Herpetological Conservation and Biology 8(1):27–36.
Submitted: 28 March 2012; Accepted: 23 January 2013; Published: 30 April 2013.

PATERNITY OF GREEN TURTLE (*CHELONIA MYDAS*) CLUTCHES LAID AT KOSGODA, SRI LANKA

E.M.L. EKANAYAKE[1,2,3], T. KAPURUSINGHE[3], M.M. SAMAN[3], D.S. RATHNAKUMARA[3], P. SAMARAWEERA[4], K.B. RANAWANA[1] AND R.S. RAJAKARUNA[1,5]

[1]*Department of Zoology, University of Peradeniya, Sri Lanka*
[2]*Postgraduate Institute of Science, University of Peradeniya, Sri Lanka*
[3]*Turtle Conservation Project, No. 11, Perera Mawatha, Madakumbura, Panadura, Sri Lanka*
[4]*Department of Molecular Biology and Biotechnology, University of Peradeniya,Sri Lanka*
[5]*Corresponding author, e-mail: rupikar@pdn.ac.lk, Tel: +9481 2394481*

Abstract.*—Adult sea turtles have multiple mates, but the frequency of multiple paternity varies between rookeries and among species. Multiple mating can influence the strength of sexual selection, the effective population size, genetic variability and introgression within a population. We determined paternity in the offspring of 19 female Green Sea Turtles (*Chelonia mydas*) nesting at Kosgoda rookery (Sri Lanka) using microsatellite markers at six loci. We collected tissue samples from the nesting female and 10 hatchlings from each clutch. We examined 24 clutches including single clutches of 17 females and three or four successive clutches of two females. Clutches of 47% of the females were sired by two (62.5%) or three (37.5%) fathers. The successive clutch analysis showed that the dominant father sired 50.0% of the total offspring followed by 33.3% by the second male. We found the same paternal alleles at all six loci in all the successive clutches. This suggests that the male or males that sired the first clutch also sired the other clutches for a given female. This provides evidence for multiple mating with the same male during a nesting season and/or sperm storage. Although the size of the females that laid clutches with multiple paternity were typically smaller than the females with single paternity clutches, this difference was not significant. There was no evidence that the same male had fathered offspring with multiple females. Although Green Turtles are highly promiscuous in their mating behavior and are known to store sperm, fewer than half of the females at Kosgoda rookery laid clutches with multiple paternity. 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.

Key Words.—Chelonia mydas; microsatellites; multiple paternity; sea turtles; Sri Lanka

INTRODUCTION

The mating system and frequency of multiple mating by male and female animals are important factors in the evolution of animal life-history (Zbinden et al. 2007). Multiple mating can influence the strength of sexual selection (Fleming and Gross 1994; Evans and Magurran 1999), the effective population size (Sugg and Chesser 1994), genetic variability and introgression within a population (Baer and Schmid-Hempel 1999). Natural selection promotes multiple matings in males as it increases the male's contribution to the next generation with the number of mates. Multiple mating in females ensures fertilization of all ova as the quantity of sperm from one male may not be sufficient to fertilize all the ova and/or multiple mating also provides access to resources from additional males (Birkhead and Moller 1993; Fedorka and Mousseau 2002; Tregenza and Wedell 2002; Zbinden et al. 2007). In populations where multiple matings occur, knowledge of its prevalence and effects on paternity distribution within a natural assemblage is vital to understand population structure (Jensen et al. 2006). This information can therefore be of great importance to the management and conservation of threatened species (Kichler et al. 1999; Moore and Ball 2002).

Turtles do not show pair bonding, cohesive social grouping, or provide parental care beyond nesting (Pearse and Avise 2001). The male contribution to offspring is limited to fertilization and genetic effects, and female turtles do not receive any direct benefits such as territory access, nuptial gifts or protection from males (Pearse and Avise 2001). Mating with one male is sufficient to fertilize all the ova of a female turtle for an entire reproductive season (FitzSimmons 1998; Pearse and Avise 2001). The physical act of mating can be dangerous to the female turtle, and the risk of predation or disease transmission increases with multiple mating (Miller 1997; Loehle 1997). Nevertheless, sea turtles are promiscuous breeders and both females and males may have multiple mates (Miller 1997; FitzSimmons 1998; Hamann et al. 2003). Several possible indirect benefits for female promiscuity have been suggested, such as insurance of egg fertilization, increased genetic

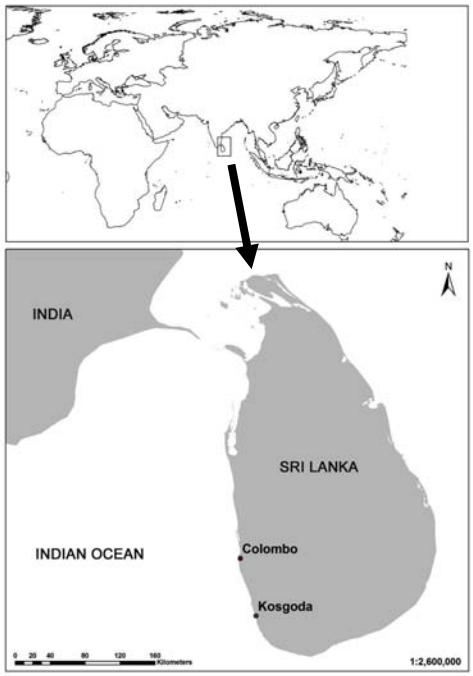

FIGURE 1. The study site of paternity of Green Turtles (*Chelonia mydas*) at Kosgoda on the southwest coast of Sri Lanka.

variability of offspring, and sperm competition (FitzSimmons 1998; Pearse and Avise 2001; Lee and Hays 2004).

Multiple paternity in sea turtles clutches can occur due to a female mating with more than one male during the same nesting season or sperm storage between consecutive nesting seasons (Uller and Olsson 2008). Most of the major reptilian taxa, including sea turtles, exhibit the capacity for sperm storage (Harry and Briscoe 1988; FitzSimmons 1998; Olsson and Madsen 1998; Kichler et al. 1999; Sever and Hamlett 2002). Sperm from multiple males are present in the reproductive tract of female sea turtles and these stored sperm may be used to fertilize ova of a single clutch, resulting in multiple paternity within that clutch (Pearse and Avise 2001). Sperm storage may also be used to separate reproductive events such as copulation, fertilization, and hatching to optimize timing of these events (Birkhead and Moller 1993). Female turtles are capable of storing sperm for multiple nesting seasons (Ewing 1943; Olsson and Madsen 1998). The incidence of multiple paternity in Green Turtles (*Chelonia mydas*) is highly variable between rookeries, with frequencies ranging from 9 to 100% among different populations (FitzSimmons 1998; Ireland et al. 2003; Lee and Hays 2004).

We investigated the paternity of the Green Turtle population nesting at the Kosgoda turtle rookery, Sri Lanka, by documenting paternal contribution to the offspring using microsatellites. If multiple paternity was present, we determined the comparative paternal contribution to offspring in a clutch sired by many males and the contribution pattern in successive clutches of a female. Furthermore, we determined the relationship of the adult female size and the presence of multiple paternity.

MATERIALS AND METHODS

We conducted our study along a 1 km stretch of beach at Kosgoda (6°33' N, 80°02' E) on the south-western coast of Sri Lanka (Fig. 1). Kosgoda turtle rookery hosts the second largest Green Turtle rookery in Sri Lanka with year-round nesting.

Sample collection from nesting females and hatchlings.—We collected tissue samples from the nesting females and hatchlings at the Kosgoda turtle rookery from May 2005 to April 2006. After completion of the nesting process, we took skin scrapings (~ 0.5 cm^2) from one of the front flippers of the female using a sterile surgical blade and stored in 1.5 ml Eppendorf® tubes (Eppendorf, Hauppaugo, New York, USA) containing 95% ethanol. We sterilized the tissue sample location with 95% ethanol prior to collection. The tag number was noted if the female had already been tagged or they were tagged before collecting the sample. We also marked the nests once the nesting process was completed. After hatchling emergence from the nest, we took skin scrapings (0.3 cm^2) from both front flippers of 10 hatchlings from each clutch. We collected samples from single clutches and successive clutches within the same nesting season.

Extraction of genomic DNA and amplification of microsatellites.—We placed approximately 0.1 g of tissue from each sample on a glass plate and cut into small pieces using a sterile surgical blade. We extracted genomic DNA by placing the tissue in 500 µl of a 5% aqueous suspension of Chelex® (BioRad, Richmond, California, USA) in a 1.5 ml Eppendorf tube and incubating for 3 h at 55 °C with continuous shaking. We vortexed samples for 30 s and placed samples in a heating block at 95 °C for 5 min. After centrifugation at 11,600 g (relative centrifugal force [RCF]) for 5 min, we removed the supernatant containing extracted DNA and stored it at -20 °C in 1.5 ml tube until use.

We amplified six microsatellite loci, namely Cm3, Cm58, Cm72, Cm84, Cc117 (FitzSimmons et al. 1995), and Cc7 (FitzSimmons 1998) by polymerase chain reaction (PCR). Four of these loci (Cm3, Cm58, Cm72, and Cm84) were developed from Green Turtle DNA, the

others (Cc117, Cc7) were from Loggerhead Turtle (*Caretta caretta*) DNA. The PCR mixture contained 3 µl of template DNA, 0.5 µM of each primer (forward and reverse), 0.2 mM dNTPs, 0.5 unit of *Taq* DNA polymerase (Bangalore Genei, Pvt Ltd., Bangalore, India), and 1.5 µl of 10 × PCR buffer with $MgCl_2$ in a final volume of 10 µl. Polymerase chain reaction conditions were optimized for use in an Flexigene® thermocycler (Techne, Cambridge, United Kingdom) with general cycling parameters consisting of an initial denaturation at 95 °C for 2 min followed by 35 cycles with primer annealing at 55 °C (Cc7), 62 °C (Cm3, Cm58, Cm72 and Cc117), or 64 °C (Cm84) for 1 min, strand synthesis at 72 °C for 1 min and denaturation at 95 °C for 45 sec, followed by final extension at 72 °C for 7 min.

We separated amplified products by electrophoresis on a 6% denaturing polyacrylamide gel. To denature the samples, 1.5 µl of loading buffer was mixed with 10 µl of PCR product and incubated for 5 min at 95 °C. Following this, we immediately placed the tubes in ice for rapid cooling. Samples were then loaded on to the gel (48 cm length) and electrophoresis was carried out at 1400 v for three hours at 45–50 °C. We used a comb with 24 wells, and we loaded the two wells on each side with the 20 bp DNA ladder (Bangalore Genei, Pvt Ltd., Bangalore, India). We loaded the PCR products from 10 hatchlings of the same clutch and the mother into a single gel. The gels were fixed in 5% acetic acid for 20 min with gentle shaking and stained with 0.4% silver nitrate for 20 min. We visualized DNA bands after developing the gels in 3% NaOH. We added a solution of 5% acetic acid to prevent further development. The size of the DNA bands was determined using a gel documentation system (BioVision, Inc. Heidelberg, Germany, 3000-WL/26M) and Vision-Capt (Ver. 14.3) software. We scored alleles of approximately one bp difference in size lengths to the nearest even number.

DNA analyses.—We determined the maternal genotypes directly from the female and in her offspring. Offspring that did not display a maternal allele were regarded as evidence of a maternal mutation. We inferred paternal alleles from the offspring after accounting for maternal alleles. If an offspring displayed an unexpected paternal allele (based on the null hypothesis of single paternity), we considered this to be a paternal mutation if observed at only one locus. We inferred multiple paternity in a clutch when we observed more than two unexpected paternal alleles in at least two loci (FitzSimmons 1998). However, this manual assignment may not be reliable when two or more fathers were present. According to the expectations of Mendelian inheritance, each allele from a parent would be at 1:1 ratio. Therefore, we used a Chi Square test ($\alpha = 0.05$) to check whether the clutch differed to Mendelian expectations.

We further assessed potential multiple paternity using the GERUD2.0 software program (Jones 2005) to calculate the minimum number of fathers in the clutch. This program calculates the number of males contributing to the fertilization of a clutch and reconstructs all possible paternal and maternal genotypes, given that all offspring are full or half siblings (Jones 2005). It ranks the parental genotype combinations by probability, based on segregation of parental alleles and their deviation from Mendelian expectations. GERUD2.0 does not use population allele frequencies or relatedness of offspring and outputs a minimum number of fathers (Jones 2001). The program does not accept missing data and therefore offspring with missing data (about 15%) could not be analyzed using this method and were deleted. A maternal mutation leads to a larger number of possible fathers in the results and sometimes the program may not give the final results. Therefore, in cases of maternal mutations, we identified the offspring and we pooled the mutant alleles with the maternal allele that was closest to it in size. The GERUD2.0 program allows only a maximum of four fathers in its analysis (Theissinger et al. 2008). We searched possible maternal genotypes and all combinations of the minimum number of fathers that were consistent with the array, including father genotypes and number of sired offspring per father and then ranked them by probability. We analyzed multiple clutches of the same female separately to determine the paternity of successive clutches. The relationship of the adult female size (curved carapace length) and the occurrence of multiple paternity was compared using a Student's *t*-test for unequal sample sizes ($\alpha = 0.05$).

RESULTS

We analyzed 19 females (Table 1) and their hatchings, including 17 single clutches and multiple clutches of two females. Altogether, we analyzed 24 clutches including three successive clutches of one female and four successive clutches of another female (Table 2).

Single clutch analysis.—We observed the presence of multiple paternity in 47% of the females according to initial manual inferences where extra paternal alleles were observed at more than one locus. Results of the chi-square test showed a significant deviation from Mendelian expectations of offspring genotype proportions in four females (21%). However, the analysis from GERUD2.0 indicated multiple paternity in 10 females (53%). Although the percentages we obtained from different analyses varied, all three methods confirmed that the Green Turtle clutches from

TABLE 1. Number of alleles and allelic range for each microsatellite locus of the adult female Green Turtles (*Chelonia mydas*).

Locus	Diversity (Number of alleles)	Allelic range
Cm58	10	122–142
Cm3	13	136–208
Cm72	18	226–296
Cc117	12	228–268
Cm84	12	310–352
Cc7	10	162–198
Mean ±SD	12.5 (±2.95)	- -

Kosgoda rookery show multiple paternity (Table 2). The presence of multiple paternity can be confirmed for each clutch if at least two of the above methods agreed in detecting more than one father. Accordingly, multiple paternity was present in clutches laid by 47% of 19 females and 54% of 24 clutches (Table 2). Two hatchlings from different females lacked the maternal alleles at loci Cm72 (#119-1) and Cm3 (#154-3). It should be noted that scoring of alleles to the nearest even number may result in missed variation among alleles that are only one bp different, and therefore reduces the probability of detecting multiple paternity.

There was no evidence that the same male had fathered offspring of more than one of the females that we studied. Clutches with multiple paternity had two or three fathers. According to single clutch analysis, hatchlings from two females had been fathered by three males while offspring of seven females had been fathered by two males. GERUD2.0 analysis provided the number and proportion of sired offspring per father (Fig. 2). In the 13 clutches that showed multiple paternity with two fathers, the mean contribution of the dominant father was 65.3% (range 50.0–90.0%) and that of the second father was 34.7% (range 10.0–50.0%). In the two clutches that showed multiple paternity with three fathers, the mean contribution of the dominant father was 50.0% (range 44.4–55.6%), and 33.3% and 16.7% (range 11.1–22.2%) were from the second and third fathers, respectively.

Pooled clutch analysis.—Data for consecutive clutches were available for two females. When these data were pooled and analyzed, it showed that offspring of both females were fathered by three males (Table 3). However, single clutch analysis (see above) showed that the offspring of one female had been fathered by three males and offspring of the other female had been fathered by two males (Fig. 2). Therefore, according to pooled analysis of the multiply sired females, a slightly higher number of clutches (37.5%) had been fathered by three males while the rest (62.5%) by two males. The same paternal alleles were observed at all six loci in all the successive clutches (Table 4). This suggests that the male or males that sired the first clutch also sired the subsequent clutches of the same female.

TABLE 2. Estimated number of fathers per clutch in the Green Turtle (*Chelonia mydas*) population at Kosgoda, Sri lanka as inferred by initial inferences, chi square test and GERUD2.0.

Clutch ID	Initial inference	χ^2	GERUD2.0	Multiple paternity present*
#100-1	1	1	1	No
#104-1	1	1	1	No
#106-1	1	1	1	No
#108-1	1	1	1	No
#110-1	1	1	1	No
#111-1	2	2	3	Yes
#111-2	2	2	2	Yes
#111-3	1	1	2	No
#112-1	2	1	3	Yes
#113-1	1	1	1	No
#119-1	2	2	2	Yes
#128-1	2	1	2	Yes
#132-1	2	1	2	Yes
#133-1	1	1	1	No
#146-1	2	1	2	Yes
#154-1	2	2	2	Yes
#154-2	2	2	2	Yes
#154-3	2	2	2	Yes
#154-4	2	2	2	Yes
#155-1	1	1	1	No
#165-1	1	1	1	No
#170-1	1	1	2	No
#171-1	2	1	2	Yes
#181-1	2	2	2	Yes
% MP	**54.0**	**33.3**	**62.5**	**54.0**
Mean ± SD	**1.5 ± 0.5**	**1.3 ± 0.5**	**1.7 ± 0.6**	**- -**

Note: The percentage multiple paternity (% MP) and mean values of inferred multiple paternity for each method across all clutches analyzed were given. *Confirmed by at least one method of analysis.

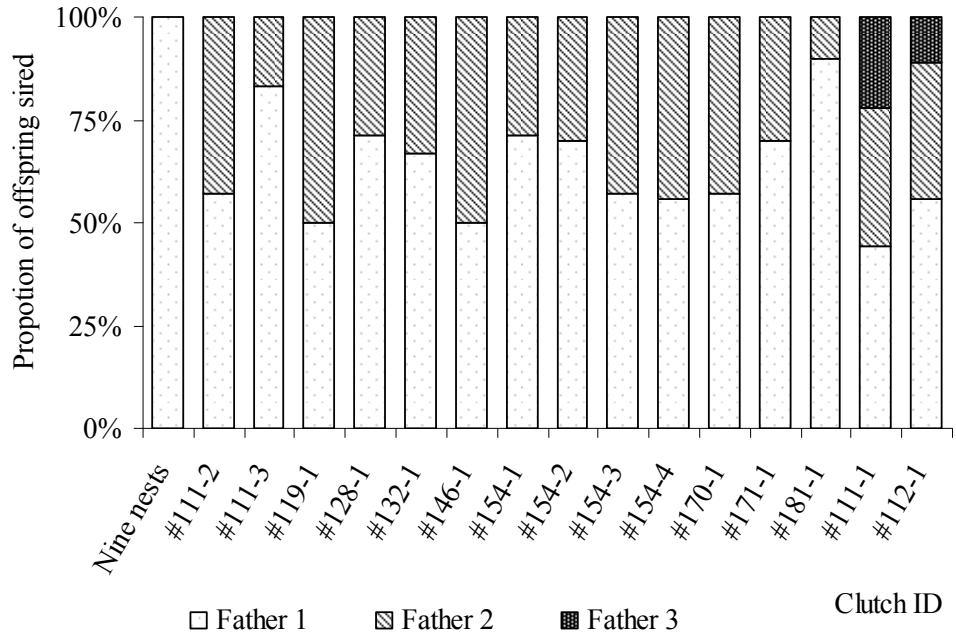

FIGURE 2. Proportion of offspring per clutch fathered by one to three male Green Turtles (*Chelonia mydas*), as inferred by GERUD2.0.

Female size and multiple paternity.—The mean curved carapace length (CCL) of female Green Turtles at the Kosgoda rookery varied from 86–120 cm (Turtle Conservation Project, unpubl. data). The mean CCL of the females with multiple paternity was 105.2 cm (± 7.1 SD; n = 8) and 107.4 cm (± 6.2 SD; n = 11) for females with single paternity. There was no significant difference between the two groups (t = -0.71, df = 17 P = 0.48).

DISCUSSION

The present study indicates that 47% of female Green Turtles nesting at Kosgoda rookery laid clutches that were sired by two or three males. Sea turtles are highly promiscuous breeders, so a high occurrence of multiple paternity was expected. Multiple paternity has been recorded for Green Turtle populations around the world but the proportions vary widely among different populations (Table 5), from 9% multiple paternity in the southern Great Barrier Reef, Australia (FitzSimmons 1998) to 100% multiple paternity of clutches laid at Ascension Island (Ireland et al. 2003). The method of analysis is important in determining the prevalence of multiple paternity in a population. Some studies have used only the manual inference method to assess paternity (Crim et al. 2002; Hoeckert et al. 2002; Moore and Ball 2002), while others have used both manual inference and one or more parentage software programs (Lee and Hays 2004; Zbinden et al. 2007; Theissinger et al. 2008).

The number of samples and the number of microsatellite loci used in the analysis can also influence results, as illustrated by two studies of multiple paternity in Green Turtles at Ascension Island (Ireland et al. 2003; Lee and Hays 2004). Ireland et al. (2003) reported 100% multiple paternity for three single clutches laid by three females, while Lee and Hays (2004) recorded 62% multiple paternity among 18 single clutches laid by 18 females. We analyzed 24 clutches laid by 19 females, used six microsatellite loci and three methods to resolve the paternity of Green Turtle clutches laid at Kosgoda.

We compared three methods to demonstrate the variation in results obtained when estimating the number of fathers contributing to a clutch, and the results from

TABLE 3. Number of fathers calculated for successive clutches of two female Green Turtles (*Chelonia mydas*) by GERUD2.0.

Adult ID	Number of fathers (number of loci)				
	Nest 1	Nest 2	Nest 3	Nest 4	Pooled data
#111	3 (6 loci)	2 (6 loci)	2 (3 loci)	-	3 (6 loci)
#154	2 (6 loci)	2 (6 loci)	2 (3 loci)	2 (2 loci)	3 (6 loci)

TABLE 4. Observed mother, offspring microsatellite genotypes per locus and genotype frequencies of the multiple clutches of two female Green Turtles (*Chelonia mydas*).

Female & Clutch ID	Locus					
	Cm58	Cm3	Cm72	Cc117	Cm84	Cc7
#111	**134/142**	**170/170**	**272/292**	**234/250**	**310/332**	**168/178**
#111 - 1	132/134 (6)	160/170 (5)	272/272 (2)	234/246 (4)	332/354(7)	178/182(4)
	134/142 (1)	166/170 (2)	272/280 (3)	228/234 (2)	310/354(2)	168/182(2)
	134/134 (1)	170/174 (2)	272/304 (2)	234/250 (2)		168/178(2)
	132/142 (1)		292/304 (1)	246/250 (1)		178/178(1)
			292/320 (1)			
#111 - 2	132/134 (5)	160/170 (3)	272/280 (6)	234/246 (6)	310/354(4)	178/182(7)
	134/142 (2)	166/170 (2)	272/304 (1)	246/250 (1)	332/354(3)	
		170/174 (2)				
#111 - 3	132/134 (3)	160/170 (3)	-	234/246 (4)	-	-
	134/142 (2)	166/170 (1)		234/250 (1)		
	134/134 (1)	170/174 (2)		246/250 (1)		
#154	**132/140**	**164/182**	**266/288**	**228/238**	**332/342**	**188/198**
#154 -1	132/136 (5)	164/182 (1)	288/310 (4)	238/252 (4)	342/360(3)	188/188(4)
	136/140 (2)	168/182 (3)	288/320 (2)	234/238 (2)	332/342(2)	184/188(3)
		164/164 (3)	266/310 (1)	228/238 (1)	332/332(2)	
#154 - 2	134/140 (7)	140/164 (8)	288/310 (6)	238/252 (5)	342/360(6)	188/192(5)
	132/134 (2)	144/164 (1)	288/320 (2)	234/238 (2)	342/350(3)	184/188(2)
			266/310 (1)	228/238 (2)		192/198(1)
						188/188(1)
#154 - 3	134/140 (2)	140/164 (6)	-	238/252 (6)	-	-
	132/136 (2)	144/160*(1)		234/238 (1)		
	132/140 (2)					
	136/140 (1)					
#154 - 4	-	-	-	-	342/360(5)	184/188(2)
					342/350(3)	188/192(4)
					332/342(1)	188/188(2)
						192/198(1)

* denotes lack of maternal allele

each method only agreed on parentage in 66% of the clutches analyzed. Our methods and results should be considered when evaluating multiple paternity detected in previous studies (Table 5).

Multiple paternity indicates that females had successful multiple matings within the current nesting season and/or stored sperm from previous breeding season(s) (FitzSimmons 1998; Uller and Olsson 2008). Female turtles have the ability to store sperm for a long period of time, up to 14 mo (Gist and Jones 1989). Ewing (1943) determined that female sea turtles are capable of storing sperm for up to 4 y, allowing them to mate with an average of less than one male per clutch (Pearse and Avise 2001). At the Kosgoda rookery, where most female Green Turtles have an inter-nesting interval of 2.5 y (Ekanayake et al. 2010), sperm stored from previous seasons could still be viable and used during fertilization, although this has not been demonstrated.

Several aspects of reproductive biology may influence the incidence of multiple paternity observed in turtle populations, such as hatchling success (Pearse and Avise 2001), population sex ratios (Bollmer et al. 1999), and sperm competition (FitzSimmons 1998). FitzSimmons (1998) discussed many hypotheses regarding the benefits of multiple mating for female sea turtles, such as: (1) fertility assurance if some males have poor-quality sperm; (2) increased offspring viability via sperm competition; (3) increased genetic diversity; and (4) reduced harassment from courting males for the duration of time a female is mounted.

The inferred number of successful mates for a female depends on whether the data from successive clutches was analyzed individually or pooled. When samples from successive clutches were pooled, a greater number of fathers were detected in comparison to single clutch analyses (Theissinger et al. 2008). In the present study, single clutch analysis showed two fathers in clutches laid by female #154 but the number of fathers increased to three when the data were pooled. Zbinden et al. (2007) studied two successive clutches each for five female Loggerheads, and clutches laid by four individuals showed an increase in the number of fathers when the data were pooled. Theissinger et al. (2008) observed only one of five females of Flatback Turtles (*Natator depressus*) had a greater number of fathers in successive clutches. Although multiple paternity has been observed for all sea turtle species, the data from the successive clutches were not pooled in many studies (Table 5). Therefore, the number of males contributing to clutches

TABLE 5. Variation of multiple paternity single and successive clutches of sea turtles.

Species	No. of females	Clutches		Mean sample size per clutch	Pooled analysis		% Multiple Paternity (No. of microsatellites)	References
		Multiple	Single		Increase in fathers*	No. of females taken		
Dermochelys coriacea	4	0	4	NA	No	-	No (2)	Rieder et al.1998
D. coriacea	4	17	-	NA	No	-	No (6)	Dutton et al. 2000
D. coriacea	20	50	0	19.5	No	-	10 (3)	Crim et al.2002
D. coriacea	12	38	0	26.8	No	-	41.7 (7)	Stewart and Dutton 2011
Caretta caretta	24	42	3	21	Yes (NA)	-	**33	Harry and Briscoe 1988
C. caretta	3	0	3	20.7	No	-	33 (2)	Bollmer et al. 1999
C. caretta	70	0	70	10	No	-	31 (4)	Moore and Ball 2002
C. caretta	15	10	10	22.8	Yes (4)	5	93 (4)	Zbinden et al. 2007
Lepidochelys kempii	26	18	17	1–10	No	-	58 (3)	Kichler et al. 1999
Lepidochelys olivacea	10	0	10	70	No	-	20 (2)	Hoekert et al. 2002
L. olivacea	13	0	13	22.6	No	-	30 (2)§	Jensen et al. 2006
L. olivacea	13	0	13	22.1	No	-	92 (2)§§	Jensen et al. 2006
Natator depressus	9	12	4	25	Yes (1)	5	67 (4)	Theissinger et al. 2008
Eretmochelys imbricata	10	4	8	31	No	-	20 (5)	Joseph and Shaw 2011
Chelonia mydas	8	0	8	NA	No	-	63 (2)	Peare et al. 1998
C. mydas	13	18	4	41	No	-	9 (5)	FitzSimmons 1998
C. mydas	3	0	3	15	No	-	100 (2)	Ireland et al. 2003
C. mydas	18	0	18	39	No	-	62 (5)	Lee and Hays 2004
C. mydas	19	7	17	10	Yes (1)	2	47 (6)	Present Study

*Number of females that increased the number of fathers when data were pooled
**Allozymes were used
NA = data not available
§Solitary nesting rookery §§Arribada rookery

may be greater than that reported in previous studies.

We found no relationship between the CCL of the female and presence of multiple paternity in Green Turtles nesting at Kosgoda rookery; this was consistent with the findings of Lee and Hays (2004) in the Green Turtle nesting population of Ascension Island. However, clutches laid by larger female Loggerheads in the Mediterranean exhibit multiple paternity more often than those of the smaller females (Zbinden et al. 2007). Zbinden et al. (2007) gave two suggestions for this relationship. First, larger females are likely to swim faster than smaller ones, and may thus arrive in the reproductive area earlier and hence spend more time mating. Secondly, average size at maturity might differ between turtles using disjoint foraging areas and mating opportunities along migratory routes might differ between migration paths. Further studies with larger sample size are necessary to confirm the relationship between multiple paternity and size of female turtles. Ireland et al. (2003) and Zbinden et al. (2007) observed that multiple paternity is likely to increase the effective population size and decrease genetic drift compared to a population where females only mate with one male. Hence, an understanding of the prevalence of multiple paternity and effects on paternity distribution can be used to evaluate the population size of Green Turtles and be of great importance to the management and conservation of this species.

Acknowledgments.—We thank the Turtle Conservation Project (TCP) staff for their technical support and the Department of Wildlife Conservation for granting permission to carry out this study. Andrea Phillott edited 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. Permission to collect tissue samples from Green Turtles was obtained from the Department of Wildlife Conservation (DWC), Sri Lanka.

LITERATURE CITED

Baer, B., and P. Schmid-Hempel. 1999. Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature 397:151−154.

Birkhead, T. R., and A.P. Moller. 1993. Sexual selection and the temporal separation of reproductive events:

sperm storage data from reptiles, birds and mammals. Biological Journal of Linnaean Society 50:295–311.

Bollmer, J.L., M.E. Irwin, J.P. Rieder, and P.G. Parker. 1999. Multiple paternity in Loggerhead Turtle clutches. Copeia 1999:475–478.

Crim, J.L., L.D. Spotila, J.R. Spotila, M. O'Connor, R. Reina, C.J. Williams, and F.V. Paladino. 2002. The Leatherback Turtle, *Dermochelys coriacea*, exhibits both polyandry and polygyny. Molecular Ecology 11:2097–2106.

Dutton, P., E. Bixby, and S.K. Davis. 2000. Tendency towards single paternity in Leatherback detected with microsatellites. Pp. 39 *In* Proceedings of the Eighteenth International Symposium on Sea Turtle Biology and Conservation. Abreu, F.A., R. Briseno, R. Marquez and L. Sarti (Compilers). NOAA Technical Memorandum, NMFS-SEFSC-436, Wahsington, D.C., USA.

Ekanayake, E.M.L., R.S. Rajakaruna, T. Kapurusinghe, M.M. Saman, D.S. Rathnakumara, P. Samarawera, and K.B. Ranawana. 2010. Nesting behaviour of the Green Turtle at Kosgoda rookery, Sri Lanka. Ceylon Journal of Science (Biological Sciences) 39:109–120.

Evans, J.P., and A.E. Magurran. 1999. Geographic variation in sperm production by Trinidadian Guppies. Proceedings of the Royal Society of London Biological Sciences 266:2083–2087.

Ewing, H. 1943. Continued fertility in female box turtles following mating. Copeia 1943:112–114.

Fedorka, K.M., and T.A. Mousseau. 2002. Material and genetic benefits of female multiple mating and polyandry. Animal Behaviour 64:361–367.

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, and S.S. Moor. 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Molecular Biology and Evolution 12:432–440.

Fleming, I.A., and M.R. Gross. 1994. Breeding competition in a Pacific Salmon (Coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. Evolution 48: 637–657.

Gist, D.H., and J.M. Jones. 1989. Sperm storage within the oviduct of turtles. Journal of Morphology 199:379–384.

Hamann, M., C.J. Limpus, and D.W. Owens. 2003. Reproductive cycles of male and females. Pp 135–161 *In* The Biology of Sea Turtles. Lutz, P.L., J.A. Musick, and J. Wyneken (Eds.). CRC Press, Boca Raton, Florida, USA.

Harry, J.L., and D.A. Briscoe. 1988. Multiple paternity in the Loggerhead Turtle (*Caretta caretta*). Journal of Heredity 79:96–99.

Hoekert, W.E.J., H. Neufeglise, A.D. Schouten, and S.B.J. Menken. 2002. Multiple paternity and female-biased mutation at a microsatellite locus in the Olive Ridley Sea Turtle (*Lepidochelys olivacea*). Heredity 89:107–113.

Ireland, J.S., A.C. Broderick, F. Glen, B.J. Godley, G.C. Hays, P.L.M. Lee, and D.O.F. Skibinski. 2003. Multiple paternity assessed using microsatellite markers in Green Turtles *Chelonia mydas* (Linnaeus, 1758) of Ascension Island, South Atlantic. Journal of Experimental Marine Biology and Ecology 291:149–160.

Jensen, M.P., F.A. Abreu-Grobois, J. Frydenberg, and V. Loeschcke. 2006. Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada Olive Ridley Sea Turtle rookeries. Molecular Ecology 15:2567–2575.

Jones, A.G. 2001. GERUD1.0: a computer program for the reconstruction of parental genotypes from progeny arrays using multilocus DNA data. Molecular Ecology Notes 1:215–218.

Jones, A.G. 2005. GERUD2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. Molecular Ecology Notes 5:708–711.

Joseph, J. and P.W. Shaw. 2011. Multiple paternity in egg clutches of Hawksbill Turtles (*Eretmochelys imbricata*). Conservation Genetics 12:601 – 605.

Kichler, K., M.T. Holder, S.K. Davis, R. Marquez, and D.W. Owens. 1999. Detection of multiple paternity in the Kemp's Ridley Sea Turtle with limited sampling. Molecular Ecology 8:819–830.

Lee, P.L.M., and G.C. Hays. 2004. Polyandry in a marine turtle: females make the best of a bad job. Proceedings of the National Academy of Science of the United States of America 101:6530–6535.

Loehle, C. 1997. The pathogen transmission avoidance theory of sexual selection. Ecological Modelling 103:231–250.

Miller, J.D. 1997. Reproduction in marine turtles. Pp. 51–81 *In* The Biology of Marine Turtles. Lutz, P.L., and J.A. Musick (Eds.). CRC Press, Washington, DC, USA.

Moore, M.K., and R.M. Ball. 2002. Multiple paternity in Loggerhead Turtle (*Caretta caretta*) nests on Melbourne Beach, Florida: a microsatellite analysis. Molecular Ecology 11:281–288.

Olsson, M., and T. Madsen. 1998. Sexual selection and sperm competition in reptiles. Pp 503–577 *In* Sperm Competition and Sexual Selection. Birkhead, T.R., and A.P. Moller. (Eds.). Academic Press, San Diego, California, USA.

Peare, T, P.G. Parker, and M.E. Irwin. 1998. Paternity analysis in the Green Sea Turtle. Pp. 116 *In* Proceedings of the Sixteenth Annual Symposium on Sea Turtle Biology and Conservation. Byles, R., and Y. Fernandez (Compilers). NOAA Technical

Memorandum, NMFS-SEFSC-412, Washington, D.C., USA.

Pearse, D.E., and J.C. Avise. 2001. Turtle mating systems: behaviour, sperm storage and genetic paternity. Journal of Heredity 92:206–211.

Rieder, J.P., P.G. Parker, J.R. Spotila, and M.E. Irwin. 1998. The mating system of the Leatherback Turtle: a molecular approach. Pp. 120–121 *In* Proceedings of the Sixteenth Annual Symposium on Sea Turtle Biology and Conservation. Byles, R., and Y. Fernandez (Compilers). NOAA Technical Memorandum, NMFS-SEFSC-412, Washington, D.C., USA.

Sever, D.M., and W.C. Hamlett. 2002. Female sperm storage in reptiles. Journal of Experimental Zoology 292:187–199.

Stewart and Dutton. 2011.

Sugg, D.W., and R.K. Chesser. 1994. Effective population sizes with multiple paternity. Genetics 137:1147–1155.

Theissinger, K., N.N. FitzSimmons, C.J. Limpus, C.J. Parmenter, and A.D. Phillott. 2008. Mating system, multiple paternity and effective population size in the endemic Flatback Turtle (*Natator depressus*) in Australia. Conservation Genetics 10:329–346.

Tregenza, T., and N. Wedell. 2002. Polyandrous females avoid costs of inbreeding. Nature 415:71–73.

Uller, T.A., and M. Olsson. 2008. Multiple paternity in reptiles: patterns and processes. Molecular Ecology 17:2566–2580.

Zbinden, J.A., C.R. Largiader, F. Leippert, D. Margaritoulis, and R. Arlettaz. 2007. High frequency of multiple paternity in the largest rookery of Mediterranean Loggerhead Sea Turtles. Molecular Ecology 16:3703–3711.

E.M. LALITH EKANAYAKE is a Research Coordinator at Turtle Conservation Project (TCP), Sri Lanka. After his B.Sc. degree he started research on sea turtles with the TCP since 1996 to date. He has completed his M.Phil. and Ph.D. degrees from the University of Peradeniya, Sri Lanka. (Photographed by A.M.D.S. Rathnakumara)

THUSHAN KAPURUSINGHE (left) started his wildlife career in 1985 and received his academic training from the Open University of Sri Lanka and the Duke University Marine Laboratory in North Carolina, USA. He has extensive work experience in marine and coastal resource management with community participation in Sri Lanka. He is the Project Leader and Co-founder of the Turtle Conservation Project (TCP) in Sri Lanka, which has operated for 18 y and employs over 100 local community members in various conservation and livelihood development projects. He completed his M.Sc. in Conservation and Tourism at Durrell Institute of Conservation and Ecology (DICE) at the University of Kent at Canterbury in the UK. His main interests are community based ecosystem conservation, diversification of community livelihoods, protected area co-management strategies, and sustainability of community based projects. (Photographed by Janaka Abeygunawardena)

M.M. SAMAN is a research officer at the TCP Sri Lanka and has been working on sea turtle research for 16 y. (Photographed by Lalith Ekanayake)

A.M.D.S. RATHNAKUMARA is a former Wildlife Officer who has been attached to the TCP for over 16 y carrying out research on sea turtle *in situ* conservation. (Photographed by Lalith Ekanayake)

PREMINDA SAMARAWEERA received his B.Sc. in Chemistry from the University of Peradeniya, Sri Lanka and Ph.D. 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. (Photographed by Nirmalie Abeyratne).

KITSIRI B. RANAWANA is a Professor in Zoology at the University of Peradeniya, Sri Lanka with a research career which spans over three decades. He has conducted numerous conservation oriented research projects in many areas all over the country. He currently serves as the Head of Department of Zoology at the University of Peradeniya. (Photographed by Lalith Ekanayake).

RUPIKA S. RAJAKARUNA received her B.Sc. (Hons.) and M.Phil degrees in Zoology from the University of Peradeniya and completed her Ph.D. from Memorial University of Newfoundland, Canada. She is a Senior Lecturer in Zoology with over 17 y of teaching and research experience at the University of Peradeniya, Sri Lanka. She has collaborated with many scientists on publications pertaining to a wide range of fields including sea turtle biology and conservation, parasitology, and frog deformities. (Photographed by Harshana Rajakaruna).

Herpetological Conservation and Biology 8(1):27–36.
Submitted: 28 March 2012; Accepted: 23 January 2013; Published: 30 April 2013.

1