green Sea Turtle (*Chelonia mydas*): analysis of microsatellite loci. *Genetics* 166: 1857–1870.

Seminoff, J.A. (2004). Guest editorial: Sea Turtles, Red Listing, and the need for regional assessments. *Marine Turtle Newsletter* 106: 4–6.

Shriver, M.D., L. Jin, E. Boerwinkle, R. Deka, R.E. Ferrell & R. Chakraborty (1995). A novel measure of genetic distance for highly polymorphic tandem repeat loci. *Molecular Biology and Evolution* 12(5): 914–920.

Slatkin, M. & L. Excoffier (1996) Testing for linkage disequilibrium in genotypic data using the EM algorithm. *Heredity* 76: 377–383.

van Oosterhout, C., W.F. Hutchinson, D.P.M. Wills & P. Shipley (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.

Wallace, B.P., A.D. DiMatteo, B.J. Hurley, E.M. Finkbeiner, A.B. Bolten, M.Y. Chaloupka, B.J. Hutchinson, F.A. Abreu-Grobois, D. Amorocho, K.A. Bjorndal, J. Bourjea, B.W. Bowen, R.B. Dueñas, P. Casale, B.C. Choudhury, A. Costa, P.H. Dutton, A. Fallabrino, A. Girard, M. Girondot, M.H. Godfrey, M. Hamann, M. López-Mendilaharsu, M.A. Marcovaldi, J.A. Mortimer, J.A. Musick, R. Nel, N.J. Pilcher, J.A. Seminoff, S. Troëng, B. Witherington & R.B. Mast (2010). Regional Management Units for marine turtles: A novel framework for prioritizing conservation and research across multiple scales. *PLoS ONE* 5(12): e15465; http://doi.org/10.1371/journal.pone.0015465

Xu, Z., J.H. Primavera, L.D. De la Pena, P. Pettil, J. Belak & A. Alcivar-Waren (2001). Genetic diversity of wild and cultured Black Tiger Shrimp *Penaeus monodon* in the Philippines using microsatellites. *Aquaculture* 199: 13–40.

Yilmaz, C., O. Turkozan & F. Bardakci (2011). Genetic structure of Loggerhead Turtle (*Caretta caretta*) populations in Turkey. *Biochemical Systematics and Ecology* 39: 266–276.

Young, A., T. Boyle & T. Brown (1996). The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution* 11(10): 413–418.

Author Details: E.M.L. Ekanayake completed his BSc, M.Phil and PhD degrees from the University of Peradeniya. He was the Research Coordinator at the Turtle Conservation Project (TCP), Sri Lanka and has been involved in sea turtle research since 1996. Currently he is the Chairman of the Bio-Conservation Society of Sri Lanka (BCSL). T. Kapurusinghe started his wildlife career in 1985 and he is the Project Leader and Co-founder of the Turtle Conservation Project (TCP) Sri Lanka. He completed his MSc in Conservation and Tourism at Durrell Institute of Conservation and Ecology (DICE) at the University of Kent at Canterbury in the UK. He has been involved in sea turtle conservation work for over three decades. M.M. Saman was a research officer at the TCP Sri Lanka and has been working on sea turtle research for 21 years. D.S. Rathnakumara was a research officer at TCP and also former Wildlife Officer. He has been carrying out research on sea turtles for 20 years. Preminda Samaraweera received his BSc in Chemistry from the University of Peradeniya, Sri Lanka and PhD in Biochemistry from the University of Arizona, USA. He is a senior lecturer in the Department of Molecular Biology and Biotechnology at the University of Peradeniya. Rupika S. Rajakaruna received her BSc (Hons.) and MPhil degrees in Zoology from the University of Peradeniya and completed her PhD at the Memorial University of Newfoundland, Canada. She currently holds the Chair of Professor of Applied Zoology at the University of Peradeniya. She has collaborated with many scientists on publications pertaining to a wide range of fields including sea turtle biology and conservation, parasitology and frog disease ecology.

Journal of Threatened Taxa

OPEN ACCESS

The Journal of Threatened Taxa is dedicated to building evidence for conservation globally by publishing peer-reviewed articles online every month at a reasonably rapid rate at www.threatenedtaxa.org. All articles published in JoTT are registered under Creative Commons Attribution 4.0 International License unless otherwise mentioned. JoTT allows unrestricted use of articles in any medium, reproduction, and distribution by providing adequate credit to the authors and the source of publication.

ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

June 2017 | Vol. 9 | No. 6 | Pages: 10249–10368
Date of Publication: 26 June 2017 (Online & Print)
DOI: 10.11609/jott.2017.9.6.10249-10368
www.threatenedtaxa.org

Articles

Communications

Short Communications

Notes

zooh! ZÜRICH

WILD ZOO Threatened Taxa