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Multiple paternity assessed using microsatellite markers, in green turtles *Chelonia mydas* (Linnaeus, 1758) of Ascension Island, South Atlantic

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Abstract

Paternity was determined for three clutches and up to 20 offspring per clutch in the green turtle (*Chelonia mydas*) from Ascension Island, South Atlantic, using microsatellite markers. All three clutches were sired by at least two different males. The results were compared with those of previous studies of multiple paternity in turtles. No significant difference among studies was observed in the mean contribution of the males siring the largest proportion of progeny per clutch. The present study also provides evidence for segregation distortion (meiotic drive) in turtles.

Keywords: Ascension Island; *Chelonia mydas*; Segregation distortion; Microsatellites; Multiple paternity

1. Introduction

Recent advances in genetic analysis have shown that multiple paternity is frequent in both invertebrates and vertebrates. Invertebrate examples include the squid *Loligo pealeii* (Buresch et al., 2001), periwinkle *Littorina obtusata* (Paterson et al., 2001), grass shrimp *Palaemonetes pugio* (Baragona et al., 2000) and vertebrate species such as guppies *Poecilia reticulata* (Evans and Magurran, 2000), garter snakes *Thamnophis sirtalis* (Mc Craken et al., 1999) and turtles (Galbraith, 1993; Hoekert et al., 1999; Peare and Parker, 1994). Paternity analysis is of particular interest for marine turtles because it provides an

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indirect approach to studying their breeding biology that would otherwise be difficult at sea.

Until recent years, there were only a few anecdotal accounts of courtship behaviour in sea turtles in wild populations (Booth and Peters, 1972; Broderick and Godley, 1997; Hendrickson, 1958). Courtship and mating generally occur immediately following migration, or very early in the nesting season close to the nesting beach (Booth and Peters, 1972; Broderick and Godley, 1997; Godley et al., 2001). Both sexes show a periodic remigration cycle to the breeding grounds with females migrating at intervals typically more than 1 year. The interval in males is thought to be shorter (Limpus, 1993). Recent molecular studies have shown that both sexes show fidelity to a particular courting area both within and between seasons (Bowen et al., 1992; FitzSimmons et al., 1997). Jessop et al. (1999) described the mating system of male turtles as scramble polygamy with males attempting to mate with as many females as possible, not maintaining territories.

Multiple mating of female green turtles (*Chelonia mydas*) has been observed (Booth and Peters, 1972; Limpus, 1993; Ulrich and Parkes, 1978). Female turtles store sperm in tubules found in the posterior part of the albumin-secreting region of the oviduct (Gist and Jones, 1989). Despite their ability to store sperm (Galbraith, 1993; Owens, 1980), the incidence of multiple paternity in marine turtles is variable between species. The incidence of multiple paternity in the loggerhead turtle (*Caretta caretta*) has varied between 33% for Melbourne beach, Florida (Bollmer et al., 1999; Moore and Ball, 2002) to 38% in a population in Mon Repos, Queensland, Australia (Harry and Briscoe, 1988). In the leatherback turtle *Dermochelys coriacea*, the frequency of multiple paternity is much reduced with two studies showing a low prevalence (Crim et al., 2000; Curtis et al., 1998). Green turtle paternity studies to date have reported 9% multiple paternity in the southern Great Barrier Reef (FitzSimmons, 1998), but 50% multiple paternity in another population observed in Tortuguero, Costa Rica (Peare and Parker, 1994). This raises the question as to why large differences in the incidence of multiple paternity could occur between populations of the same species?

The aims of the present study were: (i) to determine the level of multiple paternity in a population of green turtles from Ascension Island using microsatellite data obtained from three females and their offspring; (ii) to explore the minimum paternal contribution to offspring in a given clutch having more than one father, and (iii) to comment on evidence for what might be the first report of segregation distortion observed in a clutch of marine turtles or reptiles, generally.

2. Materials and methods

2.1. Field collection

During the 1999 season, tissue samples were taken from three nesting adult green turtles (*C. mydas*) from Ascension Island, South Atlantic (14°20′W, 7°55′S), using a sterile 6 mm skin biopsy punch (Steiffel Laboratories) along the trailing edge of the foreflipper. The location of incubating clutches was clearly marked as each nest had intranest temperature loggers that were retrieved when clutch contents were excavated at

the first sign of hatching (Broderick et al., 2001). Blood samples (maximum 0.1 ml) were obtained from the dorsal cervical sinus from live hatchlings (see Owens and Ruiz, 1980). The hatchlings were then released into the sea. Clutches were excavated and tissue samples were obtained from dead embryos in shells. The tissue were preserved in 95% ethanol. Blood samples were preserved in an approximately equal volume of buffered solution (50 mM of EDTA; 2% SDS, 10 mM of NaCl; 50 mM of Tris–HCl at pH 8). Three clutches (called X, Y and Z) were analysed.

2.2. DNA extraction and amplification

Total genomic DNA was extracted using a standard proteinase K phenol-chloroform extraction method (Towner, 1991) from each adult female and 20 offspring from each clutch. DNA concentrations were adjusted to 20 ng μl^{-1} using sterile water, and the samples were stored at 5 °C. Dinucleotide microsatellite primers Cm3 and Cm58 developed by FitzSimmons et al. (1997) were 5′ end-labelled with γ^{32P}-ATP (Amersham Pharmacia Biotech UK). Polymerase chain reactions (PCR) were carried out in a 10 μl reaction volume: 0.75 and 1 mM of forward and reverse primers, respectively; 10 × reaction buffer IV (Ab gene); 2.5 mM of Mg^{2+}; 2.5 mM of each dNTP (Pharmacia, UK); 0.03 units Red Hot *Taq* (Ab gene); 0.075 mM of γ^{32P}-ATP-labelled primer; and 20 ng of DNA. The PCR conditions were 95 °C for 2 min for denaturation; 55 °C for 30 s for annealing; 72 °C for 1 min for extension; and 95 °C for 45 s. In all, 30 cycles were used. There was a final annealing of the DNA for 30 s at 55 °C and extension for 72 °C for 1 min. PCR products were separated on 5% acrylamide gels (SequaGel™) and visualised by autoradiography using Kodak X-Omat film AR5. Allele lengths were scored using a T7 sequencing ladder (from version 2.0 of the USB DNA kit).

2.3. Data analyses

Following the technique of FitzSimmons (1998), paternal alleles were determined by eliminating the genotype of the mother from offspring in a clutch. Alleles present in hatchlings that were different from the maternal alleles and, in addition, alleles that appeared homozygous in some hatchlings were considered to be paternal. Any instance of more than two possible paternal alleles is an indication of multiple paternity. The likely number of fathers was determined in the following way. Taking into account the known female genotype, a paternal genotype was inferred that explained the maximum number of progeny in the clutch (male A). The subset of progeny thus explained was tested for agreement with Mendelian expectations. If the genotypic ratios were in agreement with Mendelian expectations, a second male genotype (male B) was chosen to explain the remaining progeny, again testing for agreement with Mendelian expectations. The possibility that mutation might be responsible for additional paternal alleles was also assessed.

For the present and, for comparison, for previous studies where paternal genotypes could be determined, the proportional contribution of each male to each clutch was estimated. The contribution of each male was calculated as a proportion of the total number of progeny sampled within a clutch. In each study, the contribution of male A (siring the most progeny) from each clutch was averaged over clutches. All the data

considered were derived from females that were sired by, and produced progeny from, two different males.

To test for genotypic association between loci and for other contingency tests, the Monte program from REAP (Roff and Bentzen, 1989) was used with 10,000 replicate simulations. A Monte Carlo approach, again with 10,000 replicate simulations, was also used to test for deviations from Mendelian expectations.

3. Results

3.1. Paternity at Ascension Island

Three or more paternal alleles were detected in all clutches at locus Cm58 and for offspring genotypes for clutch Z at locus Cm3, suggesting possible multiple paternity in green turtles from Ascension Island (Table 1).

For clutch X at locus Cm58, the father must contribute alleles 128, 126, and either 112 or 124 (Table 1). If one father has the genotype 128/126, then 13 out of 14 progeny can be accounted for without significant deviation from Mendelian proportions ($P = 0.774$). The single remaining offspring has the genotype 112/124. Such a combination is due either to a second father contributing either 112 or 124 or to a single mutational event converting one of the alleles observed in the first father. Such a mutation could involve a paternal change from 126 to 124 with the mother providing allele 112. There are 28 gene copies in the 14 progeny scored at Cm58. This would give a relatively high mutation rate of $1/28 = 0.036$.

The hypothesis of a second father receives further support when locus Cm3 is examined. Seventeen out of nineteen progeny are heterozygous 150/146 and one is 146/146. This suggests that if only a single male was responsible for these progeny, it was 150/146 or 146/146 unless it was a null heterozygote caused by mispriming (e.g. Pemberton et al., 1995). The one remaining offspring in this clutch is 146/154. Such a combination is either the result of a second father or a mutation resulting in allele 154. There are 19 progeny scored at Cm3, giving an estimated mutation rate of $1/38 = 0.026$. However, this exceptional offspring is also the one that has the odd Cm58 genotype of 112/124. Hence, either a second father fertilised eggs from clutch X or independent mutational events occurred in both loci in the one offspring. Given the mutation rate estimates for the two loci, the a priori probability of a mutation occurring at both loci in one offspring is 0.001. Given this low probability, the hypothesis that there was a second father receives strong support.

Of further note is that the high frequency of 150/146 heterozygotes at locus Cm3 in clutch X does not meet Mendelian expectations, whether the father is 150/146, 146/146, 146/null, or 150/null ($P < 0.001$ in all cases). For example, if the father is 146/146 and the mother is 150/146, then offspring have an equal probability of being either 150/146 or 146/146 if chromosomes were randomly segregating during meiosis. Such a deviant result cannot be explained by multiple fathers but could be explained by segregation distortion. For example, in the case of a 146/146 father, such a high frequency of heterozygotes could result from the overproduction of eggs carrying the 150 in preference to the 146 allele.

Table 1
Genotype frequencies at two microsatellite loci for three adult female green turtles (*C. mydas*) and a sample of their offspring from Ascension Island, South Atlantic

Maternal code (bp)			Number of offspring	Offspring genotypes (bp)		Genotype frequency
Clutch	Cm58	Cm3		Cm58	Cm3	
X	124/112	150/146	19	112/128	150/146	3
				124/126	150/146	1
				124/126	146/146	1
				124/128	150/146	5
				112/126	150/146	3
				112/124	154/146	1
				–	150/146	5
Y	112/120	–	7	112/114	–	1
				120/114	–	1
				112/120	–	1
				120/130	–	2
				112/112	–	1
				112/106	–	1
Z	112/118	140/148	20	112/114	140/138	1
				112/114	140/148	2
				112/114	–	1
				112/128	140/148	1
				112/112	140/148	3
				112/112	140/138	1
				112/118	140/148	1
				112/118	148/148	1
				118/116	140/148	1
					140/148	1
				118/114	148/148	1
				–	148/148	2
				–	140/148	2
				–	148/142	1
				–	140/138	1

Alleles are named as fragment length in base pairs (bp).

Miscoring of the female parent cannot easily explain the results. If the female parent had in fact been a 150/150 homozygote (and male A 146/146), the 146/146 and 154/146 progeny could not then be explained irrespective of the genotype of any additional male parents.

For clutch Y, the fathers must have contributed alleles 114, 112, 130, and 106 at locus Cm58. If one father is 112/114, four out of seven progeny can be accounted for with a fit to Mendelian expectations. If a second father is 130/106, the remaining three offspring can be accounted for without deviation from Mendelian expectations ($P = 0.634$). Alternatively, it could be argued that mutation in the first male, giving alleles 106 and 130 could explain the three progeny. Even if the two copies of the 130 allele required were descended from a single germ line mutation, two mutations would

be needed for two different alleles. This gives a very high estimated mutation rate of 2/14 = 0.143. Thus, a similar argument to that used for clutch X would again apply in this case. If other pairs of fathers with different genotypes are postulated, similar conclusions are reached.

For clutch Z, more than two paternal alleles were observed at both loci. For locus Cm58, the fathers must have contributed alleles 114, 128, 112, and 116. If one father is 114/112, 11/13 of the progeny can be accounted for without significant deviation from Mendelian expectations ($P = 0.587$). A second father with the genotype 116/128 could account for the remaining two progeny. If the copies of the 116 and 128 alleles were produced by mutation, this would imply a mutation rate of 2/26 = 0.077.

For clutch Z for locus Cm3, the fathers must have contributed alleles 138, 148, and 142. If one father is 148/148, 15 out of the 19 progeny could be accounted for without deviation from Mendelian expectations ($P = 0.119$). A second father with the genotype 138/142 could account for the remaining four progeny, again without significant deviation from Mendelian expectations ($P = 0.202$). If the three copies of the 138 allele and a single copy of the 142 allele were produced by mutation, this would imply a mutation rate of 2/38 = 0.053. This assumes that the three copies were derived from a single germ line mutation; otherwise, the mutation rate estimate would be doubled.

Genotypic associations between loci were not significant either for clutch X ($P = 0.068$) or for clutch Z ($P = 0.610$).

3.2. Paternal contribution of first male

The mean proportion of progeny per clutch sired by male A (that siring the most progeny) for the present and the previous paternity studies are compared in Fig. 1. The highest mean proportion for male A (0.96) was observed in the study of FitzSimmons (1998), where 9% of clutches gave evidence of multiple paternity.

In the other four studies where higher incidences of multiple paternity have been observed (20–100%), the mean proportion ranged from 0.55 to 0.82. However, a one-way

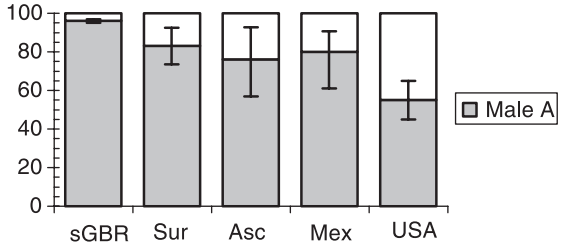

Fig. 1. Mean proportion of progeny per clutch sired by male A in turtle studies where multiple paternity has been observed. Error bars indicate the range for each study. Studies are for: southern Great Barrier Reef (sGBR, $n = 3$ clutches, FitzSimmons, 1998); Suriname (Sur, $n = 2$, Hoekert et al., 1999); Ascension Island (Asc, $n = 3$, Ireland et al., present study); Rancho Nuervo, Mexico (Mex, $n = 11$, Kichler et al., 1999); Melbourne Beach, Florida (USA, $n = 2$, Bollmer et al., 1999, Moore and Ball, 2002).

analysis of variance revealed no significance difference among studies in the mean arcsine transformed contributions of male A ($F = 1.93$, $P = 0.160$).

4. Discussion and conclusions

The results of the present study support the hypothesis that multiple paternity occurs in the green turtle population on Ascension Island. Evidence for three paternal alleles was found in all three clutches examined confirming that at least two males were responsible for siring each. This study is thus in agreement with those of Peare and Parker (1994) and Fitzsimmons (1998), where multiple paternity was also observed in the green turtle. The agreement between these studies could imply that multiple paternity is the dominant breeding strategy of the green turtle population on Ascension Island.

Despite this agreement, the three studies show widely different incidences of multiple paternity. Although the sample size of clutches is quite small in the present study, the ratio of 0:3 (single paternity clutches: multiple paternity clutches) is significantly different from the equivalent 20:2 ratio in the FitzSimmons (1998) study ($P = 0.000$), but not from the 9:9 ratio in the Peare and Parker (1994) study. Moreover, the three ratios are highly significantly heterogeneous ($P < 0.001$).

Several factors have been related to the incidence of multiple paternity observed in individual turtle populations, e.g., sex ratio (Bollmer et al., 1999), an increase in hatchling success (Pearse and Avise, 2001), and sperm competition (FitzSimmons, 1998). In multiple paternity studies of other species, e.g., the guppy *P. reticulata*, the female mate choice has been associated with courtship intensity and sperm number (Matthews et al., 1997) resulting from variation in population density (Jirotkul, 1999).

Population density should in turn be related to population size. A lower-breeding population size is likely to reduce density and, thus, reduce the chance of a female meeting and mating with more than one male. Incidence of low population densities has been reported in several turtle studies (Crim et al., 2000; Dutton et al., 1998; Reider et al., 1998). The distance a male turtle has to travel in order to find a female should also be a function of female density. In larger estimated female breeding populations, males show fidelity to particular courting sites moving very little during the mating period (FitzSimmons et al., 1997; Hays et al., 2001; Limpus, 1993). This would also increase the opportunity for multiple mating. The hypothesis that the incidence of multiple paternity might thus increase with increasing female breeding population size was tested by combining data from this and previous studies. Female breeding population size was estimated as the number of clutches laid by all females in a given population, divided by the mean clutch frequency of the population, adjusted by the estimated remigration interval. The results are given in Fig. 2. A trend in increasing incidence of multiple paternity with increasing female population size is evident but neither linear nor polynomial regression gives a significant fit. Also suggested is that variation in the incidence of multiple paternity increases with population size. Caution is required in interpreting Fig. 2 because of the heterogeneity of the data and the large measurement errors inherent in the plotted points; a better test of the hypothesis awaits further studies.

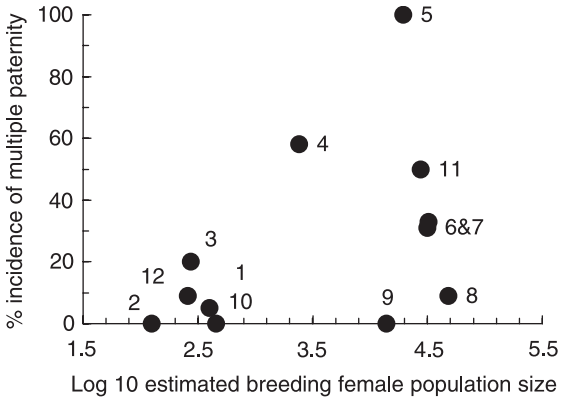

Fig. 2. Incidence of multiple paternity plotted against $\log_{10}$ transformed estimated female breeding population size of all paternity studies to date. The data sources for the numbered plotted points are given in the Appendix A.

In sea turtle studies to date where multiple paternity has resulted in two males siring the offspring of a given clutch, the contribution of male A shows wide variation, from 0.96 (FitzSimmons, 1998) to 0.55 (Bollmer et al., 1999; Moore and Ball, 2002). Differential use of sperm stored from different males with which a female has mated is one factor that might explain this variation. It is known that females store sperm whether or not their clutches exhibit multiple paternity (Dutton et al., 1998; FitzSimmons, 1998; Harry and Briscoe, 1988; Kichler et al., 1999). In the FitzSimmons' (1998) study, male A sired 40 of 42 offspring in the first clutch from one female and all the offspring in her second clutch. A number of 2 of 42 offspring were sired by a different male for the first clutch, suggesting the possibility of differential sperm use. In other cases, sperm from all males with which a female mate is used in all clutches, e.g., for two clutches laid by separate females in the study of Kichler et al. (1999) and for four consecutive clutches studied by Crim et al. (2000), three paternal alleles were observed in all consecutive clutches.

Three alleles in a clutch may be attributed to one father if the mutation rate in microsatellites were particularly high (Kichler et al., 1999). Dutton et al. (1998) when examining paternity in the leatherback turtle *D. coriacea* in St.Croix concluded that this was the case when three paternal alleles were observed in only one locus. Kichler et al. (1999) proposed that for their data to be explained by mutation alone required a mutation rate of 0.098. In the present study, summing gene copies and putative mutational events over clutches and loci gives an estimated mutation rate of 0.056. This would be higher (0.076) if it were assumed that multiple copies of the same alleles were derived from different independent mutational events. These values are an order of magnitude greater than those estimated in green turtles for five loci (FitzSimmons,1998), also dinucleotide loci, where values ranged from 0.00054 to 0.0096. This implies that mutation is an unlikely explanation for our observations.

For clutch X, the unexpectedly high frequency of genotype (150/146) at locus Cm3 is evidence for what could be the first recorded incidence of segregation

distortion in reptiles. Segregation distortion, the gametic production of segregating alleles in non-Mendelian proportions can be due to paternal or maternal 'distorter' alleles that 'sabotage' gametes carrying alternative alleles (Pardo-Manuel de Villena et al., 2000; Zeh and Zeh, 1996). A familiar example of paternal segregation is the SD locus in *Drosophila melanogaster* (Mc Lean et al., 1994) and the t-complex region of the mouse *Mus musculus* (Charlesworth, 1994). In such cases, embryo death, sterility, or the inability of some sperm to fertilise ova are common (Pardo-Manuel de Villena, 2000; Zeh and Zeh, 1996). Maternal segregation distortion has been observed in the *Om* locus in the mouse (Pardo-Manuel de Villena et al., 2000), humans (Evans et al., 1994), and other mammals, where not only can the effects be similar to that of paternal segregation distortion but also the unequal segregation of chromosomes in the polar bodies during meiosis may be observed (Pardo-Manuel de Villena et al., 2000).

If maternal transmission ratio distortion was responsible, there was no evidence of embryonic death (unlike the *Om* locus in the mouse (Pardo-Manuel de Villena et al., 2000). In the clutch of 102 eggs, only one embryo was found dead in the shell and no dead full-term hatchlings were present following the excavation of the clutch. Segregation distortion has not been reported previously in turtles nor in reptiles, generally. Clearly, further analysis of clutches is needed to establish whether it is caused by a high frequency of a segregation distorter gene specific to the population under study.

The numbers of clutches and loci examined in the present study are comparable with the numbers in some other turtle studies (see Appendix A). The data has been sufficient to provide evidence of both multiple paternity and segregation distortion. Further research is needed to assess the nature and causes of differential contributions of males to a clutch. There are also gaps in our knowledge as to whether paternity is similar for different species of marine turtles and population sizes and density. However, further work should lead to a better understanding of the paternity patterns seen in marine turtles and their ecological and genetic significance.

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Appendix A. Turtle studies contributing data to Fig. 2. Replicate values for the same population are represented by * and +

No.	Species	Population	Reference	Samples taken	No. of clutches	No. of offspring analysed
1	*D. coriacea*	La Baulas, Costa Rica	Reider et al. (1998)	1996	4	not known
2	*D. coriacea*	St. Croix, US Virgin I.	Dutton et al. (1998)	1992–1994	not known	not known
3	*L. olivacea*	Suriname	Hoekert et al. (1999)	1995	10	70
4	*L. kempi*	Rancho Nuevo, Mexico	Kichler et al. (1999)	?	26	2–14
5	*C. mydas*	Ascension Island, UK	Ireland et al. (present study)	1999	3	7–20
6	*C. caretta*	Melbourne Beach, Florida	Moore and Ball (2002)	1996	not known	not known
7	*C. caretta*	Melbourne Beach, Florida	Bollmer et al. (1999)	1994	3	20–22
8	*C. mydas*	sGBR, Australia	FitzSimmons (1998)	1991–1994	22	22–58
9	*D. coriacea*	Suriname, W. Atlantic	Hoekert (unpublished data)	1996–1997	not known	not known
10	*D. coriacea*	Playe Grande, Costa Rica	Crim et al. (2000)	1998	80	Total = 1600
11	*C. mydas*	Tortuguero, Costa Rica	Peare and Parker (1994)	1991–1992	18	30

Num	Female breeding population size references
1/10	Spotila et al. (2000); Steyermark et al. (1996)
2	Boulon et al. (1996)
3	Schultz (1975)
4	Heppell (personal communication); Turtle Expert Work Group Report, 1998
5	Godley (personal communication)
6/7	Moore (personal communication); Dodd (1988)
8	FitzSimmons (personal communication)
9	Hoekert, Biotopic, STANASU, (personal communication)
11	Bjorndal et al. (1999)

References

Baragona, M.A., Haig-Ladewig, L.A., Wang, S.Y., 2000. Multiple paternity in the grass shrimp *Palaemonetes pugio*. Am. Zool. 40, 935.

Bjorndal, K.A., Wetherall, J.A., Bolten, A.B., Mortimer, J.A., 1999. Twenty-six years of green turtle nesting at Tortuguero, Costa Rica: an encouraging trend. Conserv. Biol. 13, 126–134.

Bollmer, J.L., Irwin, M.E., Reider, J.P., Parker, P.G., 1999. Multiple paternity in loggerhead turtle clutches. Copeia 2, 475–478.

Booth, J., Peters, J.A., 1972. Behavioural studies on the green turtle (*Chelonia mydas*) in the sea. Anim. Behav. 20, 808–812.

Boulon, R.H., McDonald, D.M., Dutton, P.H., 1996. Leatherback turtles (*Dermochelys coriacea*) on St. Croix, U.S. Virgin Islands: fifteen years of conservation. Chelonian Conserv. Biol. 2, 141–147.

Bowen, B.W., Meylan, A.B., Ross, J.P., Limpus, C.J., Balaazs, G.H., Avise, J., 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of maternal phylogeny. Evolution 46, 865–881.

Broderick, A.C., Godley, B.J., 1997. Observations of reproductive behaviour of male green turtles (*Chelonia mydas*) at a nesting beach in Cyprus. Chelonian Conserv. Biol. 2, 615–616.

Broderick, A.C., Godley, B.J., Hays, G.C., 2001. Metabolic heating and the prediction of sex ratios for green turtles (*Chelonia mydas*). Physiol. Biochem. Zool. 74 (2), 161–170.

Buresch, K.M., Hanlon, R.T., Maxwell, M.R., Ring, S., 2001. Microsatellite DNA markers indicate a high frequency of multiple paternity within individual field-collecting egg capsules of the squid *Loligo pealeii*. Mar. Ecol. Prog. Ser. 210, 161–165.

Charlesworth, B., 1994. The evolution of lethals in the t-haplotype system of the mouse. Proc. R. Soc. Lond., B 258, 101–107.

Crim, J., Spotila, J.R., Spotila, L., Williams, C., Paladino, F.V., Reina, R., 2000. In press: genetic variation and multiple paternity in the leatherback turtle (*Dermochelys coriacea*). Proceedings of the 20th Annual Symposium on Sea Turtle Biology and Conservation, The Delta Orlando Resort, Orlando, Florida USA, pp. 20–21.

Curtis, C., Williams, C.J., Spotila, J.R., 1998. Mating system of Caribbean leatherback turtles as indicated by analysis of microsatellite DNA from hatchlings and adult females. Proceedings of the 18th Annual Symposium on Sea Turtle Biology and Conservation. Compilers: Abreau-Grobois, F.A., Briseño-Dueñas, R., Márques, R., Sarti, L., NOAA Tech. Memo. US Department of Commerce-SEFC-241, p. 155.

Dodd, C.K., 1988. Synopsis of the biological data on the loggerhead sea turtle *Caretta caretta* (Linnaeus, 1958). US Fish Wildl. Serv. Biol. Rep. 88 (14), 1–130.

Dutton, P., Bixby, E., Davis, S.K., 1998. Tendency towards single paternity in leatherbacks detected with microsatellites. Proceedings of the 18th Annual Symposium on Sea Turtle Biology and Conservation. Compilers: Abreu-Grobois, F.A., Briseño-Dueñas, R., Márques, R., Sarti, L., NOAA Tech. Memo. US Department of Commerce-SEFC-241, p. 39

Evans, J.P., Magurran, A.E., 2000. Multiple benefits of multiple mating in guppies. Proc. Natl. Acad. Sci. U. S. A. 97, 10074–10076.

Evans, K.A., Fryer, C., Inglehearn, J., Duvall-Young, J.L., Whittaker, J.L., Gregory, C.Y., Butler, R., Ebenezer, H., Hunk, D.M., Bhattacharya, S., 1994. Genetic-linkage of cone-rod dystrophy to chromosome 19q and evidence for segregation distortion. Nat. Genet. 6, 210–213.

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

FitzSimmons, N.N., Limpus, C.J., Norman, J.A., Goldizen, A.R., Miller, J.D., Moritz, C., 1997. Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proc. Natl. Acad. Sci. U. S. A. 94, 8912–8917.

Galbraith, D.A., 1993. Multiple paternity and sperm storage in turtles. Herpetol. J. 3, 117–123.

Gist, D.H., Jones, J.M., 1989. Sperm storage within the oviducts of turtles. J. Morph. 199, 379–384.

Godley, B.J., Broderick, A.C., Hays, G.C., 2001. Nesting of green turtles *Chelonia mydas* at Ascension Island, South Atlantic. Biol. Conserv. 97, 151–158.

Harry, J.L., Briscoe, D.A., 1988. Multiple paternity in the loggerhead turtle (*Caretta caretta*). J. Heredity 79, 96–99.

Hays, G.C., Broderick, A.C., Glen, F., Godley, B.J., Nicholls, W.J., 2001. The movements and submergence behaviour of male green turtles at Ascension Island. Mar. Biol. 139, 395–399.

Hendrickson, J.R., 1958. The green turtle, *Chelonia mydas* (Linnaeus) in Malaya and Sarawak. Proc. R. Soc. Lond., B 130, 455–535.

Hoekert, W.E.J., Neuféglise, H., Schouten, A.D., Menken, S.B.J., 1999. Multiple paternity in the olive ridley sea turtle. Proceedings of the 19th Annual Symposium of Sea Turtle Biology and Conservation 2000. Compilers: Kalb, H., Wibbells, T., NOAA Natl. Mar. Fish. Serv. Tech. Memo. US Department of Commerce-SEFC-241, p. 13.

Jessop, T., FitzSimmons, N.N., Limpus, C.J., Whittier, J.M., 1999. Interactions between behaviour and plasma steroids within the scramble mating system of the promiscuous green turtle (*Chelonia mydas*). Horm. Behav. 36, 86–97.

Jirotkul, M., 1999. Population density influences male–male competition in guppies. Anim. Behav. 58, 1169–1175.

Kichler, K., Holder, M.T., Davis, S.K., Marquez, M.R., Owens, D.W., 1999. Detection of multiple paternity in the Kemp's Ridley sea turtle with limited sampling. Mol. Ecol. 8, 819–830.

Limpus, C.J., 1993. The green turtle, *Chelonia mydas*, in Queensland: breeding males in the southern Great Barrier Reef. Wild Res. 20, 513–523.

Matthews, I.M., Evans, J.P., Magurran, A.E., 1997. Male display rate reveals ejaculate characteristics in the Trinidadian guppy *Poecilia reticulata*. Proc. R. Soc. Lond., 695–700.

Mc Craken, G.F., Burghard, G.M., Houts, S.E., 1999. Microsatellite markers and multiple paternity in the garter snake *Thamnophis sirtalis*. Mol. Ecol. 8, 1475–1479.

Mc Lean, J.R., Merrill, C.J., Powers, P.A., Ganetzky, B., 1994. Functional identification of the segregation distorter locus of *Drosophila melanogaster* by germline transformation. Genetics 137, 201–209.

Moore, M.K., Ball, R.M., 2002. Multiple paternity in loggerhead turtle (*Caretta caretta*) nests on Melbourne Beach: a microsatellite analysis. Mol. Ecol. 11, 281–288.

Owens, D.W., 1980. The comparative reproductive physiology of sea turtles. Am. Zool. 20, 549–563.

Owens, D.W., Ruiz, G.J., 1980. New methods of obtaining blood and cerebrospinal fluid from marine turtles. HPTGA 36, 17–20.

Pardo-Manuel de Villena, F., de La Casa-Esperõn, Briscoe, T.L., Sapienza, C., 2000. A genetic test to determine the origin of maternal transmission ratio distortion: meiotic drive at the mouse *Om* locus. Genetics 154, 333–342.

Paterson, I.G., Partridge, V., Buckland-Nicks, J., 2001. Multiple paternity in *Littorina obtusata* (Gastropoda, Littorinidae) revealed by microsatellite analyses. Biol. Bull. 20, 261–267.

Peare, T., Parker, P.A., 1994. Multiple paternity in the green turtle (*Chelonia mydas*). Proceedings of the 14th Annual Symposium on Sea Turtle Biology and Conservation. Compilers: Bjorndal, K.A., Bolten, A.B., Johnson, D.A., Eliazar, P.J., NOAA Tech. Memo. NMFS-SEFSC-351, pp. 115–118.

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

Pemberton, J.M., Bancroft, D.R., Barrett, A., Slate, J., 1995. Non amplifying alleles at microsatellite loci: a caution for parentage and population studies. Mol. Ecol. 4, 249–252.

Reider, J.P., Parker, P.G., Spotila, J.R., Irwin, M.E., 1998. The mating system of the leatherback turtle: a molecular approach. Proceedings of the 16th Symposium on Sea Turtle Biology and Conservation. Compilers: Byles, R., Fernandez, Y., NOAA Tech. Memo. US Department of Commerce-SEFC-241, p. 120.

Roff, D.A., Bentzen, P., 1989. The statistical analysis of mitochondrial DNA polymorphisms: chi-squared and the problem of small samples. Mol. Biol. Evol. 6, 539–545.

Schultz, J.P., 1975. Sea turtles nesting in Suriname. Zool. Verh. 143, 143 (Rijksmuseum van Natuurlijke Historie, Leiden).

Spotila, J.R., Reina, R.D., Steyermark, A.C., Plotkin, P.T., Paladino, F.V., 2000. Pacific leatherbacks face extinction. Nature (Lond.) 405, 529.

Steyermark, A.C., Williams, K., Spotila, J.R., Paladino, F.V., Rostal, D.C., Morreale, J., Koberg, M.T., Arauz, R., 1996. Nesting leatherback turtles at Las Baulas National Park, Costa Rica. Chelonian Conserv. Biol. 2, 173–183.

Towner, P., 1991. Purification of DNA. Essential molecular biology: a practical approach. In: Brown, T.A. (Ed.), The Practical Approach Series, vol. 1. Oxford Univ. Press, Oxford, pp. 47–68.

Turtle Expert Working Group, 1998. An assessment of the Kemp's ridley turtle (*Lepidochelys kempii*) and loggerhead (*Caretta caretta*) sea turtle populations in the western north Atlantic. NOAA Tech. Memo. NMFS-SEFSC-409, pp. 1–96.

Ulrich, G.F., Parkes, A.S., 1978. The green sea turtle (*Chelonia mydas*): further observations on breeding in captivity. J. Zool. 185, 237–251.

Zeh, J.A., Zeh, D.W., 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. Proc. R. Soc. Lond., B 263, 1711–1717.