2010-12-16

Population Structure and the Mating System of Loggerhead Turtles (Caretta caretta)

Janne Thoft Nielsen
University of Miami, jnielsen@bio.miami.edu

UNIVERSITY OF MIAMI

POPULATION STRUCTURE AND THE MATING SYSTEM OF LOGGERHEAD TURTLES (CARETTA CARETTA)

By

Janne Thoft Nielsen

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

December 2010

UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

POPULATION STRUCTURE AND THE MATING SYSTEM OF LOGGERHEAD TURTLES (CARETTA CARETTA)

Janne Thoft Nielsen

Approved:

_____	_____
Michael S. Gaines, Ph.D.	Terri A. Scandura, Ph.D.
Professor of Biology	Dean of the Graduate School
_____	_____
Robert Cowen, Ph.D.	Barbara Whitlock, Ph.D.
Professor of Biology	Associate Professor of Biology

_____
Alberto Abreu-Grobois, Ph.D.
Research Scientist
Unidad Academica Mazatlan
Universidad Nacional Autónoma de México

NIELSEN, JANNE THOFT (Ph.D., Biology)

Population Structure and the Mating System of Loggerhead Turtles (*Caretta caretta*) (December 2010)

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Professor Michael S. Gaines.
No. of pages in text. (231)

Studies of the maternally inherited mitochondrial DNA in loggerhead sea turtles (*Caretta caretta*) demonstrate that females are philopatric, returning to nest in the region where they hatched. Eleven genetic stocks of maternal lineages have been identified in the Atlantic Ocean. An analysis of the conventionally-used 380 bp of the mitochondrial control region of a sample of individuals from the genetic stock of loggerheads in Mexico (N = 175) revealed 13 haplotypes. When a longer sequence read of 815 bp was analyzed, 17 haplotypes were uncovered. In the genetic stock of loggerheads in northwestern Florida (N = 25), three haplotypes were identified with both control region sequence lengths. Based on the currently known distributions of the three long CC-A1 and CC-A2 haplotypes, two of each are unique to Mexico. This makes the longer sequence reads useful for stock identification. Within Mexico, there was evidence of significant population structuring between Cozumel and the northern region of the sampling area on mainland Mexico (pairwise $\varphi_{ST} = 0.1003$, $p = 0.0197$), but not after Bonferroni correction. A direct comparison of female and male nuclear microsatellite genotypes indicated male-biased dispersal between Mexico and northwestern Florida. Within Mexico, microsatellite analysis indicated significant structuring of females between

sampling years and between the northern and the southern region of the sampling area on the mainland. Consequently, this genetic stock, while perhaps not in equilibrium, shows signs of female natal homing. An analysis of clutches indicated that significantly more clutches in Mexico had multiple paternity compared to the northwestern Florida (66% and 23%, respectively). The frequency of multiple paternity was not correlated with female abundance, nest density or sex ratio of reproductively successful individuals. There was no evidence of females benefiting through increased reproductive success from multiple paternity. This is consistent with other studies of sea turtles.

Acknowledgements

I would like to thank my advisor Dr. Michael Gaines for his support and guidance with my project. Also, my outside committee member Dr. F. Alberto Abreu-Grobois has been instrumental during the course of this project and whose insight and guidance I greatly appreciate. I thank Dr. Barbara Whitlock and Dr. Robert Cowen on my committee for their comments throughout the process. I am grateful to Flora, Fauna y Cultura de México, AC whose staff has been extremely accommodating and helpful with the field portion and logistics of my dissertation work in Mexico. Especially Alejandro Martinez, Iñaky Iturbe, and Leonel Gómez helped me with sampling design and sampling and were extremely supportive during my stays at the Xcacel turtle camp in Mexico. Without their help, this project would not have been possible. I would also like to acknowledge the help of Rafael Chacón and his staff from the marine turtle protection program of the Municipio de Cozumel with sampling on Cozumel. On St. George Island in northwestern Florida, I would like to acknowledge the help of the Apalachicola National Estuarine and River Research station as well as Bruce Drye for all of their logistic support.

Table of Contents

Page

List of Figures.....v

List of Tables.....vii

Chapter 1 Introduction.....1

Chapter 2 Population Structure of Loggerhead Turtles: A Mitochondrial DNA Analysis.....8

Chapter 3 Population Structure of Loggerhead Turtles: A Nuclear DNA Analysis.....77

Chapter 4 The Mating System of Two Populations of Loggerhead Turtles.....138

Chapter 5 Conclusions.....209

References.....215

List of Figures

Page

Chapter 2

Figure 2.1....68

Figure 2.2....69

Figure 2.3....70

Figure 2.4....71

Figure 2.5....72

Figure 2.6....73

Figure 2.7....74

Figure 2.8....75

Figure 2.9....76

Chapter 3

Figure 3.1....129

Figure 3.2....130

Figure 3.3....131

Figure 3.4....136

Figure 3.5....137

Chapter 4

Figure 4.1....192

Figure 4.2....197

Figure 4.3....198

Figure 4.4....199

Figure 4.5.....200

Figure 4.6.....201

Figure 4.7.....202

Figure 4.8.....203

Figure 4.9.....204

Figure 4.10.....205

Figure 4.11.....206

Figure 4.12.....207

Figure 4.13.....208

List of Tables

Chapter 2

Table 2.1....44
Table 2.2....45
Table 2.3....46
Table 2.4....47
Table 2.5....48
Table 2.6....49
Table 2.7....50
Table 2.8....51
Table 2.9....52
Table 2.10....53
Table 2.11....55
Table 2.12....56
Table 2.13....57
Table 2.14....58
Table 2.15....59
Table 2.16....60
Table 2.17....61
Table 2.18....62
Table 2.19....63
Table 2.20....64
Table 2.21....65

Table 2.22....66
Table 2.23....67
Chapter 3
Table 3.1....112
Table 3.2....113
Table 3.3....114
Table 3.4....116
Table 3.5....117
Table 3.6....118
Table 3.7....119
Table 3.8....120
Table 3.9....121
Table 3.10....122
Table 3.11....123
Table 3.12....124
Table 3.13....125
Table 3.14....126
Table 3.15....127
Table 3.16....128
Chapter 4
Table 4.1....180
Table 4.2....181
Table 4.3....183

Table 4.4.....184

Table 4.5.....185

Table 4.6.....186

Table 4.7.....187

Table 4.8.....189

Table 4.9.....191

Chapter 1

Introduction

The application of molecular tools to study the ecology, behavior and evolution of sea turtles has been instrumental in the development of the field because the highly migratory and mostly oceanic nature of these animals make direct observation extremely difficult (Lee 2008). Loggerhead sea turtles (*Caretta caretta*) belong to the ancient order Testudines and are globally distributed in warm temperate and subtropical oceans. Comparative phylogeographic analyses of sea turtles (Cheloniidae and Dermochelyiidae) indicate that patterns of global genetic structuring are shaped by the habitat and temperature preferences of each species (Bowen and Karl 2007). Green turtles (*Chelonia mydas*), which are more tropical in distribution than loggerheads, have two divergent mitochondrial DNA lineages that correspond to the Atlantic and the Indo-Pacific ocean basins with little evidence of genetic exchange (Bowen et al. 1992, Encalada et al. 1996, Bourjea et al. 2007) Loggerheads also have two distinct lineages corresponding to the ocean basins, but with less geographic partitioning than green turtles (Encadala et al. 1998). Their tolerance of cooler water has enabled loggerheads, more so than green turtles, to bypass southern Africa, a geographic barrier to dispersal and genetic exchange between the Atlantic and Indian Oceans for many tropical marine species (Bowen and Karl 2007).

While loggerhead turtles spend most of their lives at sea, gravid females come up onto beaches where they lay an average of 4.1 clutches of eggs, at ca. two-week intervals, during a nesting season (Dodd 1988, TEWG 2000). Because of the relatively easy access to females and hatchlings on the nesting beaches, research has focused on this aspect of

their life history. Early mark-recapture studies detected that females return consistently to the same beach to nest. Two hypotheses were proposed to account for this behavior. The natal homing hypothesis suggests that females return to their natal beach to reproduce (Carr 1967). Under an alternative scenario, the social facilitation hypothesis proposes that first-time female breeders follow experienced females to a nesting beach and subsequently return to that same beach to nest (Hendrickson 1958, Owens et al. 1982). MtDNA analyses are able to distinguish between these two scenarios, because natal homing will result in genetic partitioning among nesting populations and social facilitation will result in genetic admixture. Natal homing in loggerheads first received genetic support in the early 1990s (Bowen et al. 1993) and has been repeatedly been confirmed since then. To date at least 11 genetic stocks of maternal lineages have been identified in the Atlantic Ocean (Bowen et al. 1993, Schroth et al. 1996, Encalada et al. 1998, Laurent et al. 1998, Bowen et al. 2004, Garofalo et al. 2009, Reis et al. 2010, Monzón-Argüello et al. in press).

Immediately after hatching, hatchlings enter the ocean where they spend their time amidst seaweed (*Sargassum*) and in convergence zones high in nutrients from upwelling (Carr 1987). Juvenile loggerheads remain in an oceanic habitat for several years (Bjorndal et al. 2000). Genetic analyses based on mtDNA haplotypes have demonstrated that juveniles may cross entire ocean basins (Bowen et al. 1995, Bolten et al. 1998). After the oceanic stage, sub-adults recruit to neritic habitats where they remain for about a decade until maturity (Carr 1987), which is estimated at 21-35 years of age (Frazer and Ehrhart 1985, Frazer et al. 1994). As adults, loggerheads may engage in extensive migrations of thousands of kilometers between foraging and reproductive areas

(Musick and Limpus 1997). While nesting populations are characterized by specific mtDNA haplotype frequencies, genetic analyses have revealed that individuals from various genetic stocks converge in foraging areas (e.g. Bolten et al. 1998, Bass et al. 2004). These mixed stock analyses estimate the relative contribution of genetic stocks to the foraging area. Because mtDNA haplotypes are shared among stocks, although occur at different frequencies, confidence limits tend to be large and individual stock assignment is difficult with the resolution of the currently-used mtDNA marker (Chapman 1996, Pella and Masuda 2001, Bolker et al. 2003, Bolker et al. 2007). Also, the variability of the size of nesting populations can confound the analyses. Very small populations may go undetected because their contributions to a foraging area can be limited in comparison to very large populations. Another limitation is "orphan" haplotypes, which are found in a foraging area but have not been detected in any nesting population due to incomplete sampling (Bowen and Karl 2007).

Because mtDNA is maternally inherited and females are more easily are studied than males, we know much less about male migratory and reproductive behavior. The different inheritance patterns of chromosomes and genomes provide gender-specific genetic markers that lend themselves well to studies of sex-biased dispersal (e.g. Allendorf and Seeb 2000, Buonocarsi et al. 2001, Lu et al. 2001). For example, in most mammals male dispersal can be detected with surveys of the Y chromosome because it is passed down from father to sons (Zegura et al. 2004), while the mtDNA tracks female movement (Bonatto and Salzano 1997). Sea turtles, like many reptiles, do not have sex-discerning genes because (Bull 1980, Janzen and Paukstis 1991). The incubation temperature during embryonic development determines their sex (Yntema and

Mrosovsky 1980). In such cases, the use of bi-parentally inherited nuclear DNA (nDNA) markers is necessary to illustrate the influence of male-mediated gene flow on population structure. Discordance in genetic structuring detected by mtDNA and nDNA may indicate sex-biased dispersal. Recently, studies using nDNA microsatellite markers have illustrated that males tend to provide an avenue for gene flow among genetic stocks more so than females, although the patterns appear to vary among regions (Bowen et al. 2005, Carreras et al. 2007). Male migration and homing remain poorly understood (Bowen and Karl 2007).

To address some of these gaps in knowledge, my research focused on two genetic stocks of loggerhead turtles, one in the Yucatan Peninsula, Mexico and one in northwestern Florida, USA. Mexico hosts an intermediate-sized nesting population (ca. 2300 nests per year, Ehrhart et al. 2003), which harbors high genetic diversity, likely a vestige of the region's role in providing refugia during the last glacial maxima (Bowen et al. 1993). The Mexican stock is in the southern range of the species in the northern hemisphere, and thus may provide a link to the loggerheads nesting in the south Atlantic (e.g. Brazil) which are relatively divergent from loggerheads north of the equator. Despite the Yucatan Peninsula's central role in providing essential nesting habitat, maintaining genetic diversity of the species, and potentially serving as a stepping stone for gene flow between populations in the northern and southern hemispheres, little research has been conducted on this genetic stock. To date, the mtDNA haplotype frequency distribution of Mexican genetic stock is based on a mere 20 samples. The northwestern Florida genetic stock is a minor nesting population (ca. 600 nests per year, Ehrhart et al. 2003). This is in sharp contrast to the neighboring southern Florida genetic

stock, which is one of two largest nesting populations in the world (Baldwin et al. 2003, Ehrhart et al. 2003). State monitoring programs and research have focused on the larger more "important" population. The small size of this stock makes it susceptible to local extirpation due to the genetic effects of inbreeding and genetic drift (Frankham 1996). Recent trends suggest a continued decrease in nesting (Florida Fish and Wildlife Conservation Commission *unpublished data*). In addition to the inherent conservation value of focusing both on small and large nesting populations to maintain intraspecific genetic variation throughout the species range, mechanisms that enable small populations to persist may be identified (Bell et al. 2010).

I begin in Chapter 2 with an analysis of the mtDNA diversity of these two genetic stocks of loggerhead turtles in Mexico and in northwestern Florida. By increasing the sample size of these two genetic stocks, compared to the sample size of previous studies, my goal was to characterize the genetic diversity and provide a more accurate genetic profile of loggerheads nesting in these regions. This genetic data is instrumental to understanding the interconnectedness of the various genetic stocks as they overlap in foraging areas. In addition, I analyzed a longer segment of the control region used to define each genetic stock. The previously used shorter control region sequence can distinguish haplotypes but many of them are shared among different genetic stocks. The frequency of these haplotypes varies among stocks, however the use of a larger mtDNA marker may be able to distinguish haplotypes unique to each genetic stock. I sought to determine the utility of analyzing a longer segment of the control region for resolving unique haplotypes that will aid in stock identification. Stock identification is critical in mixed stock analyses where the contribution of each stock to foraging areas is estimated.

Currently, only relative contributions from each stock can be estimated due to the high number of shared haplotypes.

In Chapter 3, I used bi-parentally inherited microsatellite nDNA markers to examine the role of males in mediating gene flow between the Mexican and northwestern Florida stocks. I directly compared male and female genetic data to test predictions about sex-biased gene flow. Also, I used these highly polymorphic markers to look at fine-scale structuring to understand how precisely females are returning to their natal beach.

In Chapter 4, I investigated the mating system of loggerhead turtles. The mating system ties in with the two previous chapters because ultimately mate choice will influence population structure and gene flow. Mating with individuals from other populations result in gene flow and genetic homogenization. If individuals of both sexes are philopatric and only mate within their own natal population genetic differentiation will arise among populations. I identified full and half sibling groups of hatchlings, reconstructed parental genotypes, and assigned single or multiple paternity to clutches. Multiple paternity is the fertilization of a single clutch of eggs by multiple males resulting in full and half siblings. I sought to elucidate the relationship between the frequency of multiple paternity and population parameters, such as female abundance and operational sex ratio. Additionally, I tested the female benefit hypothesis by comparing various estimators of reproductive success from clutches with single and multiple paternity. According to Bateman's principle (Bateman 1948), females are expected to mate multiply only if the benefits outweigh the costs. Benefits that pertain to sea turtles may be limited to indirect genetic benefits such increased reproductive success (Pearse and

Avise 2001). By analyzing paternity across successive clutches laid by four females, I made inferences about the timing of mating and elusive male migratory behavior.

In the final chapter I summarize my findings and discuss the broader impacts of my research.

Chapter 2

Population Structure of Loggerhead Turtles: A Mitochondrial DNA Analysis

Background

Species are often geographically structured into populations which may remain interconnected by migration and dispersal. Dispersal and subsequent reproduction between individuals from different units allows for gene flow and homogenizes genetic differences. Gene flow is promoted when there is high spatial connectivity in the absence of dispersal (Bowen and Grant 1997), and when costs of reproducing outside of one's natal area are low (Perrin and Mazalov 2000).

Mechanisms that limit gene flow and generate population structure include isolation due to geographic or physical barriers, isolation by distance as well as social structure and resource specialization (Hoelzel et al. 1998, Whitehead et al. 1998, Engelhaupt et al. 2009). Another mechanism is natal homing (Meylan et al. 1990), in which individuals return to reproduce at their own place of birth. Natal homing has been documented most notably in birds and mammals (Greenwood 1980, Clutton-Brock 1989). If most individuals in a population only breed at or near their natal sites, then genetic partitioning results due to limited gene flow. Natal homing will be favored by natural selection when the costs of dispersal and finding new breeding sites are high (Perrin and Mazalov 2000).

Natal homing can, and often does, vary between the sexes. In birds, females tend to be the dispersers, whereas males remain faithful to the natal area (Greenwood 1980, Clarke et al. 1997). However, in other species such as great white sharks (*Carcharadon carcharias;* Pardini et al. 2001), southern elephant seals (*Mirounga leonina;* Fabiani et al.

2003) and sperm whales (*Physeter macrocephalus*; Engelhaupt et al. 2009) females are philopatric and males disperse great distances.

The dispersal tendency of one sex, while homogenizing genetic differences among populations, may reduce local mate and resource competition (Hamilton 1967, Clarke 1978, respectively). Also it may lower the risk of inbreeding (Clutton-Brock 1989). In turn, the philopatric sex promotes genetic differentiation among populations. In this way, the behavior of individuals either to be faithful to their natal site or to disperse simultaneously shapes the genetic structure of populations.

Mark and recapture studies of sea turtles have demonstrated that adult females return to the same region to nest, not just within a season, but from year to year, and has been termed "nest site fidelity" (Limpus et al. 1992, Plotkin 2003). With the application of molecular methods in the early 1990's, mitochondrial DNA (mtDNA) studies revealed that females from different rookeries were highly differentiated (Meylan et al. 1990, Bowen et al. 1993, Bass et al. 1996). Individuals from different rookeries overlap in foraging areas, but the structuring found in the maternally-inherited mtDNA among rookeries implies that females return to their natal region to nest (natal homing, Koch et al. 1969), likely guided by geomagnetic imprinting (Lohmann et al. 2008).

To date, at least 11 discrete genetic stocks of loggerheads have been identified in the Atlantic Ocean based on a ca. 380-bp segment of the control region (Figure 2.1) (Encalada et al. 1998, Laurent et al. 1998, Pearce 2001, Garofalo et al. 2009, Reis et al. 2010, Monzón-Argüello et al. in press). Each genetic stock is characterized by significantly different frequencies of mtDNA control region haplotypes. Many haplotypes are shared among populations, and while haplotype-sharing may indicate

current gene flow among stocks, in this case shared haplotypes more likely represent historical contact or common ancestry. Mark and recapture data indicate very little movement of females among populations (Bjorndal et al. 1983). Also, the pattern of diversity seen today – a gradual loss of haplotype diversity from south to north – suggests range expansion accompanied by population bottlenecks after the last glacial maxima 12,000 years ago (Bowen et al. 1993, Lee 2008).

The 380-bp segment of the control region has been used widely in mixed stock analyses to estimate the proportion of loggerhead turtles from different genetic stocks in adult and juvenile foraging areas where individuals from different stocks converge (e.g. Engstrom et al. 2002, Bowen et al. 2005, Casale et al. 2008). However, the accuracy of mixed stock analyses is limited both by insufficient resolution of the genetic marker and incomplete sampling of the source populations (Chapman 1996, Pella and Masuda 2001, Bolker et al. 2003, Bolker et al. 2007). Haplotype-sharing among populations creates the need for increased marker resolution, which may resolve additional haplotypes and reduce the number of shared haplotypes. Levels of genetic diversity within and genetic differentiation among populations can be assessed more accurately with markers with higher resolution. Insufficient sampling of some nesting populations is another concern. Low sample sizes may mask the true genetic diversity of a population. Other nesting populations may not have been genetically characterized, or even located. Until recently, the genetic diversity of the second largest loggerhead nesting population in the Atlantic Ocean, in Cape Verde, had not been quantified (Monzón-Argüello et al. in press).

In this study, by testing three hypotheses, I sought to characterize the genetic profile of two sparsely-sampled populations of loggerhead turtles, nesting in the Yucatan

Peninsula, Mexico and in northwestern Florida. While each of these has been identified as discrete genetic stocks based on previous studies (Encalada et al. 1998, Pearce 2001), analyses suffer from relatively small sample sizes (Mexico n = 20; northwestern Florida n = 49) compared to other populations in the northwestern Atlantic Ocean (Dry Tortugas n = 58, southern Florida = 109, northern Florida-North Carolina = 105). Mexico, in the southern part of the nesting range of loggerheads, is thought to have provided refugia during glacial maxima and consequently may harbor much genetic diversity (Bowen et al. 1993). It is unlikely that the small sample size of the previous study in Mexico accurately can portray the true level of diversity in this region, which hosts an "intermediate"-sized nesting population (Ehrhart et al. 2003). Despite the relatively small sample size, Mexico had the highest haplotype diversity (h = 0.65) of Atlantic loggerheads (Encalada et al. 1998), although this likely may be an underestimate of the true diversity of this population. This high diversity of loggerheads in Mexico makes it an ideal region to study fine-scale structure.

Loggerheads nest throughout the Florida panhandle, with most areas hosting less then five nests per km (Florida Fish and Wildlife Conservation Commission *unpublished data*). This genetic stock represents a "minor" nesting population (Ehrhart et al. 2003), which is in stark contrast to the neighboring southern Florida stock – a "major" nesting population – which is host to over 10,000 female nesters annually, and is one of the world's two largest nesting populations; the other one is in Masirah, Oman (Baldwin et al. 2003, Ehrhart et al. 2003). For this reason, the scant nesting in northwestern Florida has been overlooked by state monitoring programs. Florida began a coordinated program of nest counts in 1979 and in 1989 established a subset of beaches (index beaches) to

represent a standardized index of sea turtle nesting that would allow for reliable analyses of trends across years. Nesting beaches in northwestern Florida were not added to this program until 1997 (Witherington et al. 2009). From the period 1997 to 2010, the annual number of nests declined by over 50% on these index beaches (Florida Fish and Wildlife Conservation Commission *unpublished data*). In the rest of the state, a similar trend was detected in the years 1998 to 2006 with a decline of 43-44% (Witherington et al. 2009). Since then, the rest of the state has seen and upswing in nesting activity, that is not apparent in northwestern Florida where the population continues its decline (Florida Fish and Wildlife Conservation Commission *unpublished data*). The small population size, recent colonization history likely associated with founder effects cause this genetic stock to be vulnerable to genetic drift and as a result have low genetic diversity (Frankham 1996). In fact, haplotype diversity in northwestern Florida (h = 0.38 - 0.44) was lower than in Mexico (h = 0.65) and southern Florida (h = 0.57 - 0.66) (Encalada et al. 1998, Bowen et al. 2005). Low population size, if accompanied by low density, may lead to reduced fertility and breeding success (Liermann and Hilborn 2001, Berec et al. 2007), which additionally puts this population at risk for local extirpation. Further research of this vulnerable population is warranted to determine the potential genetic effects of its continued decline and its connectivity to other populations, which could supply much-needed genetic variation.

First, I hypothesized that increasing the sample size of these two stocks would change their genetic profile. I predicted that larger sample sizes would uncover increased genetic diversity compared to the prior datasets. Small sample sizes can lead to significantly inaccurate estimates of genetic diversity (Nei 1978, Bashalkhanov 2009). I

also predicted that genetic structuring among loggerheads from rookeries in Mexico and the southeastern United States would be more pronounced with the larger sample sizes compared to the smaller samples sizes from prior studies.

Second, I hypothesized that analyzing a longer segment of the control region than conventionally analyzed would increase resolution between loggerheads in Mexico and northwestern Florida. I predicted that polymorphisms in the extended part of the control region sequence would resolve additional haplotypes compared to the shorter sequence (Tamura and Nei 1993). Long control region sequences from loggerheads from Cape Verde generated additional haplotypes compared to the number of haplotypes from the short sequence. (Monzón-Argüello et al. in press). Due to increased polymorphism, I predicted that the long sequence would describe increased genetic diversity compared to the short sequence and that population differentiation between Mexico and northwestern Florida would be more pronounced.

Finally, I hypothesized that there would be fine-scale structuring within Mexico. I predicted that sites within Mexico would be genetically differentiated from one another due to precise natal homing of females. While mtDNA evidence supports females returning to nest within their natal region, I predicted they return to nest on or near their natal beach. With the shorter control region sequence, the finest level of structure shows genetic differentiation among rookeries separated by as little as 120 km in Japan and 50 to 100 km in Florida (Hatase et al. 2002, Bowen and Karl 2007). With the longer control region sequence, structure may be resolved at an even finer scale.

Methods

Study sites

The loggerhead rookeries in Quintana Roo (QR), Mexico (20°33'N, 87°38'W) belong to the Yucatan Peninsula genetic stock, as defined by Encalada et al. (1998). I collected samples from nine nesting sites spread out along 52.4 km of coastline on the mainland as well as from Cozumel (20°40'N, 86°86'W), an island about 20 km off the coast (Figure 2.2). Seven of the mainland sites (Aventuras, Chemuyil, Xcacel, XelHa, Tankah, Kanzul, Cahpechen) are patrolled nightly, where nearly all nesting turtles are observed and tagged. This allows each turtle to be tracked throughout the season. At the remaining two mainland sites (Paamul and Punta Cadena) and on Cozumel females are not tagged, but nests are located and marked. I collected samples during the nesting seasons in 2006 and 2008, which I will refer to as QR2006 and QR2008, and collectively as QR. Sample sizes and number of nests per nesting site are listed in Table 2.1.

The loggerhead rookeries on St. George Island (SGI), Florida (29°68N, 84°80W) belong to the northwestern Florida genetic stock, as defined by Encalada et al. (1998) (Figure 2.3). Nests are located and marked throughout the nesting season, but females are not tagged. I collected samples in SGI during the 2007 and 2008 nesting seasons (SGI2007 and SGI2008) (Table 2.1). Alligator Point (29°89'N, 84°38'W), a peninsula about 35 km northeast of SGI, is within the northwestern Florida population (Figure 2.3). I collected a sample from one female I encountered nesting in 2007. For the sake of simplicity, I grouped the Alligator Point sample with the samples from SGI and refer to these two locations jointly as SGI. The remigration interval (the time between two consecutive nesting seasons) for female loggerheads ranges from one to nine years, with

an average interval of two and a half to three years (Schroeder et al. 2003). Thus, chances of sampling the same matrilines in SGI in 2007 and 2008 were minimal. See Chapter 2 for details on how microsatellite genotypes were analyzed to verify individuals sampled in different years were not related (full or half siblings, or female – offspring).

Field methods

At sites where females were observed and tagged (Table 2.1), I collected samples from females after nesting, using a 3 or 6 mm biopsy punch to biopsy a skin plug from the posterior edge of the female's fore flipper. At sites where females were not observed or tagged, I collected samples from hatchlings that were assumed to be unrelated (i.e. no full or half siblings). Hatchlings inherit their mtDNA from their mother, so this sampling scheme does not affect the genetic analysis. The inter-nesting interval (the time between oviposition cycles) typically lasts 13 to 14 days (Broderick et al. 2002, Schroeder et al. 2003), although it can be shorter (10 days) especially when the water is warmer (Hays et al. 2002). Thus to minimize pseudoreplication by sampling hatchlings from successive clutches laid by a single female, I sampled only hatchlings from clutches laid within a 10-day window at each site every nesting season. Hatchlings, if dead, were sampled by cutting a small piece of tissue from the front flipper. Live hatchlings were sampled by drawing 0.1 cc blood using a ½ cc 28 gauge disposable insulin syringe (Kendall) from the dorsal cervical sinus following the method of Owens and Ruiz (1980). I released hatchlings at the nest site immediately after sampling. Skin plugs and flipper clips were stored in 1 ml DMSO buffer (20% DMSO and 6M NaCl) and blood was stored in 1 ml of Longmire's lysis buffer (Longmire et al. 1992) in 1.5-2 ml tubes.

All procedures were approved by University of Miami Institutional Animal Care and Use Committee # 07-114, and samples in Mexico were collected under permit # 07656 issued by Secretaría de Medio Ambiente y Recursos Naturales, and in Florida under marine turtle permit # 189 issued by Florida Wildlife Conservation Commission. Mexican samples were imported into the United States under CITES permit # 124476.

DNA extraction, amplification, and sequencing

I extracted DNA from tissue samples using a standard ethanol precipitation protocol. I amplified part of the mtDNA control region using primers LCM15382 (forward) and H950 (reverse) (5' - GCT TAA CCC TAA AGC ATT GG - 3' and 5'-GTCTCGGATTTAGGGGTTTG -3', respectively; Abreu-Grobois et al. 2006) with the polymerase chain reaction (PCR) (Saiki et al. 1988). These primers flank 782 bp of the 922-980 bp of the control region (Abreu-Grobois et al. 2006), leaving out a highly repetitive segment at the 3' end, that has a variable number of repeats and heteroplasmy (Laurent et al. 1998). This amplification encompasses the entire 380-bp segment amplified by the TCR5/TCR6 primers (Norman et al. 1994) that have been used conventionally for loggerhead population structure analysis.

The total PCR volume was 20 µl consisting of ca. 50 ng of template DNA, 0.2 µM dNTP's, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.5 µM of each primer, and 0.5 unit *Taq* DNA polymerase (Promega Corporation). PCR cycling conditions on an Eppendorf Mastercycler Gradient PCR cycler (Eppendorf AG) consisted of an initial cycle at 94°C for 5 min, followed by 36 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min, followed by a final cycle of 72°C for 10 min.

The PCR products were incubated with Shrimp Alkaline Phosphatase (SAP) and Exonuclease I (Exo) enzymes (USB Corporation) to phosphorylate the amplified fragments and degrade excess primer following the manufacturer's recommendations.

Cycle sequencing was carried out in 10 µl reaction volumes consisting of 0.5 µl PCR product purified with the SAP and Exo enzymes (10-50 ng), 0.5 µl BigDye version 3.1 reaction mix, 2 µl 5X reaction buffer, and 1 µM of either the forward LCM15382 or reverse H950 primer, using the following thermal profile: initial cycle at 95^{o}C for 1 min, then 40 cycles at 95^{o}C for 1 min, 50^{o}C for 20 sec, and 60^{o}C for 4 min. Sequencing reaction products were run through Sephadex multiscreen plates (Millipore) to remove unincorporated nucleotides. Purified products were then dried for 45 minutes in a vacuum centrifuge and subsequently resuspended in 12 µl of Hi-Di Formamide (Applied Biosystems). The fragments were sequenced in both directions on an ABI 3130XL automated sequencer (Applied Biosystems).

MtDNA sequence data analysis

Contigs of nucleotide sequence data were assembled in SEQUENCHER version 4.6 (GeneCodes). Forward and reverse sequences for each individual were checked for consistency. Final sequences were imported into DNASP version 5 (Librado and Rozas 2009) to identify unique haplotypes.

Haplotypes were designated according to the standardized nomenclature of the Archie Carr Center for Sea Turtle Research (ACCSTR: http://accstr.ufl.edu). Names for short haplotypes for Atlantic loggerheads follow the format of CC-AX (where X is any integer designating the specific haplotype), and long haplotype names include the name

of the short haplotypes they encompass plus a haplotype-specific suffix (e.g. CC-A1.1 is a long haplotype that encompasses the short haplotype of CC-A1). Novel haplotypes were submitted to ACCSTR to receive a name.

To quantify genetic diversity, the following indices for each site and each dataset (short and long control region sequences) were calculated in ARLEQUIN version 3.5 (Excoffier and Lischer 2010): the average nucleotide diversity over all nucleotide sites (π), (Tajima 1983, Nei 1987), haplotype diversity (h) (Nei 1987), and number of polymorphic sites (s).

To test for genetic differences among years, for differences in resolution between the short and long control region sequence, and for fine-scale structure among sites within QR, I assessed the level of genetic structuring using three different methods as implemented in ARLEQUIN. First, I used the exact test of population differentiation, which tests the hypothesis that haplotypes are distributed randomly among populations. I ran this test with 100,000 steps in the Markov chain and 10,000 dememorization steps. Second, I ran an analysis of molecular variance (AMOVA) with 99,999 permutations to assess the amount of genetic diversity that was attributable to within and among sites or seasons. An AMOVA is a hierarchical analysis of variance which partitions the total genetic variance into covariance components due to differences at the different levels of analysis. I selected the Tamura-Nei model of nucleotide substitutions to estimate sequence divergence. This model is designed for control region sequences and takes into account differences in transition and transversion rates as well as differences in transition rates between purines and between pyrimidines (Tamura and Nei 1993). Finally, I calculated Wright's fixation index of population subdivision (pairwise φ_{ST}) with

statistical significance obtained over a minimum of 99,999 permutations as my third measure of population structure. Wright's fixation index computes genetic distances either by following a user-specified model of nucleotide substitution rate to calculate distances between haplotypes (φ_{ST}) or by considering all distances between non-identical haplotypes as equal (F_{ST}). I chose to the Tamura-Nei model of nucleotide substitutions to calculate φ_{ST} as using a distance model compared to the haplotype frequency model provides greater resolution. φ_{ST} values range from 0 to 1, with 0 indicating panmixia (negative values should be considered as 0), and 1 indicating complete genetic differentiation. To help interpret φ_{ST} values, the following scale has been suggested as a rough guideline: 0 - 0.05 indicates little genetic differentiation; 0.05 - 0.15 moderate differentiation; 0.15 - 0.25 great differentiation; and values greater than 0.25 very great genetic differentiation (Wright 1978, Hartl and Clark 1997).

The exact test of population differentiation and Wright's fixation index of population subdivision both involve multiple pairwise tests at the same significance level which increases the type I error rate (Zaykin et al. 2002). Typically, this problem is circumvented by applying a sequential Bonferroni correction which maintains an experiment-wise significance level while adjusting the significance level of individual tests to reduce the probability of spurious results (Holm 1979, Rice 1989). Although its use has been debated as it increases the type II error rate (Moran 2003), I applied the sequential Bonferroni correction modified by Jaccard and Guilamo-Ramos (2002) as implemented in MACBONFERRONI (Watkins 2002) for all cases of multiple comparisons and I report results both with and without the correction.

For population structure analysis to test the sample size hypothesis, in addition to my data, I used previously published short control region haplotypes from QR (n = 20; Encalada et al. 1998, which I will refer to as EncMX) and northwestern Florida (n = 49; compiled in Bowen et al. 2004, which I will refer to as BowNWFL), southern Florida (n = 109; compiled in Bowen et al. 2004), Dry Tortugas (n = 58; Pearce 2001), and northeastern Florida-North Carolina (n = 105; compiled in Bowen et al. 2004).

For population structure analysis to test the sequence length hypothesis, in addition to my data, I used previously published datasets that included both short and long haplotypes from Georgia, USA (n = 17; Monzón-Argüello et al. in press), Cape Verde (n = 128; Monzón-Argüello et al. in press) and Italy (n = 38; Garofalo et al. 2009).

I constructed a statistical parsimony network (Templeton et al. 1992) of the long control region sequences to depict phylogenetic relationships among haplotypes in QR and SGI using the software TCS version 1.21 (Clement 2000).

To assess fine-scale structure, I compared all sites in QR with one another. Females who nested at multiple sites were included in the sample for each of those sites (Table 2.1). Although this led to replication of some individuals, I felt it was more accurate initially to test for structure among all the sites, rather than combine the sites that were used by individual females. Combining sites would potentially conceal any structuring that may exist among females. Also, I calculated genetic distances among regions by grouping sites in the following way: 1) Paamul, 2) north - consisting of Aventuras, Chemuyil, Xcacel, and XelHa, 3) central - consisting of Punta Cadena and Tankah, 4) south - consisting of Kanzul and Cahpechen, and 5) Cozumel.

Sample sizes per site were uneven due to logistic reasons (uneven distribution of personnel and effort among sites) and because nesting at some sites was scarcer than at others. To compare haplotypes and haplotype richness among sites while standardizing for the effect of sample size, I constructed two rarefaction curves (one for short and one for long haplotypes) using ANALYTIC RAREFACTION 1.3 developed by Steven Holland (available at http://www.uga.edu/strata/software/) based on equations by Raup (1975) and Tipper (1979). While rarefaction is generally used to estimate species richness, haplotypes in this case are substituted for species.

To test for a correlation between genetic distance and geographic distance (isolation-by-distance) among the10 nesting sites in QR, I ran a Mantel test with 1,000 permutations in ARLEQUIN. Geographic distances were calculated as the shortest distance between the latitude and longitude coordinates for each pair of sites, except for Cozumel. To calculate distances from Cozumel to each of the mainland sites, I determined the distance from the middle of the 22-km stretch of coastline on Cozumel where I collected samples (20.435N, 86.83W) to the southern tip of the island (20.27N, 86.99W) and from there the distance to each of the other sites. In this way I simulated a turtle swimming south of the island in order to reach the mainland. I also ran the Mantel test on the five regions (Paamul, north, central, south, and Cozumel). The center of each region was used to calculate distances between each pair of regions, except for Cozumel, where I used the southern tip of the island.

I performed a spatial analysis of molecular variance first of the 10 nesting sites and then of the five regions in QR with the program SAMOVA 1.0 (Dupanloup et al. 2002). This program defines groups of populations that are maximally differentiated

from one another by maximizing the proportion of total genetic variance due to differences among groups (F_{CT}). The number of groups is specified *a priori*, and the correct number of groups tends to correspond with the larger F_{CT}. Identifying the correct number of groups relies on the degree of differentiation among groups and the absence of isolation-by-distance (Dupanloup et al. 2002).

Results

Characterization of mtDNA sequence data

I obtained a total of 816 bp of high quality sequence data for the control region that was confirmed in both the forward and reverse directions. This segment resulted in 19 unique long haplotypes. When the sequences were trimmed to the 380-bp segment corresponding to the published short haplotypes, 14 unique haplotypes were distinguished. The long sequence had 40 transitions and one transversion and the short sequence 21 transitions and no transversions. Nucleotide frequencies were the same across both the long and the short sequences: A = 32.37%, C = 20.33%, G = 14.05%, T = 33.24%.

Mexico

The exact test of population differentiation comparing QR2006, QR2008 and EncMX (short sequence only), indicated that both the short and long haplotypes were randomly distributed (short haplotypes: exact $p \geq 0.3345$ for all pairwise comparisons; long haplotypes: exact $p = 0.5103$) (Table 2.2).

With the short control region sequence, the AMOVA among QR2006, QR2008, and EncMX revealed that most of the variance was due to genetic variation within each dataset (V_b = 2.5490, 97.7%) (Table 2.3). The three datasets were not genetically differentiated (φ_{ST} = 0.0230, p = 0.0696).

Pairwise φ_{ST} between EncMX and QR2006 (short sequence) indicated slight, but significant, genetic differentiation (φ_{ST} = 0.0838, p = 0.0266) before sequential Bonferroni correction but not after. Neither EncMX and QR2008 (short sequence) or QR2006 and QR2008 (short and long sequences) were genetically differentiated ($\varphi_{ST\ (EncMX\text{-}QR2008)}$ = 0.0434, p = 0.0965; short: $\varphi_{ST\ (QR2006\text{-}QR2008)}$ = 0.0020, p = 0.2540, long: $\varphi_{ST\ (QR2006\text{-}QR2008)}$ = 0.0007, p = 0.2943) (Table 2.4).

Because no structure was found between QR2006 and QR2008 with either the short or long control region sequences, I grouped the two sampling years for subsequent analyses. Two out of the three tests for genetic structure indicated no structure between EncMX and either of the sampling years in QR. When QR2006 and QR2008 were grouped, no genetic differentiation was observed with EncMX with either the exact test of population differentiation (exact p = 0.6518) nor with pairwise φ_{ST} (φ_{ST} = 0.0621, p = 0.0582) (Table 2.5). Thus, when applicable, I combined EncMX with the QR dataset to obtain a total Mexican dataset (totalMX).

Northwestern Florida

The exact test of population differentiation indicated a random distribution of short and long haplotypes between the two sampling years in SGI and of short haplotypes

between BowNWFL and each of the two years in SGI (exact $p \geq 0.3873$ for all pairwise comparisons) (Table 2.6).

The AMOVA among the two sampling years in SGI and BowNWFL revealed that most of the variance was attributable to within site variation ($V_b = 2.9454$, 101.43%) resulting in lack of genetic differentiation ($\varphi_{ST} = -0.01430$, $p = 0.4671$) (Table 2.7).

Pairwise φ_{ST} was not significant between any pairwise comparisons with either the short or the long sequences ($\varphi_{ST} \leq 0.0192$, $p \geq 0.2490$) (Table 2.8).

Because none of the three tests indicated genetic differentiation between seasons, I combined the two sampling years in SGI for subsequent analyses. No genetic differentiation was observed between the combined SGI dataset (n = 25) and BowNWFL with either measure of genetic structure (exact $p = 0.5052$; $\varphi_{ST} = 0.0036$, $p = 0.3258$) (Table 2.9). Thus, when applicable, I combined BowNWFL with SGI to obtain a total northwestern Florida dataset (totalNWFL).

Sample size

There were 13 short control region sequence haplotypes in QR, one of which was novel, compared to five haplotypes previously described from this region in EncMX (Table 2.10). In both datasets, CC-A2 was the most common haplotype found in about half of the individuals, followed by CC-A10 found in about a quarter of the individuals. Haplotype diversity was not significantly greater in QR compared to EncMX (h_{QR} = 0.7250 ±0.0271 and h_{EncMX} = 0.6526 ±0.0927, respectively) (Table 2.11). However, nucleotide diversity was greater in QR compared to EncMX (π_{QR} = 0.0146 ±0.0078 and π_{EncMX} = 0.0024 ±0.0019). When the two datasets were combined, totalMX, no measures

of genetic diversity were greater than for QR alone. Nucleotide diversity was greater in totalMX than in EncMX ($\pi_{totalMX}$ = 0.0136 ±0.0073 and π_{EncMX} = 0.0024 ±0.0019).

In SGI, I detected three haplotypes, one of which was novel, compared to four from northwestern Florida previously described in BowNWFL (Table 2.2). In both SGI and BowNWFL, CC-A1 was the most common haplotype, present in more than three quarters of the sampled individuals. Two additional haplotypes were previously detected by BowNWFL, but were not found in this dataset. When the two datasets were combined, totalNWFL, a total of five haplotypes were described. Haplotype and nucleotide diversity were not significantly different between SGI and BowNWFL, nor between totalNWFL and BowNWFL (h_{SGI} = 0.2900 ±0.1095, $h_{BowNWFL}$ = 0.3827 ±0.0805, $h_{totalNWFL}$ = 0.3491 ±0.0658, π_{SGI} = 0.0108 ±0.0062, $\pi_{BowNWFL}$ = 0.0177 ±0.0094, $\pi_{totalNWFL}$ = 0.0154 ±0.0082) (Table 2.11).

The exact test of population differentiation returned highly significant nonrandom distributions of haplotypes in pairwise comparisons of EncMX, QR, and totalMX with BowNWFL, SGI, and totalNWFL (exact $p << 0.0001$) (Table 2.12). All comparisons remained significant after Bonferroni correction. In pairwise comparisons with other northwestern Atlantic populations, QR and totalMX returned higher levels of significance in pairwise comparisons with Dry Tortugas (exact $p << 0.0001$) than EncMX (exact p = 0.0011). Pairwise comparisons of SGI and Dry Tortugas, and SGI and northeastern Florida-North Carolina returned lower levels of significance (exact $p_{Dry\ Tortugas}$ = 0.0286, exact $p_{northeastern\ Florida\text{-}North\ Carolina}$ = 0.0050) than did BowNWFL and totalNWFL compared to Dry Tortugas and North Carolina (BowNWFL: exact $p_{Dry\ Tortugas}$ = 0.0055, exact $p_{northeastern\ Florida\text{-}North\ Carolina} << 0.0001$; totalNWFL: exact $p << 0.0001$ for both).

Wright's test of population subdivision indicated that EncMX was more genetically differentiated from BowNWFL, SGI, and totalNWFL ($\varphi_{ST\ (BowNWFL)} = 0.6767$, $\varphi_{ST\ (SGI)} = 0.8363$, $\varphi_{ST\ (totalNWFL)} = 0.7064$, $p < 0.001$ for all) compared to QR and totalMX (QR: $\varphi_{ST\ (BowNWFL)} = 0.5358$, $\varphi_{ST\ (SGI)} = 0.6288$, $\varphi_{ST\ (totalNWFL)} = 0.5710$, $p < 0.001$ for all; totalMX: $\varphi_{ST\ (BowNWFL)} = 0.5639$, $\varphi_{ST\ (SGI)} = 0.5643$, $\varphi_{ST\ (totalNWFL)} = 0.5963$, $p < 0.001$) (Table 2.13). In comparisons with southern Florida and northeastern Florida-North Carolina, EncMX was also more genetically differentiated than QR and totalMX (southern Florida: $\varphi_{ST\ (EncMX)} = 0.3421$, $\varphi_{ST\ (QR)} = 0.2243$, $\varphi_{ST\ (totalMX)} = 0.2510$, $p < 0.001$ for all; northeastern Florida-North Carolina: $\varphi_{ST\ (EncMX)} = 0.9760$, $\varphi_{ST\ (QR)} = 0.7740$, $\varphi_{ST\ (totalMX)} = 0.7869$, $p < 0.001$ for all). Conversely, the genetic distance was greater between Dry Tortuga and QR than between either Dry Tortuga and EncMX or totalMX ($\varphi_{ST\ (QR)} = 0.0378$, $p < 0.05$; $\varphi_{ST\ (EncMX)} = 0.0228$, $p > 0.05$; $\varphi_{ST\ (totalMX)} = 0.0290$, $p > 0.05$), although after sequential Bonferroni correction, Dry Tortugas was not significantly differentiated from either QR or totalMX. Genetic distances were greater between SGI and Dry Tortugas and SGI and southern Florida compared to BowNWFL and totalNWFL (Dry Tortugas: $\varphi_{ST\ (SGI)} = 0.7959$, $\varphi_{ST\ (BowNWFL)} = 0.6683$, $\varphi_{ST\ (totalNWFL)} = 0.6922$, $p < 0.001$ for all; southern Florida: $\varphi_{ST\ (SGI)} = 0.2199$, $\varphi_{ST\ (BowNWFL)} = 0.1264$, $\varphi_{ST\ (totalNWFL)} = 0.1711$, $p < 0.001$ for all). However, SGI was the least differentiated from northeastern Florida-North Carolina compared to BowNWFL and totalNWFL ($\varphi_{ST\ (SGI)} = 01658$, $p < 0.01$; $\varphi_{ST\ (BowNWFL)} = 0.2579$, $p < 0.001$; $\varphi_{ST(totalNWFL)} = 0.1791$, $p < 0.001$).

Long versus short control region sequences

To test the prediction that the longer control region sequence would describe greater genetic diversity than the shorter sequence, I compared genetic diversity indices obtained with the shorter and longer sequences from QR and SGI. In QR, the longer sequence contained 50 polymorphic sites resulting in 17 long haplotypes compared to 30 polymorphic sites resulting in 13 haplotypes with the shorter sequence (Table 2.11). Haplotype diversity and nucleotide diversity in QR were greater for the long sequence than for the short (h_{long} = 0.7984 ±0.0217, h_{short} = 0.7250 ±0.0271, π_{long} = 0.0138 ±0.0070, π_{short} = 0.0146 ±0.0078) (Table 2.11).

In SGI the long sequence contained 41 polymorphic sites compared to 25 in the short sequence (Table 2.11). However, the polymorphisms resulted in three haplotypes with both sequence lengths. There were no significant differences in either haplotype or nucleotide diversity between the short and long sequences in SGI (h_{short} = 0.2900 ±0.1095, h_{long} = 0.2800 ±0.1070, π_{short} = 0.0108 ±0.0062, π_{long} = 0.0092 ±0.0050) (Table 2.11).

The short haplotypes CC-A1, CC-A2, and CC-A11 each split into additional long haplotypes when the long sequence was analyzed. CC-A1 resulted in CC-A1.1 which was only present in SGI, and CC-A1.3 and CC-A1.4 that were only found in QR (Figures 2.4 and 2.5). Individuals from QR with the CC-A2 haplotype bore the CC-A2.1, CC-A.2.3 and CC-A2.5 long haplotypes, with CC-A2.1 being the most common (Figures 2.4 and 2.6). All CC-A2 individuals from SGI bore the CC-A2.1 long haplotype. CCA-11, only found in QR, split into CC-A11.1 and CC-A11.3 with the latter being the most common (Figures 2.4 and 2.7).

There was no detectable difference between short and long haplotypes in the level of significance of the nonrandom haplotype distribution in QR and SGI (exact $p << 0.0001$ for both) (Table 2.14). There was a slight difference in the level of significance between SGI and Cape Verde (exact $p_{short} = 0.0002$; exact $p_{long} << 0.0001$). All significant comparisons remained significant after Bonferroni correction.

The AMOVA among QR, SGI, Georgia, Cape Verde, and Italy indicated that long sequence described almost twice the amount of variance as the short sequence ($V_{a+b(long)} = 12.57$, $V_{a+b(short)} = 6.39$) (Table 2.15). However, while highly significant, the φ_{ST} was very similar for the short and long sequences ($\varphi_{ST\ (short)} = 0.7477$, $\varphi_{ST\ (long)} = 0.7440$, $p << 0.0001$ for both).

Genetic distances between QR and SGI calculated with the short and long sequences were very similar ($\varphi_{ST\ (short)} = 0.6248$, $\varphi_{ST\ (long)} = 0.6288$, $p << 0.0001$ for both) (Table 2.16). The pairwise comparison of SGI and Cape Verde returned different genetic distances for the short and long sequences ($\varphi_{ST\ (short)} = 0.1458$, $p = 0.0025$; $\varphi_{ST\ (long)} = 0.3435$ $p << 0.0001$). In this comparison, the long sequence indicated greater genetic distance between the populations. Regardless of sequence length, SGI and Georgia were not significantly differentiated from one another ($\varphi_{ST\ (short)} = 0.0592$, $p = 0.1725$; $\varphi_{ST\ (long)} = 0.0578$, $p = 0.1730$).

Fine-scale structure

Of the 175 samples from QR, 143 were collected from tagged females who were tracked throughout the nesting season (data collected by Flora, Fauna y Cultura de Mexico, A.C. staff and volunteers). Fifty-two (36%) of these females nested at more than

one site within the same season. However, only eight females overall (5.6%) nested both in the northern and the southern region, separated by a minimum of 22 km. From the central region, mark-recapture data is only available from Tankah, and the only two females recorded from Tankah also nested in the south, a distance of 11 km. Females are not monitored or tagged on Cozumel, thus no data is available on movement between the island and the mainland. Of the 52 (36%) females that nested at multiple sites, the average distance traveled between sites was 6.7 km. The majority (n = 37, 71%) of these females nested at sites within 5 km of one another, and a few (n = 8, 15%) were long-distance nesters who nested at sites separated by more than 20 km (Figure 2.8). The distribution of distances between all pairs of sites is plotted in the same figure. The distribution of distances between studied sites and the frequency distribution of distances traveled by females nesting at more than one site are not correlated (Pearson correlation coefficient = 0.666, p = 0.148).

The steepness of the rarefaction curves indicated that the number of haplotypes observed per site fell within the 95% confidence interval of the expected number of haplotypes based on their respective sample sizes (Figure 2.9). This assumes that each site harbored the same level of haplotype diversity as indicated by the entire totalMX dataset (short haplotypes) or QR (long haplotypes). The exception was Cahpechen, which had a greater number of haplotypes than expected. Larger sample sizes for most sites would likely result in more haplotypes. This is especially true for Paamul (n = 2), Punta Cadena (n = 5), and Tankah (n = 2), which had extremely low sample sizes. For the analysis of genetic structure, I assumed that the frequencies of the observed haplotypes approached the true haplotype frequency distribution for each site based on

the rarefaction curves. The haplotypes observed at each nesting site are listed in Table 2.17.

The exact test of population differentiation among the 10 sites in QR indicated significant genetic differentiation based on a nonrandom distribution of long haplotypes between six pairs of sites (Paamul and Chemuyil: $p = 0.0367$; Paamul and Cozumel: $p = 0.0269$; Cahpechen and Chemuyil: $p = 0.0134$; Cahpechen and XelHa: $p = 0.0441$; Punta Cadena and Chemuyil: $p = 0.0105$; and Punta Cadena and XelHa: $p = 0.0137$) (Table 2.18). None of these comparisons remained significant after Bonferroni correction.

The AMOVA revealed that most of the variation was attributable to variation within sites ($V_b = 5.3612$, 91.89%), compared to variation among sites ($V_a = 0.4731$, 8.11%) (Table 2.20). However, the overall φ_{ST} indicated significant, although slight, structure among sites ($\varphi_{ST} = 0.0801$, $p = 0.0016$) (Table 2.19).

Pairwise φ_{ST} indicated significant differentiation between Paamul and all other sites ($\varphi_{ST} \geq 0.4905$, $p \leq 0.0477$), except Tankah ($\varphi_{ST} = 1.000$, $p = 0.3341$) (Table 2.18). Cahpechen and Cozumel were significantly differentiated from Aventuras, Chemuyil, Xcacel, and XelHa (Cahpechen: $\varphi_{ST} \geq 0.0626$, $p \leq 0.0323$; Cozumel: $\varphi_{ST} \geq 0.0962$, $p \leq 0.0485$). After applying Bonferroni correction, no significant differences remained.

The Mantel test for isolation by distance among all 10 sites did not detect a significant correlation between genetic and geographic distances (correlation coefficient $r = 0.31$, $p = 0.10$).

The SAMOVA detected the most abrupt genetic change between Paamul and all other sites ($F_{CT} = 0.66922$, $p = 0.09677$), with 66.92% of the variation attributable to among group variation, when a two-group structure was evaluated (Table 2.20). When a

three-group structure was selected for the analysis, Paamul and Tankah each formed independent groups and the rest of the sites were grouped together (F_{CT} = 0.4849, p = 0.02835) with 48.49% of the variation attributable to among group variation. With four groups specified, Paamul, Tankah, and Punta Cadena were each grouped separately and the rest of the sites were grouped together (F_{CT} = 0.2986, p = 0.01369) with 29.86% of the variation attributable to among-group variation. Specifying additional number of groups did not result in a higher F_{CT}, which remained highest with the bipartite structure.

I reran the SAMOVA without Paamul, Tankah, and Punta Cadena because of their small sample sizes. When a two-group structure was evaluated, Cahpechen and Cozumel grouped together and the rest of the sites formed a second group (F_{CT} = 0.10235, p = 0.04790 ± 0.00754) with 10.23% of the variance attributable to among group variation (Table 2.20). Chemuyil and Kanzul split from the large group to form a third unit, when a three-group structure was specified (F_{CT} = 0.09014, p = 0.0088) with 9.01% of the variance attributable to among group variation. Again, the highest F_{CT} resulted from the bipartite structure, although it was not quite significant.

When grouping sites into five regions (Paamul, north, central, south, and Cozumel) the exact test of population differentiation indicated that haplotypes were randomly distributed (p > 0.05) except for Paamul and Cozumel (p = 0.0325), although this was not significant after Bonferroni correction (Table 2.21). The AMOVA indicated significant structure among the regions (φ_{ST} = 0.1180, p = 0.0015), although most variation was attributable to within sites (V_b = 5.15, 88.21%) compared to among sites (V_a = 0.69, 11.79%) (Table 2.22). Pairwise φ_{ST} suggested significant genetic distances between Paamul and all other regions (north: φ_{ST} = 0.5955, p = 0.0100; central: φ_{ST} =

0.9477, p = 0.0289; south: φ_{ST} = 0.7578, p = 0.0052; Cozumel: φ_{ST} = 0.9546, p = 0.0041) and between north and Cozumel (φ_{ST} = 0.1003, p = 0.0197) (Table 2.21). Only distances between Paamul and south and between Paamul and Cozumel remained significant after Bonferroni correction. The Mantel test among the five regions indicated no correlation between genetic and geographic distances (correlation coefficient r = 0.0647, p = 0.2552).

When the SAMOVA was applied to the five regions, the two-group structure had the highest F_{CT} suggesting this partitioning of the regions is the most accurate, with Paamul by itself and all other regions together (F_{CT} = 0.6849, p = 0.2004) with 68.49% of the variance attributable to among group variation (Table 2.23). When three groups were specified, the analysis grouped north, south, and Cozumel grouped together, and Paamul and the central region each grouped separately (F_{CT} = 0.3276, p = 0.0978) with 32.76% of the variance attributable to among group variation. When four groups were indicated, Cozumel and the central region grouped together and all other sites grouped separately (FCT = 0.1979, p = 0.1007) with 19.79% of the variance attributable to among group variation. I ran the SAMOVA excluding Paamul and the central region from the analysis due to low sample size, and specified a two-group structure. This analysis suggested grouping the north and south regions together and grouping Cozumel by itself (F_{CT} = 0.0581, p = 0.3157) with 5.81% of the variance attributable to among group variation.

Discussion

The aim of this study was to quantify the genetic diversity of the Mexican and the northwestern Florida genetic stocks of loggerhead turtles as well as to explore the utility

of analyzing a longer sequence of the control region than conventionally examined, both for inter- and intra-population analyses. I predicted that increased sample sizes from these two populations would reveal higher levels of genetic diversity when compared to the original datasets. Also, I predicted that the longer control region sequence would be useful in providing greater resolution of stocks, and as a by-product, increase the genetic distance among stocks compared to the genetic distance measured by the shorter control region sequence. The expected increase in resolution provided by the longer sequence led me to hypothesize that fine scale structure could be detected.

Sampling years

The two sampling years in QR were not genetically differentiated with any test of genetic structure when either the short or the long control region sequence was analyzed. EncMX was significantly, although only slightly, differentiated from QR2006 with pairwise φ_{ST}, but not after Bonferroni correction. This suggests that the datasets most likely represent the same genetically cohesive population with no differences across years. There were also no signs of temporal variation in northwestern Florida comparing the two samplings years on SGI and the BowNWFL dataset. These results are consistent with studies from other regions where no genetic differences have been observed across years (e.g. Garofalo et al. 2009, Monzón-Argüello et al. in press).

Effects of sample size

I detected seven short haplotypes in QR in addition to the five that previously had been described for this region in EncMX. Despite the greater number of haplotypes,

haplotype diversity was not greater in QR than EncMX, because haplotype diversity takes into account sample size. However, in support of my prediction, nucleotide diversity and the number of polymorphic sites were greater for QR than EncMX because QR described more divergent haplotypes. In SGI I detected one short haplotype in addition to two out of three others that previously had been described for northwestern Florida in BowNWFL. Contrary to my prediction, all measures of genetic diversity were the same for SGI and BowNWFL, and even the combined dataset totalNWFL, with the exception of the number of polymorphic sites which was greater for BowNWFL (and totalNWFL), due to the greater number of haplotypes. I was able to greatly increase the sample size from QR compared to EncMX due to the relatively high density of nesting and the logistic support from Flora, Fauna y Cultura de Mexico, A.C. which runs a state-wide (Quintana Roo) sea turtle conservation program. In contrast, I was only able to collect about half as many samples from northwestern Florida compared to BowNWFL. Loggerhead nesting is much less dense in northwestern Florida compared to Mexico, which made it logistically difficult to obtain a large sample size. This was confounded by lack of tagging of the females, which drastically reduced the potential sample size as I had to limit myself to sampling nests laid within a10-day window. Despite the small sample size and the fact that I detected fewer haplotypes than BowNWFL, I described a novel haplotype adding to the known genetic diversity of the loggerheads nesting in northwestern Florida.

The discovery of CC-A1 in QR caused this population to be less differentiated from the populations in northeastern Florida-North Carolina, northwestern Florida, and southern Florida than EncMX. CC-A1 is common throughout Florida, but was not

detected in EncMX. This was contrary to my prediction of more pronounced structure. However, the genetic distance between Dry Tortugas and QR was greater than that indicated by EncMX. Differences in haplotype composition became more pronounced with the greater sample size provided by QR than EncMX. The absence of haplotypes in SGI compared to BowNWFL resulted in less genetic distance between SGI and southern Florida compared to BowNWFL. The missing haplotypes (CC-A3 and CC-A7) were shared between BowNWFL and southern Florida. The absence of these same haplotypes likely also caused the greater genetic similarity between SGI and northeastern Florida-North Carolina compared to BowNWFL. Absence or presence of key haplotypes can either reduce or increase genetic structuring, but larger sample sizes more accurately represent the true genetic profile of a population. The combined datasets for Mexico (totalMX) and northwestern Florida (totalNWFL) provide intermediate genetic distances between the other populations and are likely more accurate compared to the genetic distances obtained with the individual datasets (EncMX, QR, BowNWFL, and SGI), assuming no pseudoreplication across datasets from the same region.

Resolution with longer control region sequences

One of the seven newly detected haplotypes in QR, CC-A1, happens to be the predominant haplotype in the southeastern United States (Encalada et al. 1998, Bowen et al. 2004). This study demonstrates that CC-A1 is ubiquitous in loggerhead populations in the northwestern Atlantic Ocean. At first glance this does not bode well for mixed stock analyses. However the long control region sequence does show promise for resolving this common haplotype. The long haplotypes of CC-A1 that are present in QR (CC-A1.3

and 1.4) are not present in SGI nor in Georgia (Monzón-Argüello et al. in press), which both have the CC-A1.1 haplotype (Figure 2.5). The southern Florida genetic stock has a large proportion of individuals with the CC-A1 haplotype (48%). The haplotype make-up of CC-A1 in southern Florida will be informative as to how mixed stock analyses will be affected. CC-A2, which is the most common haplotype in QR (46%) and Italy (58%, Garofalo et al. 2009), and the second most common in southern Florida (41%, Bowen et al. 2004) and SGI (12%), also shows promise for increasing the resolving power of mixed stock analyses. In QR, three long haplotypes of CC-A2 are present, two of which are unique to QR (CC-A2.3 and 2.5) (Figure 2.6). However the most common of the long sub-haplotypes in QR (CC-A2.1, 80%) is also the only one observed in SGI and Italy. CC-A11, while a relatively rare haplotype, shows much diversity, splitting into three long haplotypes, two of which are unique to Mexican loggerheads (CC-A11.1 and 11.3) and one of which is unique to Cape Verde (CC-A11.2) (Figure 2.7). Because CC-A11 is rare, this haplotype will not have much of an impact on mixed stock analyses, but its geographic distribution and long haplotypic diversity make it very useful for assigning population origin to the few turtles that do have this haplotype.

The degree of structure among the five populations (QR, SGI, Cape Verde, Georgia, and Italy) did not change with the use of the long control region sequence compared to the short, except in two pairwise comparisons (φ_{ST}), Cape Verde and SGI, and Cape Verde and Georgia, thus supporting my prediction of greater resolution. In both comparisons, the significance of the degree of structure was greater with the long sequence by two and three orders of magnitude, respectively. These populations all share the short CC-A1 haplotype, but not its long haplotypes; Cape Verde loggerheads are CC-

A1.3 while SGI and Georgia loggerheads are CC-A1.1. Only when shared short haplotypes are split into multiple longer haplotypes, some of which are not shared among populations, does the resolution of structure improve, and most markedly with φ_{ST} as compared to the exact test of population differentiation. This is not surprising as the exact test treats all haplotypes equally, whereas Wright's fixation index takes into account all the polymorphism present in a nucleotide sequence when calculating genetic distance.

Fine-scale structure

The rarefaction curve based on totalMX shows that if the number of short haplotypes for the entire region is representative of what is present at each site then the number of haplotypes per site is within the expected number given the sample size (Figure 2.9). For a population with diversity similar to totalMX, a sample size of 22 should be the minimum to be confident that at least half of the haplotypes are uncovered. The rarefaction curve based on the number of long haplotypes detected in QR is similar (Figure 2.9). However, the minimum sample size to uncover half of the haplotypes is now 26, due to the higher number of long haplotypes compared to short haplotypes. Thus, the greater the diversity of a population, the larger a sample size is needed to describe the diversity. Sample sizes for several sites in QR are on the lower end, such as Paamul, Tankah, and Punta Cadena. Interpretation of results regarding these sites should therefore be made with caution.

Precise natal homing of females in QR was not consistently supported, contrary to my prediction. Prior to Bonferroni correction, the exact test of population differentiation

and pairwise φ_{ST} agreed on significant structuring between four pairs of sites: Paamul and Chemuyil, Paamul and Cozumel, Cahpechen and Chemuyil, and Cahpechen and XelHa. After Bonferroni correction was applied, these differences were no longer significant, questioning the validity of this structuring. Additionally, differences between Paamul and other sites are not reliable based on the small sample size, which was fixed for a rare haplotype (CC-A1.4). Without a larger sample size from Paamul, it is difficult to say how isolated this site really is.

Based on φ_{ST} alone, Cozumel and Cahpechen were significantly differentiated from four other sites (Aventuras, Chemuyil, Xcacel, and XelHa: $\varphi_{ST} = 0.0626$ to 0.1889, $p < 0.05$, not significant after Bonferroni correction) (Table 2.18) with genetic distances that exceed the lowest level of separation among genetic stocks of loggerheads in the Mediterranean (Israel n = 20, Greece n = 60; $\varphi_{ST} = 0.043$, $p < 0.01$, Carreras et al. 2007). Despite these relatively high values of genetic differentiation within QR, they were not significant after Bonferroni correction, and there was not consistency between φ_{ST} and the exact test. For these reasons, structuring within QR should be interpreted with caution and verified by other means, such as with additional genetic studies, satellite tracking or mark-recapture data.

The SAMOVA indicated that the most rapid genetic change was between females at Paamul and all other sites, and this two-group structure had the most support based on the highest F_{CT} value. Paamul is the northernmost site on the mainland, almost 23 km from the next closest site, Aventuras. This result is consistent with the genetic distances indicated by pairwaise φ_{ST}, however the small sample size may bias the results.

To avoid potentially biasing the SAMOVA, I reran the analysis without Paamul, Tankah, and Punta Cadena, which each had sample sizes of five individuals or less. Without these three sites, the SAMOVA identified the most rapid genetic change between Cozumel and Cahpechen as one unit and all other sites grouped. This corresponds with the pairwise φ_{ST}, as these two sites are not genetically differentiated, and each of them are significantly differentiated (but not after Bonferroni correction) from the remaining sites of Aventuras, Chemuyil, Xcacel and XelHa. This bi-partite structure had the most statistical support.

The overall pattern of structure based on the SAMOVA does not suggest that any of the sites are significantly different, perhaps with exclusion of Paamul. However, a larger sample sizes from Paamul is necessary to evaluate this hypothesis with a greater level of confidence. Also, there was no consistency in the grouping sites. SAMOVA's grouping of sites and placement of genetic barriers were consistent with the lack of isolation by distance found by the Mantel test.

I then analyzed genetic structuring among five regions in QR. The only agreement between the exact test and pairwise φ_{ST} was differentiation between Cozumel and Paamul. After Bonferroni correction, only significant differentiation between Paamul and Cahpechen, and Paamul and Cozumel based on φ_{ST} were retained. Again, results pertaining to Paamul should be interpreted with caution. The SAMOVA of the five regions suggested grouping Paamul by itself. The general trend of the regional SAMOVA analysis does not correspond with the individual site analysis in the placement of Cozumel. The regional analysis (excluding Paamul and central) identifies the starkest genetic change between Cozumel and the mainland (north and south regions). The site

analysis suggested grouping Cozumel with Cahpechen, in the southern region, instead of grouping Cahpechen with the north. The site analysis also split up Kanzul and Cahpechen, which I grouped together as part of the southern region due to their geographic proximity, but there may not be a genetic basis for this association. The lack of genetic similarity within regions is further supported by non-significant correlations of geographic distance with genetic distance determined by the Mantel test.

In the different runs of the SAMOVA, it is not clear whether the rates of gene flow are higher between Cozumel and the southern or the northern region. Considering the other tests for genetic structuring, the overall tendency is for Cozumel to be slightly differentiated, although not consistently significantly differentiated, from the mainland. The prevailing currents in the Cozumel Channel tend to flow northward between Cozumel and the mainland (Chávez et al. 2003), which should enable the turtles easily to reach the mainland sites in the lee of the island (the northern region and further north to Paamul), assuming they swim south of the island. It may be more energetically costly to swim to nesting sites in the southern region as this would be against the direction of the current. Swimming the other direction, from the southern region to Cozumel may be less energetically costly than swimming from the northern region to Cozumel. However, the direction of gene flow cannot be evaluated. The pattern of gene flow that we see can be a result of several scenarios, either in the past or ongoing. Turtles can hatch in one region and nest outside of their natal region for their entire reproductive lifespan. Or they could nest in one region one season, and the next season nest in another, or nest in multiple regions within a season.

Mark-recapture data provide valuable information on female nesting patterns. Tagging data from individuals sampled in this study indicated considerable movement among nesting sites, with most of the movement among sites within a few km of one another. Very few females moved among sites separated by distances between 10 and 23 km, but more moved among sites separated by distances greater than 24 km. This pattern of movement explains why the genetic structure did not fit an isolation by distance model. Movement to and from the centrally located sites of Punta Cadena and Tankah, provide the intermediate distances that the turtles travelled less frequently. Only two of the sampled females were observed nesting in both Tankah and Kanzul, a distance of 11 km. Punta Cadena and Tankah are not in and of themselves unfavorable; in the 2006 season Tankah had the second highest number of loggerhead nests out of the central Quintana Roo nesting beaches. An analysis of the total available mark-recapture data spanning two decades from the mainland Quintana Roo nesting beaches (Flora, Fauna y Cultura de Mexico, A.C., unpublished data) will be extremely telling in terms of female nest site fidelity in these regions. Unfortunately, female turtles are not monitored or tagged on Cozumel, so no information regarding nesting patterns between the island and the mainland is available. Future tagging or satellite tracking will provide key information on an individual's pattern of nesting on a regional scale.

Absolute natal homing likely is not favored by selection on an evolutionary time scale as it would not allow populations to adapt to a changing environment, thereby leading to extinction (Carr et al. 1978, Bowen and Karl 2007). Contemporary mark-recapture studies have identified this pattern of most females nesting on the same beach and few individuals nesting far outside the range of their previous nest site (LeBuff 1974,

Dodd 1988, Bjorndal et al. 1983). Colonization of the Mediterranean and the northern nesting sites in the southeastern United States, as well the northward expansion of Japanese nesting colonies provide historical evidence of instances where long-distance female nesting behavior was successful (Bowen et al. 1993, Hatase et al. 2002, Reece et al. 2005, Bowen and Karl 2007).

Broader impacts

Studies of the genetic structuring of loggerhead nesting populations have been ongoing since the early 1990's, but gaps in our knowledge remain. Some nesting areas lack any genetic characterization (e.g. see Monzón-Argüello et al. in press) and the genetic profile of others is based on small sample sizes (e.g. see Carreras et al. 2007, Garofalo et al. 2009). As I have demonstrated in this study, increasing the sampling effort of a nesting population can uncover additional genetic variation, although the genetic diversity quantified may also decrease. The larger sample size, the more representative of the population it will be. The longer control region sequence harbors more genetic variation than the short sequence, and the long sequence is especially useful in some cases, such as in distinguishing among populations carrying the common and shared CC-A1 haplotype. This haplotype diverged into three when the long sequence was analyzed, with two of the long haplotypes unique to QR and the third only present in the southeastern United States populations (northwestern Florida and Georgia). Of course, the currently available information on the geographic distribution of long derivatives of the CC-A1 haplotype will change as other studies apply the long sequence analysis to regional nesting populations.

Genetic characterization of the nesting stocks with greater resolution provided by the longer control region sequence will significantly improve the accuracy of mixed stock analyses in foraging areas. Mixed stock analyses can reveal aspects of migratory behavior which preclude direct observation, as well as elucidate which regional rookeries are affected by mortality caused by commercial and artisanal fisheries (Bowen and Karl 2007). This is of urgent concern due to the endangered status of the loggerhead turtle on a global scale (IUCN 2010) and the continuing decline of many populations (Witherington et al. 2009).

Loggerhead rookeries in QR are not consistently substructured, indicating that the population likely has reached equilibrium after extreme population lows as a result of human exploitation of sea turtles on a global scale (McClenachan et al. 2006). With the joining of the Convention on International Trade in Endangered Species in 1991 (http://www.cites.org), Mexico started to implement monitoring and conservation programs, and subsequently has seen a rise in the numbers of nesting females on the Yucatan Peninsula (Garduno-Andrade et al. 1999), although they are nowhere near historic abundances (McClenachan et al. 2006). This insight into female homing behavior and the use of nesting beaches, made possible with mark-recapture studies and the application of highly polymorphic markers, make it imperative that beaches on a regional scale are protected in order to conserve the entire range of genetic variation of this vulnerable species.

Table 2.1. Sample sizes and sample type by site and year. Sample sizes for individual sites in Quintana Roo (QR) reflect the number of females who nested there. Some females nested at multiple sites, in which case they are represented multiple times. Total sample size for QR and all sample sizes for St. George Island (SGI) reflect the actual number of individuals sampled. N/A: not available. Nests numbers in Mexico are provided by Flora, Fauna y Cultura de Mexico, A.C. Nest numbers on SGI are provided by B. Drye (the marine turtle permit holder for the area) and do not include the area monitored by St. George Island State Park (14.5 km). * Franklin County, Florida nest numbers averaged over 2007 and 2008 are obtained from Florida Fish and Wildlife Conservation Commission. SGI and Alligator Point are located in Franklin County.

Site	Sample size	Nests	Year	Sample type
QR-total	175	2436	2006, 2008	
Paamul	2	N/A	2008	hatchling
Aventuras	25	114	2006	female
Aventuras	5	248	2008	female
Chemuyil	16	44	2006	female
Chemuyil	3	54	2008	female
Xcacel	46	153	2006	female
Xcacel	15	258	2008	female
XelHa	24	106	2006	female
XelHa	7	102	2008	female
Punta Cadena	5	N/A	2008	hatchling
Tankah	1	144	2006	female
Tankah	1	119	2008	female
Kanzul	11	86	2006	female
Kanzul	22	130	2008	female
Cahpechen	12	94	2006	female
Cahpechen	15	175	2008	female
Cozumel	21	N/A	2008	hatchling
SGI-total	25			
Alligator Point	1	257*	2007	female
SGI	11	106	2007	hatchling
SGI	13	133	2008	hatchling

Table 2.2. Exact test of population differentiation with significance at $p \leq 0.05$ obtained with 100,000 steps in the Markov chain and 10,000 dememorization steps among the two sampling years in Quintana Roo: QR2006 (n = 99), QR2008 (n = 76), and the dataset from Encalada et al. (1998): EncMX n = 20). Significance values for the short and long control region sequences are below and above the diagonal, respectively.

	QR2006	QR2008	EncMX
QR2006	---	0.51027	N/A
QR2008	0.33454	---	N/A
EncMX	0.43104	0.79822	---

Table 2.3. Analysis of molecular variance with 99,999 permutations of the short control region sequence among the two sampling years in Quintana Roo: QR2006 (n = 99), QR2008 (n = 76) and the dataset from Encalada et al. (1998): EncMX (n = 20). φ_{ST} = 0.02301, p = 0.0696.

Source of Variation	df	Sum of Squares	Variance Components	Percent of Variation
Among sites	2	11.887	0.06004 (V_a)	2.30
Within sites	192	489.410	2.54901 (V_b)	97.70
Total	194	501.297	2.60905	

Table 2.4. Wright's fixation index of population subdivision (pairwise φ_{ST} and (p-value)) among the two sampling years in Quintana Roo: QR2006 (n = 99), QR2008 (n = 76) and the dataset from Encalada et al. (1998): EncMX (n = 20, short sequence only) with the short and long control region sequences below and above the diagonal, respectively. Significance was obtained over a minimum of 99,999 permutations. * indicates significance at $p \leq 0.05$, and **bold** indicates significance at $p \leq 0.05$ after sequential Bonferroni correction.

	QR2006	QR2008	EncMX
QR2006	---	0.0007 (0.2943)	N/A
QR2008	0.0020 (0.2540)	---	N/A
EncMX	0.0838 (0.0266*)	0.0434 (0.0965)	---

Table 2.5. Exact test of population differentiation with significance at $p \leq 0.05$ obtained with 100,000 steps in the Markov chain and 10,000 dememorization steps above the diagonal. Wright's fixation index of population subdivision (pairwise φ_{ST} and (p-value)) with statistical significance obtained over a minimum 99,999 permutations below the diagonal. QR: Quintana Roo from this study (n = 175); EncMX: dataset from Encalada et al. (1998) (n = 20).

	QR	EncMX
QR	---	0.6518
EncMX	0.0621 (0.0582 ±0.0008)	---

Table 2.6. Exact test of population differentiation with significance at $p \leq 0.05$ obtained with 100,000 steps in the Markov chain and 10,000 dememorization steps among the sampling years on St. George Island: SGI2007 (n = 12), SGI2008 (n = 13), and the dataset from Bowen et al. (2004): BowNWFL (n = 49). Significance values for the short and long control region sequences are below and above the diagonal, respectively.

	SGI2007	SGI2008	BowNWFL
SGI2007	---	0.3948	N/A
SGI2008	0.3873	---	N/A
BowNWFL	0.4286	0.8712	---

Table 2.7. Analysis of molecular variance of the short control region sequence with 99,999 permutations among the sampling years in St. George Island: SGI2007 (n = 12) and SGI2008 (n = 13), and the dataset from Bowen et al. (2004): BowNWFL: dataset from Bowen et al. (2004) (n = 49). φ_{ST} = -0.0143, p = 0.4671.

Source of Variation	df	Sum of Squares	Variance Components	Percent of Variation
Among sites	2	4.34	-0.0415 (V_a)	-1.43
Within sites	71	209.12	2.9454 (V_b)	101.43
Total	73	213.47	2.9034	

Table 2.8. Wright's fixation index of population subdivision (pairwise φ_{ST} and (*p*-value)) among sampling years on St. George Island: SGI2007 (n = 12), SGI2008 (n = 13), and the dataset from Bowen et al. (2004): BowNWFL (n = 49). Results from the short and long control region sequences are below and above the diagonal, respectively. Significance ($p \leq 0.05$) was obtained over a minimum of 99,999 permutations.

	SGI2007	SGI2008	BowNWFL
SGI2007	------	-0.0436 (0.3908)	N/A
SGI2008	-0.0408 (0.3923)	------	N/A
BowNWFL	-0.0442 (0.7530)	0.0192 (0.2490)	------

Table 2.9. Exact test of population differentiation with significance at $p \leq 0.05$ obtained with 100,000 steps in the Markov chain and 10,000 dememorization steps above the diagonal. Wright's fixation index of population subdivision (pairwise φ_{ST} and (p-value)) with statistical significance ($p \leq 0.05$) obtained over 99,999 permutations below the diagonal. SGI: combined sampling years from St. George Island (n = 25); BowNWFL: dataset from Bowen et al. (2004) (n = 49).

	SGI	BowNWFL
SGI	---	0.5052
BowNWFL	0.0036 (0.3258)	---

Table 2.10. The number of individuals of each short and long control region haplotype detected in Quintana Roo (QR) and St. George Island (SGI) from this study, and Mexico (EncMX), northwestern Florida (BowNWFL), Georgia (GA), Cape Verde (CV), and Italy (IT) from other studies (references are indicated below table). * indicates novel haplotypes.

Haplotypes								
short	long	QR	EncMX	SGI	Bow NWFL	GA	CV	IT
CC-A1		14		21	38	17	88	
	1.1			21		17		
	1.2							
	1.3	1					79	
	1.4	13					6	
	1.5						3	
CC-A2		80	11	3	7		2	22
	2.1	64		3			2	22
	2.3	6						
	2.5	10						
CC-A3		3	2		2			
	3.1	3						
CC-A5		2						
	5.1	2						
CC-A7					2			
CC-A8		7	1					
	8.1	7						
CC-A9		8	1					
	9.1	8						
CC-A10		41	5					
	10.1	41						
CC-A11		8					1	
	11.1	1						
	11.2						1	
	11.3*	7						
CC-A12		2						
	12.1*	2						
CC-A14		7						
	14.1	7						

CC-A17							36	
	17.1						30	
	17.2						6	
CC-A20								14
	20.1							14
CC-A31								2
	31.1							2
CC-A36		1						
	36.2*	1						
CC-A42		1						
	42.1*	1						
CC-A47							1	
	47.1						1	
CC-A59*				1				
	59.1*			1				
CC-A60*		1						
	60.1*	1						
Total		175	20	25	49	17	128	38
Reference[¥]		1	2	2	3	4	4	5

[¥]1. This study, 2. Encalada et al 1998, 3. Bowen et al. 2004, 4. Monzón-Argüello et al. in press, 5. Garofalo et al. 2009.

Table 2.11. Sample sizes (n), number of haplotypes (*k),* haplotype diversity (*h*), nucleotide diversity (π), and number of polymorphic sites (*s*) for the Quintana Roo (QR) and St. George Island (SGI) datasets from this study and for the datasets from Encalada et al. (1998) from Mexico (EncMX) and from Bowen et al. (2004) from northwestern Florida (BowNWFL), combined datasets (totalMX, totalNWFL).

	n	*k*	*h*	π	*s*
short haplotypes					
EncMX	20	5	0.6526 ±0.0927	0.0024 ±0.0019	5
QR	175	13	0.7250 ±0.0271	0.0146 ±0.0078	30
totalMX	195	13	0.7174 ±0.0263	0.0136 ±0.0073	30
BowNWFL	49	4	0.3827 ±0.0805	0.0177 ±0.0094	27
SGI	25	3	0.2900 ±0.1095	0.0108 ±0.0062	25
totalNWFL	74	5	0.3491 ±0.0658	0.0154 ±0.0082	27
long haplotypes					
QR	175	17	0.7984 ±0.0217	0.0138 ±0.0070	50
SGI	25	3	0.2800 ±0.1070	0.0092 ±0.0050	41

Table 2.12. Exact test of population differentiation with significance at $p \leq 0.05$ obtained with 100,000 steps in the Markov chain and 10,000 dememorization steps. The analysis was performed three times: once with the datasets from Encalada et al. (1998) (EncMX) and Bowen et al. (2004) (BowNWFL), once with QR (Quintana Roo, this study) and SGI (St. George Island, this study), and once with totalMX (EncMX and QR combined) and totalNWFL (BowNWFL and SGI combined), with pairwise comparisons with three other Atlantic populations: Dry Tortugas (DT, n = 58), southern Florida (SFL, n = 109), northeastern Florida-North Carolina (NEFLNC, n = 104). * indicates significance at $p \leq 0.05$, and **bold** indicates significance at $p \leq 0.05$ after sequential Bonferroni correction.

	Mexico			northwestern Florida		
	EncMX	QR	totalMX	BowNWFL	SGI	totalNWFL
BowNWFL	**0.0000***	**0.0000***	**0.0000***	---	---	---
SGI	**0.0000***	**0.0000***	**0.0000***	---	---	---
totalNWFL	**0.0000***	**0.0000***	**0.0000***	---	---	---
DT	**0.0011***	**0.0000***	**0.0000***	**0.0055***	**0.0286***	**0.0000***
SFL	**0.0000***	**0.0000***	**0.0000***	**0.0000***	**0.0000***	**0.0000***
NEFLNC	**0.0000***	**0.0000***	**0.0000***	**0.0000***	**0.0050***	**0.0000***

Table 2.13. Genetic partitions as measured by pairwise φ_{ST} (p-value) between the datasets for Mexico and northwestern Florida and other Atlantic populations. Mexican datasets: Encalada et al. (1998) (EncMX, n = 20), this study (QR, n = 175), combined (totalMX, n = 195). Northwestern Florida datasets: compiled in Bowen et al. (2004) (BowNWFL, n = 49), this study (SGI, n = 25), combined (totalNWFL, n = 74). Significance ($p \leq 0.05$) was obtained over a minimum of 99,999 permutations. * indicates significance at $p \leq 0.05$, and **bold** indicates significance at $p \leq 0.05$ after sequential Bonferroni correction.

	Mexico			northwestern Florida		
	EncMX	QR	totalMX	BowNWFL	SGI	totalNWFL
BowNWFL	**0.6767 (0.0000*)**	**0.5358 (0.0000*)**	**0.5639 (0.0000*)**	---	---	---
SGI	**0.8363 (0.0000*)**	**0.6288 (0.0000*)**	**0.6543 (0.0000*)**	---	---	---
totalNWFL	**0.7064 (0.0000*)**	**0.5710 (0.0000*)**	**0.5963 (0.0000*)**	---	---	---
DT	0.0228 (0.0677)	0.0378 (0.0239*)	0.0290 (0.0363*)	**0.6683 (0.0000*)**	**0.7959 (0.0000*)**	**0.6922 (0.0000*)**
SFL	**0.3421 (0.0001*)**	**0.2243 (0.0000*)**	**0.2510 (0.0000*)**	**0.1264 (0.0012*)**	**0.2199 (0.0005*)**	**0.1711 (0.0000*)**
NEFLNC	**0.9760 (0.0000*)**	**0.7740 (0.0000*)**	**0.7869 (0.0000*)**	**0.2579 (0.0000*)**	**0.1658 (0.00548)**	**0.1791 (0.0000*)**

Table 2.14. Exact test of population differentiation with significance at $p \leq 0.05$ obtained with 100,000 steps in the Markov chain and 10,000 dememorization steps among sites. Quintana Roo (QR, n = 175); St. George Island (SGI, n = 25); Georgia (n = 17); Cape Verde (n = 128); Italy (n = 38). P-values for the short and long control region sequences are below and above the diagonal, respectively. * indicates significance at $p \leq 0.05$, and **bold** indicates significance at $p \leq 0.05$ after sequential Bonferroni correction.

	QR	SGI	Georgia	Cape Verde	Italy
QR	---	**0.0000***	**0.0000***	**0.0000***	**0.0000***
SGI	**0.0000***	---	0.2606	**0.0000***	**0.0000***
Georgia	**0.0000***	0.2509	---	0.0473	**0.0000***
Cape Verde	**0.0000***	**0.0002***	**0.0000***	---	**0.0000***
Italy	**0.0000***	**0.0000***	**0.0000***	**0.0000***	---

Table 2.15. Analysis of molecular variance with 99,999 permutations among Quintana Roo (n = 175), St. George Island (n = 25), Georgia (n = 17), Cape Verde (n = 128), and Italy (n = 38). Results for the short and long control region sequences are before and after the slash, respectively. φ_{ST} = 0.7477 / 0.7440, p << 0.0001 for both.

Source of Variation	df	Sum of Squares	Variance Components	Percent of Variation
Among sites	4	1219.65 / 2389.97	4.77 / 9.35 (V_a)	74.77 / 74.40
Within sites	378	609.08 / 1216.79	1.61 / 3.22 (V_b)	25.23 / 25.60
Total	382	1828.73 / 3606.75	6.39 / 12.57	

Table 2.16. Wright's fixation index of population subdivision (pairwise φ_{ST} and (p-value)) among sites: Quintana Roo (QR, n =175); St. George Island (SGI: n = 25); Georgia (n = 17); Cape Verde (n = 128); Italy (n = 38) with the short and long control region sequences below and above the diagonal, respectively. Significance ($p \leq 0.05$) was obtained over a minimum of 99,999 permutations. * indicates significance at $p \leq 0.05$, and **bold** indicates significance at $p \leq 0.05$ after sequential Bonferroni correction.

	QR	SGI	Georgia	Cape Verde	Italy
QR	---	**0.6288 (0.0000*)**	**0.7046 (0.0000*)**	**0.7632 (0.0000*)**	**0.1042 (0.0018*)**
SGI	**0.6248 (0.0000*)**	---	0.0578 (0.1730)	**0.3435 (0.0000*)**	**0.8853 (0.0000*)**
Georgia	**0.7109 (0.0000*)**	0.0592 (0.1725)	---	**0.4215 (0.0000*)**	**0.9883 (0.0000*)**
Cape Verde	**0.7712 (0.0000*)**	**0.1458 (0.0025*)**	0.0575 (0.0404*)	---	**0.9496 (0.0000*)**
Italy	**0.1027 (0.0018*)**	**0.8721 (0.0000*)**	**0.9772 (0.0000*)**	**0.9490 (0.0000*)**	---

Table 2.17. The number of each short and long haplotype by nesting site in Quintana Roo. Some females nested at multiple sites, in which case they are represented multiple times. Actual sample size is 171 (site information for four females is unknown due to labeling error). PL: Paamul, AV: Aventuras, CH: Chemuyil, XC: Xcacel, XH: XelHa, PC: Punta Cadena, TK: Tankah, KZ: Kanzul, CP: Cahpechen, CZ: Cozumel.

Haplotypes short	long	PL	AV	CH	XC	XH	PC	TK	KZ	CP	CZ
CC-A1	1.1										
	1.3					1					
	1.4	2	3		7	5			1	1	
CC-A10	10.1		5	1	13	5	3		6	9	6
CC-A11	11.3		2	1	5				2		
	11.1				1						
CC-A12	12.1				1					1	
CC-A14	14.1		1	2	3	3			1		
CC-A2	2.1		9	9	22	13		1	15	7	11
	2.3						1		3	3	1
	2.5		4	4	1	2			2	1	1
CC-A3	3.1		1	1	1				1	1	
CC-A36	36.2				1						
CC-A5	5.1				1			1	1		
CC-A8	8.1		2	1	2		1			1	1
CC-A9	9.1		2		2	2			1	2	
CC-A42	42.1										1
CC-A59	59.1										
CC-A60	60.1		1		1					1	

Table 2.18. Genetic partitions among 10 sites in Quintana Roo. P-values from the exact test of population differentiation with significance obtained with 100,000 steps in the Markov chain and 10,000 dememorization steps are above the diagonal. Wright's fixation index of population subdivision (pairwise φ_{ST} and (p-value)) with statistical significance obtained over a minimum of 99,999 permutations are below the diagonal. Paamul (PL, n = 2); Aventuras (AV, n = 30); Chemuyil (CH, n = 19); Xcacel (XC, n = 61); XelHa (XH, n = 31); Punta Cadena (PC, n = 5); Tankah (TK, n = 2); Kanzul (KZ, n = 33); Cahpechen (CP, n = 32); and Cozumel (CZ, n = 21). * indicates significance at $p \leq 0.05$, and **bold** indicates significance at $p \leq 0.05$ after sequential Bonferroni correction

	PL	AV	CH	XC	XH	PC	TK	KZ	CP	CZ
PL	---	0.4248	0.0367*	0.317	0.312	0.0958	0.3344	0.0995	0.0801	0.0269*
AV	0.6138 (0.0203*)	---	0.6717	0.767	0.5448	0.1892	0.4536	0.5125	0.2548	0.2997
CH	0.7169 (0.0047*)	-0.0292 (0.5604)	---	0.1215	0.1201	0.0105*	0.4936	0.4291	0.0134*	0.0925
XC	0.5303 (0.0174*)	-0.0162 (0.6232)	0.0036 (0.2363)	---	0.6785	0.106	0.4197	0.5417	0.0818	0.4113
XH	0.4905 (0.0397*)	-0.0147 (0.5500)	0.0093 (0.1961)	-0.0206 (0.8047)	---	0.0137*	0.2599	0.2695	0.0441*	0.1042
PC	0.9492 (0.0477*)	0.0476 (0.2128)	0.0574 (0.1054)	0.0780 (0.0998)	0.1084 (0.0993)	---	0.287	0.1368	0.6166	0.1203
TK	1.000 (0.3341)	-0.1617 (0.7560)	-0.2231 (0.7865)	-0.0939 (0.6118)	-0.0784 (0.4452)	0.2849 (0.2887)	---	0.5665	0.3982	0.4005
KZ	0.7494 (0.0052*)	-0.0085 (0.4019)	0.0315 (0.7435)	0.0292 (0.1178)	0.0465 (0.0920)	0.0091 (0.1128)	-0.2261 (0.7373)	---	0.5815	0.8628
CP	0.8824 (0.0055*)	0.0823 (0.0243*)	0.0626 (0.0323*)	0.1164 (0.0065*)	0.1625 (0.0037*)	-0.0469 (0.4155)	-0.1536 (0.4786)	0.0165 (0.2398)	---	0.6123
CZ	0.9546 (0.0035*)	0.1115 (0.0485*)	0.0962 (0.0241*)	0.1381 (0.0107*)	0.1889 (0.0052*)	0.0801 (0.1574)	-0.0730 (0.3803)	0.0393 (0.1857)	-0.0196 (0.8461)	---

Table 2.19. Analysis of molecular variance with 99,999 permutations among 10 sites in Quintana Roo. Paamul (n = 2); Aventuras (n = 30); Chemuyil (n = 19); Xcacel (n = 61); XelHa (n = 31); Punta Cadena (n = 5); Tankah (n = 2); Kanzul (n = 33); Cahpechen (n = 32); and Cozumel (n = 21). $\varphi_{ST} = 0.0801$, $p = 0.0016$.

Source of Variation	df	Sum of Squares	Variance Components	Percent of Variation
Among sites	9	141.81	0.4731 (V_a)	8.11
Within sites	226	1211.63	5.3612 (V_b)	91.89
Total	235	1354.44	5.8343	

Table 2.20. Spatial analysis of molecular variance of 10 sites in Quintana Roo: Paamul (PL, n = 2); Aventuras (AV, n = 30); Chemuyil (CH, n = 19); Xcacel (XC, n = 61); XelHa (XH, n =31); Punta Cadena (PC, n = 5); Tankah (TK, n = 2); Kanzul (KZ, n = 33); Cahpechen (CP, n = 32); and Cozumel (CZ, n = 21). The proportion of total genetic variance due to differences among groups is indicated by F_{CT} and the percentage of the variance attributable to among-group variation by % for each number of groups specified.

# Groups	Groups	F_{CT}	p	%
all 10 sites				
2	PL / all others	0.66922	0.09677	66.92
3	PL / TK / all others	0.4849	0.02835*	48.49
4	PL / TK / PC / all others	0.2986	0.01369*	29.86
5	PL / TK / PC / CZ : all others	0.15760	0.00782*	15.76
6	PL / AV / XC / XH / CH,KZ / PC,TK,CP,CZ	0.13960	0.0000*	13.96
Excluding PL, TK, PC				
2	CP,CZ / CH,KZ,AV,XC,XH	0.10235	0.04790 ±0.00754	10.23
3	CP,CZ / CH,KZ / AV,XC,XH	0.09014	0.00880*	9.01

Table 2.21. Wright's fixation index of population subdivision (pairwise φ_{ST} and (p-value)) over a minimum of 100,000 permutations among five regions in Quintana Roo below the diagonal. Exact test of population differentiation with significance at $p \leq 0.05$ obtained with 100,000 steps in the Markov chain and 10,000 dememorization steps five regions in Quintana Roo above the diagonal. PL: Paamul (n = 2), north: Aventuras, Chemuyil, Xcacel, and XelHa (n = 101), central: Punta Cadena and Tankah (n = 7), south: Kanzul and Cahpechen (n = 46), and CZ: Cozumel (n = 21). * indicates significance at $p \leq 0.05$, and **bold** indicates significance at $p \leq 0.05$ after sequential Bonferroni correction.

	Paamul	North	Central	South	Cozumel
Paamul	---	0.2490	0.1933	0.1277	0.0325*
North	0.5955 (0.0100*)	---	0.1221	0.3186	0.4724
Central	0.9477 (0.0289*)	0.0582 (0.1951)	---	0.6615	0.1989
South	**0.7578 (0.0052*)**	0.0189 (0.1322)	-0.0209 (0.4515)	---	0.8653
Cozumel	**0.9546 (0.0041*)**	0.1003 (0.0197*)	-0.0511 (0.6413)	0.0259 (0.2173)	---

Table 2.22. Analysis of molecular variance among five regions in Quintana Roo. Paamul (n = 2); North: Aventuras, Chemuyil, Xcacel, and XelHa (n = 101); Central: Punta Cadena, Tankah (n = 7); South: Kanzul, Cahpechen (n = 46); and Cozumel (n = 21). Fixation index φ_{ST} = 0.1180, p = 0.0015.

Source of Variation	df	Sum of Squares	Variance Components	Percent of Variation
Among sites	4	92.64	0.69 (V_a)	11.79
Within sites	172	885.75	5.15 (V_b)	88.21
Total	176	978.40	5.84	

Table 2.23. Spatial analysis of molecular variance of five and three regions in Quintana Roo: Paamul (PL, n = 2); north (n = 101); central (n = 7); south (n = 46); and Cozumel (CZ, n = 21). The proportion of total genetic variance due to differences among groups is indicated by F_{CT} and the percentage of the variance attributable to among-group variation by % for each number of groups specified.

# Groups	Groups	F_{CT}	p	%
5 regions				
2	PL / north, central, south, CZ	0.6849	0.2004	68.49
3	PL / central / north, south, CZ	0.3276	0.0978	32.76
4	PL / north / south / central, CZ	0.1979	0.1007	29.86
3 regions (excl. PL and central)				
2	CZ / north, south	0.0581	0.3157	5.81

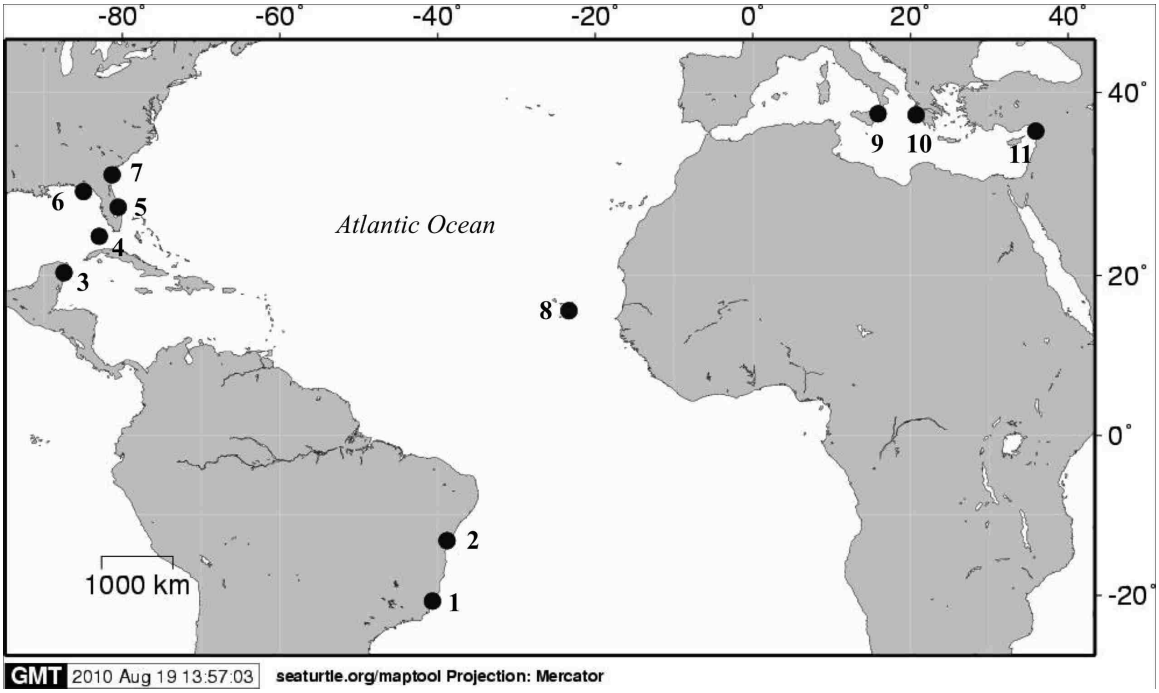


Figure 2.1. Loggerhead turtle genetic stocks identified in the Atlantic Ocean. 1) Southern Brazil, 2) northern Brazil, 3) Yucatan Peninsula, Mexico, 4) Dry Tortugas, Florida, USA, 5) southern Florida, USA, 6) northwestern Florida, USA, 7) northern Florida- North Carolina, USA, 8) Cape Verde, Portugal, 9) Italy, 10) Greece, and 11) eastern Turkey (Encalada et al. 1998, Laurent et al. 1998, Garofalo et al. 2009, Pearce 2001, Reis et al. 2010, Monzón-Argüello et al. in press).

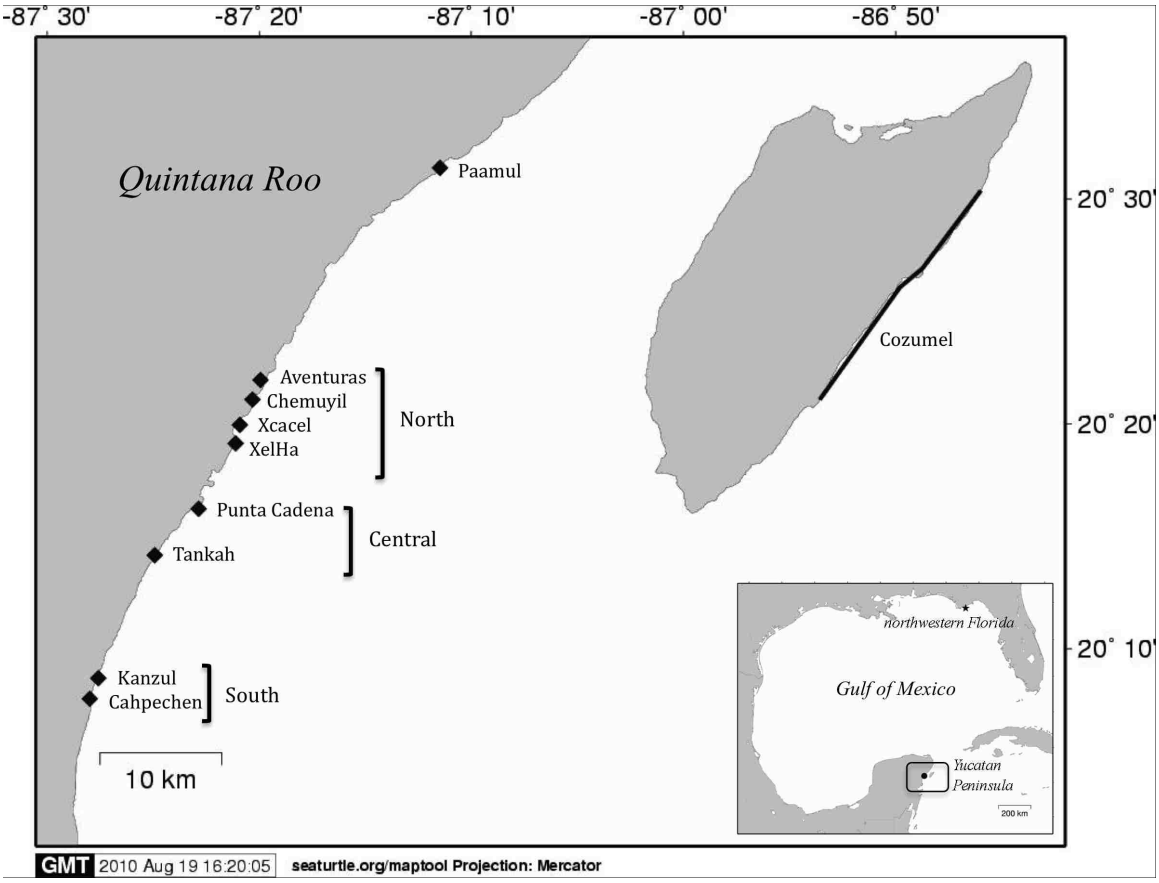


Figure 2.2. Sampling sites and regional groupings of rookeries in Quintana Roo, Mexico, which are part of the Yucatan Peninsula genetic stock.

Figure 2.3. Sampling sites from rookeries on St. George Island and Alligator Point in Florida, which belong to the northwestern Florida genetic stock.

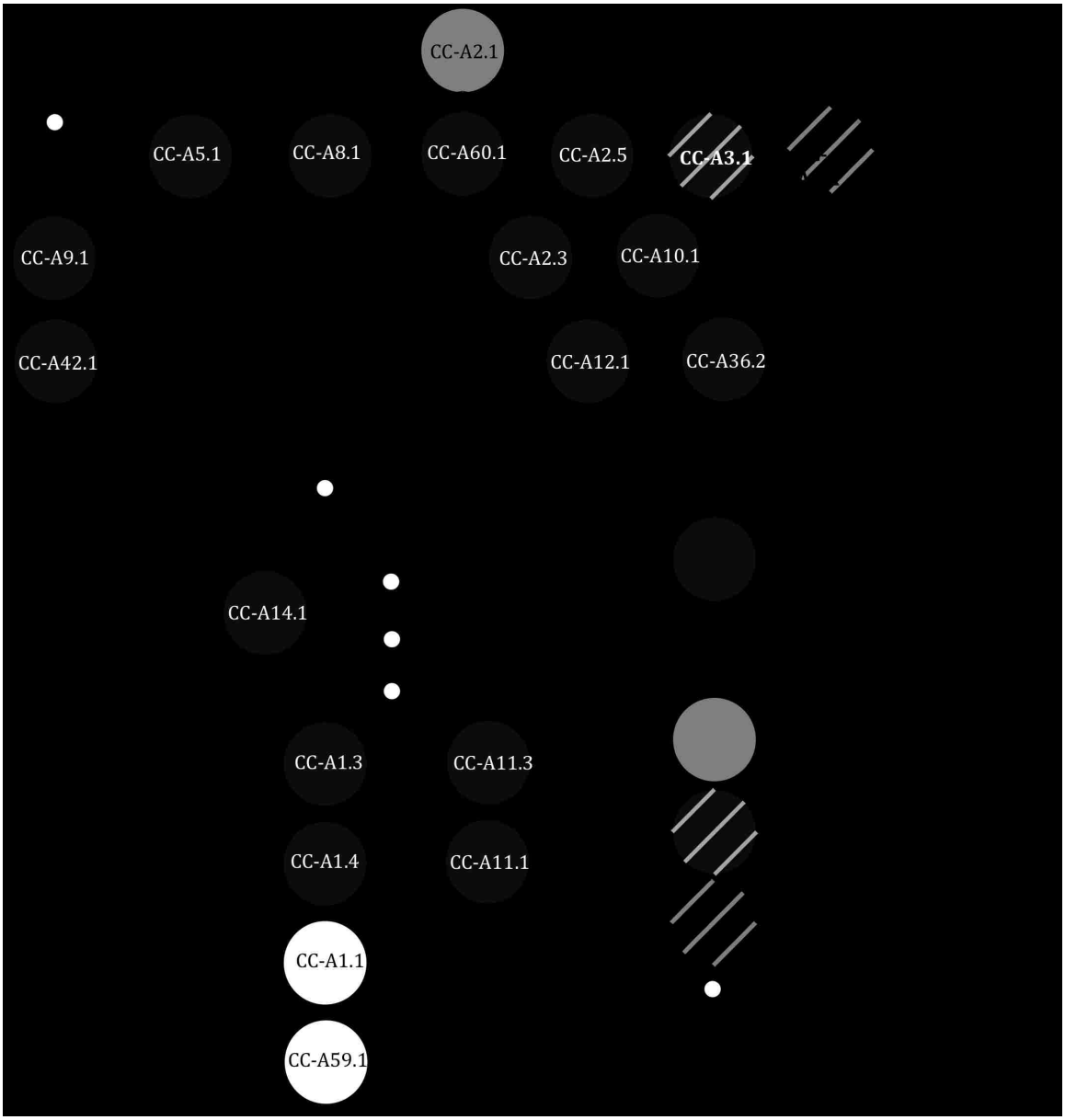


Figure 2.4. Statistical parsimony network of the long control region haplotypes (817bp). Colors of the haplotypes denote their geographical location. Each line represents one nucleotide substitution, except where indicated. QR: Quintana Roo (this study); SGI: St. George Island (this study): BowNWFL: northwestern Florida (compiled in Bowen et al. 2004). Note that CC-A7.X is short haplotype CC-A7 from BowNWFL, but its long variant is unknown.

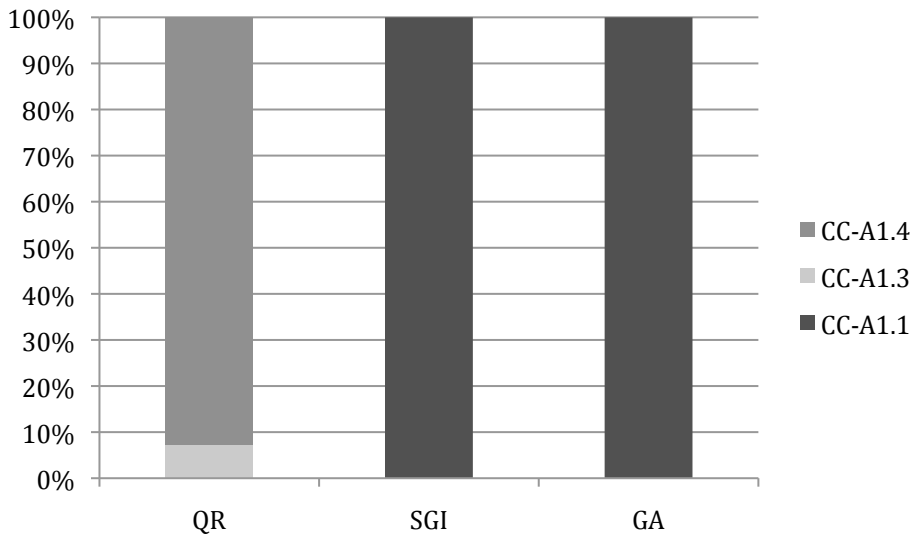


Figure 2.5. Distribution of the long CC-A1 haplotypes in the Atlantic Ocean. The number of individuals with the CC-A1 haplotype is indicated by n. QR: Quintana Roo (n = 14, this study), SGI: St. George Island (n = 21, this study), GA: Georgia, USA (n = 17, Monzón-Argüello et al. in press).

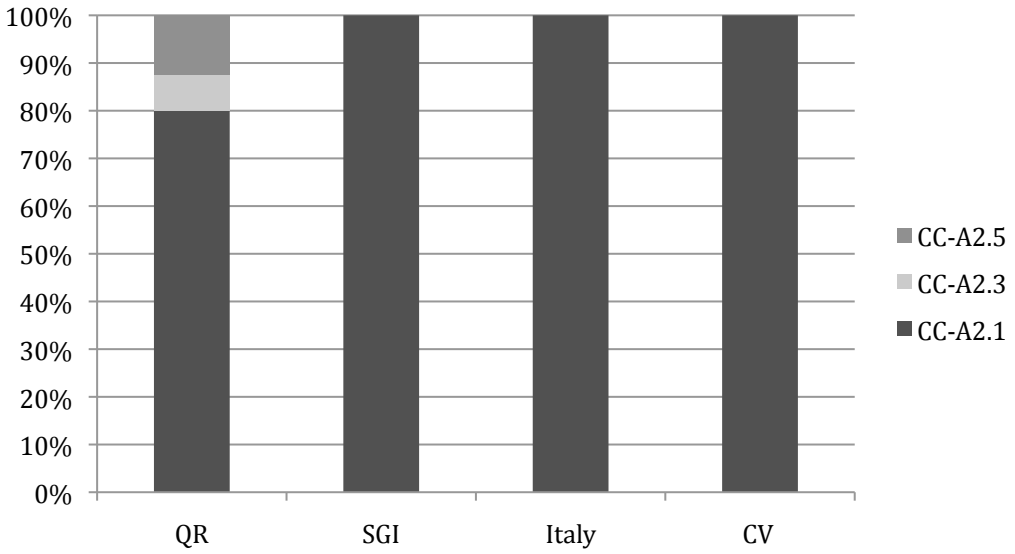


Figure 2.6. Distribution of the long CC-A2 haplotypes in the Atlantic Ocean. The number of individuals with the CC-A2 haplotype is indicated by n. QR: Quintana Roo (n = 80, this study), SGI: St. George Island (n = 3, this study), Italy (n = 22, Garofalo et al. 2009), CV: Cape Verde (n = 2, Monzón-Argüello et al. in press).

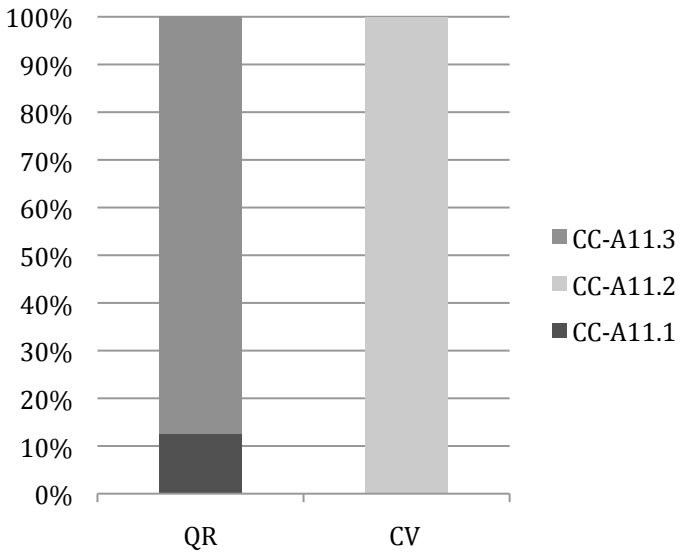


Figure 2.7. Distribution of the long CC-A11 haplotypes in the Atlantic Ocean. The number of individuals with the CC-A11 haplotype is indicated by n. QR: Quintana Roo (n = 8, this study), CV: Cape Verde (n = 1, Monzón-Argüello et al. in press).

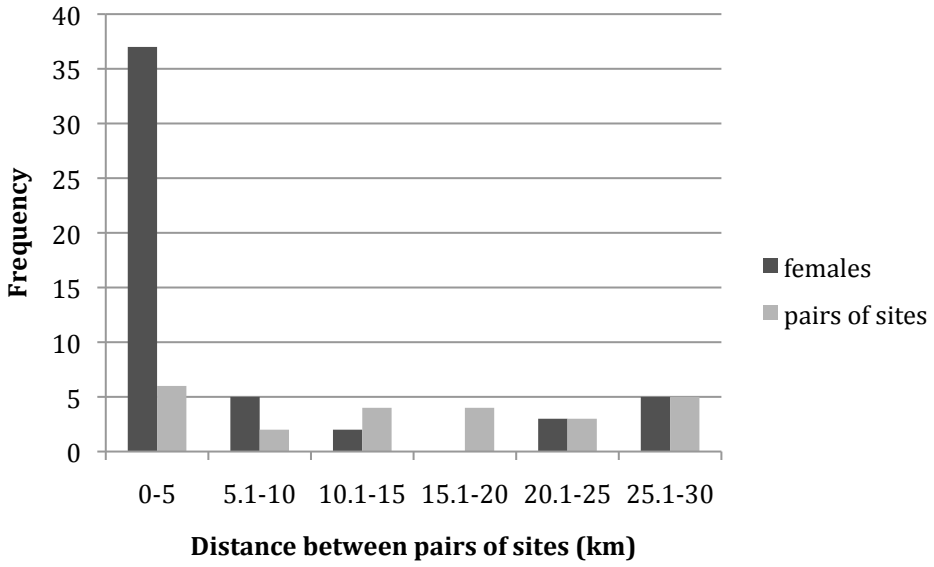


Figure 2.8. The number of pairs of sites at incremental distances from one another, and the number of females (n = 52) who laid nests, at a minimum of two sites, separated by these distances. The majority of females (n = 37, 71%) nested on beaches within 5 km of one another. The two frequency distributions (females and pairs of beaches) are not correlated (Pearson correlation coefficient = 0.666, p = 0.148).

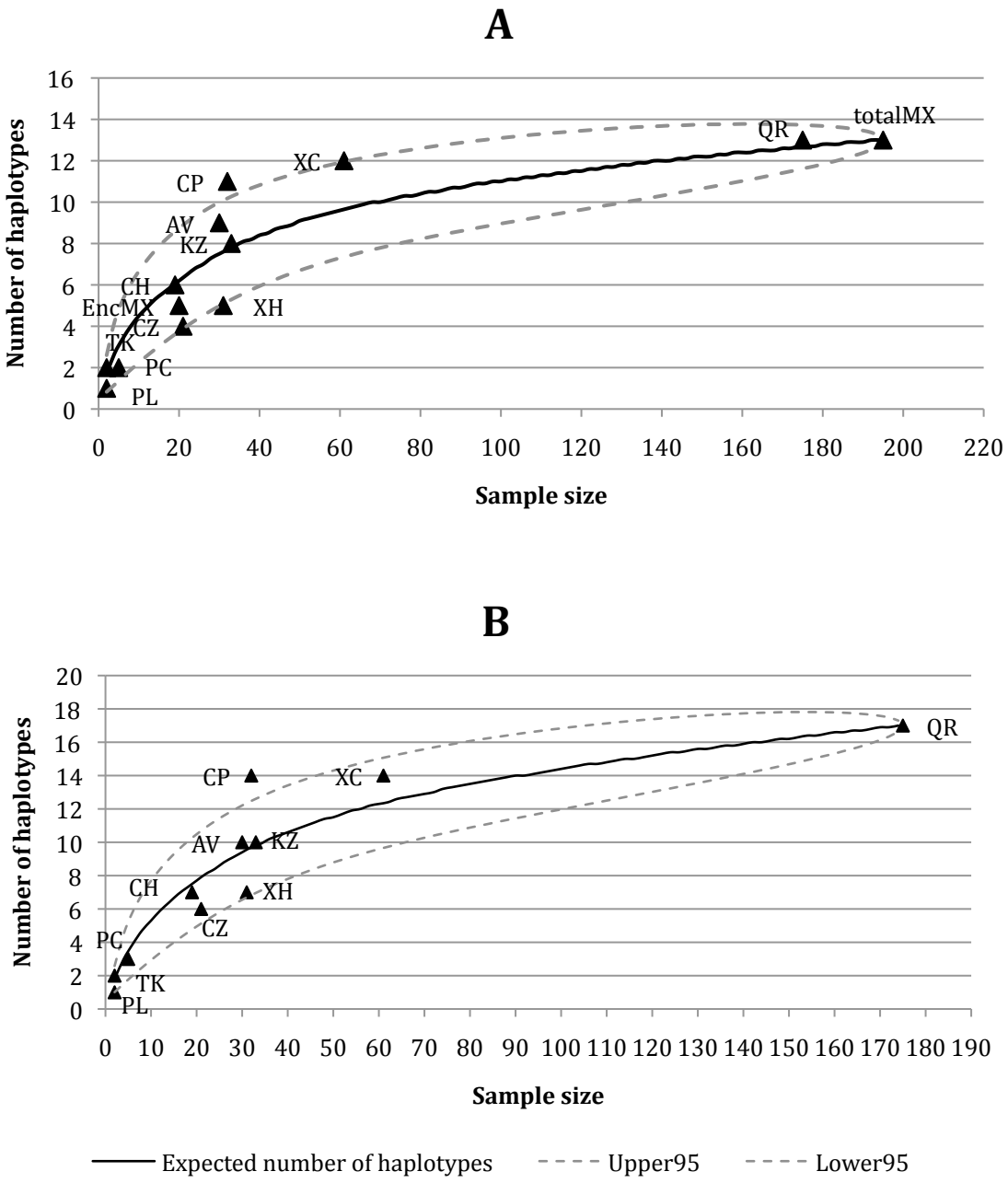


Figure 2.9. Analytic rarefaction of haplotype richness based on the total diversity observed in Quintana Roo with the short control region sequence (panel A) and the long control region sequence (panel B). Number of haplotypes and sample size are plotted for individual beaches. Panel A only – EncMX: dataset from Encalada et al. 1998, totalMX: combined datasets from this study and Encalada et al. 1998. Both panels – QR: Quintana Roo, PL: Paamul, AV: Aventuras, CH: Chemuyil, XC: Xcacel, XH: XelHa, PC: Punta Cadena, TK: Tankah, KZ: Kanzul, CP: Cahpechen, CZ: Cozumel.

Chapter 3

Population Structure of Loggerhead Turtles: A Nuclear DNA Analysis

Background

The patterns of genetic diversity within and among populations are affected by gene flow, as well as mutation, natural selection, and genetic drift (Avise 2000). Gene flow can be inhibited or promoted by the life history characteristics of a species. Dispersal, or genetic exchange without dispersal (gamete dispersal, Hoelzel et al. 2007), is a prerequisite for gene flow, which results in genetically homogenizing populations across a species' range. Restriction of gene flow causes populations to become isolated and vulnerable to inbreeding and genetic drift, which increases the amount of genetic variance across populations and results in population divergence (Wright 1943, Wright 1946). In some species, returning to breed in natal areas, or philopatry, and nesting site fidelity are behavioral traits that inhibit gene flow and increase genetic structuring among sites.

The tendency in breeding behavior to remain philopatric or when dispersal varies between the sexes, results in sex-biased gene flow results. Birds and mammals provide many examples of species where one sex is more philopatric than the other (Greenwood 1980, Pusey 1987). In birds, females tend to disperse from their natal site, while in mammals generally males disperse. Examples of sex-biased dispersal also exist in reptiles (e.g. freshwater crocodiles, *Crocodylus johnstoni* – Tucker et al. 1998) and invertebrates (e.g. honeybees, *Apis melliferia* – Clarke et al. 2002). Evolutionary explanations of sex-biased gene flow are generally centered on either avoidance of inbreeding or sex-differences in competition (Pusey 1987).

In migratory animals, temporary geographic overlap of reproductively active individuals from different populations may lead to genetic exchange, and male-mediated gene flow when females remain philopatric. This phenomenon can result in complex population structuring, which can confound the resolution of populations (Bowen et al. 2005). Many marine species undertake long-range breeding and/or foraging migrations that result in population overlap and sex-biased gene flow (hawksbill sea turtle, *Eretmochelys imbricata* – Bowen et al. 1996; great white shark, *Charcaradon caracarias* – Pardini et al. 2001; sperm whale, *Physeter macrocephalus* – Engelhaupt et al. 2009; rainbow trout, *Oncorhynchus mykiss* – Brunelli et al. 2010).

Loggerhead sea turtles (*Caretta caretta*) also embark on long-range migrations between foraging and breeding areas, experience population overlap, and there is evidence of male-mediated gene flow in some areas (Schroth et al. 1996, Bowen et al. 2005). Our understanding of male-biased gene flow and fine-scale population structuring is poor. Because of this and the listing of loggerheads as endangered by the IUCN Red List of Threatened Species (IUCN 2010), it remains a particularly relevant species for studying complex population structure.

Female loggerhead turtles exhibit natal philopatry based on analyses of maternally-inherited mitochondrial DNA (mtDNA) markers, resulting in divergent maternal lineages among regional nesting aggregations (Encalada et al. 1998). These divergent lineages are referred to as genetic stocks. Female loggerheads are more easily studied than males. Every year females emerge from the ocean to nest on sub-tropical to temperate beaches worldwide (Schroeder et al. 2003), providing an opportunity for researchers to study them. Males are more elusive, remaining in the ocean throughout

their lives, which means direct observation and sampling of males for genetic analysis is more difficult than for females. Therefore, most of what is known about loggerhead turtles, and sea turtles in general, is gleaned from females.

The use of biparentally-inherited nuclear DNA (nDNA) markers has provided insights on differences in female and male migratory behavior. Discordance in population structure detected by mtDNA and nDNA, after taking into account the four-fold lower effective population size imparted by mtDNA compared to nDNA and the mutation rate of each class of marker (Birky et al. 1983), is indicative of sex-biased gene flow. Loggerhead turtles in the southeastern United States show evidence of male-biased gene flow based on a lack of microsatellite-based genetic structuring contrary to the highly structured maternal lineages of the nesting populations in this area (Bowen et al. 2005). But in the eastern Mediterranean both females and males are philopatric, with limited male-mediated gene flow (Carreras et al. 2007).

To date, at least 11 genetic stocks of loggerhead turtles have been identified in the Atlantic Ocean, with females homing to their natal region to nest (Encalada et al. 1998, Laurent et al. 1998, Pearce 2001, Garofalo et al. 2009, Reis et al. 2010, Monzón-Argüello et al. in press) (Figure 3.1). The precision of female site-fidelity within each region is undetermined. Tagging studies have revealed that most females nest within a 5-km range (Schroeder et al. 2003, and see chapter 2). However, this nesting site fidelity will only result in a geographic genetic pattern if it is correlated with each female's *natal* nest site. And the marker used to look for genetic patterns needs to show sufficient resolution. Some genetic stocks of loggerheads are dominated by a single mtDNA haplotype (Encalada et al. 1998, Bowen et al. 2005), which renders this marker inadequate for

resolving fine-scale structuring in these populations. Microsatellites tend to be highly variable due to their relatively high mutation rate (Balloux and Lugon-Moulin 2002) and therefore often, but not always, are more powerful than mtDNA for resolving population structure (Goudet et al. 1996, Buonaccorsi et al. 1999). In a study using microsatellite markers, female green turtles produced a faint genetic signal of natal homing to sites separated by only 15 km (Lee et al. 2007).

The two main objectives in this part of my study were to characterize the nDNA diversity, with which to interpret male-mediated gene flow and fine-scale structuring. I focused on two genetic stocks of loggerhead turtles, in the Yucatan Peninsula in Mexico and in northwestern Florida. The Mexico stock has demonstrated high genetic diversity of mtDNA control region haplotypes (Encalada et al. 1998, also see Chapter 2), but nDNA diversity has never been assessed. Mexico is in the southern part of the loggerheads' nesting range north of the equator, which is subtropical to temperate beaches. The high genetic mtDNA diversity found in this region is thought to stem Mexico's role as refugia during glacial maxima when contemporary nesting areas further north would have been too cold (Hedgpeth 1954, Bowen et al. 1993). The high genetic diversity and the "intermediate" (Ehrahrt et al. 2003) size of this genetic stock resulting in fairly dense nesting along over 100 km of the Caribbean coastline provide an excellent opportunity to examine fine-scale structuring of loggerheads.

Northwestern Florida likely represents an area that was colonized within the last 12,000 as the climate warmed and became more suitable for loggerhead nesting (Hedgpeth 1954, Bowen et a. 1993). Due to its recent history, this genetic stock likely has experienced the founder effect leading to low genetic diversity, as seen in the mtDNA

control region haplotypes (Encalada et al. 1998, also see Chapter 2). nDNA may likewise be similarly effected. Additionally, this area hosts a relatively "minor" (Ehrhart et al. 2003) nesting population. The small size further puts this genetic stock at risk for genetic drift, inbreeding, reduced breeding success, all leading to low genetic diversity (Frankham 1996, Liermann and Hilborn 2001, Berec et al. 2007). Also, local extirpation is threatened by a steep decrease in nest numbers since 1997, when annual surveys were implemented, which were controlled for effort to enable trend analysis (Florida Fish and Wildlife Conservation Commission *unpublished data*, Witherington et al. 2009). Foraging areas for loggerhead turtles in the Gulf of Mexico and throughout the Caribbean most likely support turtles from both Mexico and northwestern Florida (Engstrom et al. 2002, Bass et al. 2004, Reece et al. 2006). Foraging areas comprise genetically mixed individuals in contrast to the divergent mtDNA lineages of females that define different genetic stocks. The areas of population convergence provide an opportunity for gene flow among stocks. A nDNA analysis of loggerheads from the Mexico and the northwestern Florida stocks will be able to identify gene flow between the two stocks.

With the first objective, I sought to assess sex-biased gene flow between the two maternally-structured genetic stocks of loggerhead turtles in Mexico and northwestern Florida, by directly comparing female and male microsatellite data. I predicted that there would be more gene flow between males from the two populations than between females, as a result of males mating opportunistically with females from other populations at foraging areas coupled with female natal homing behavior. I predicted high levels of male-mediated gene flow following the pattern found in the southeastern United States, and not restricted levels as was found in the Mediterranean Sea (Bowen et al. 2005,

Carreras et al. 2007). These two studies examined population structure based on microsatellite DNA, but did not specifically compare female and male datasets, which I did in this study.

The second objective of this study was to evaluate fine-scale structuring within the Mexican population. I predicted that females home precisely to their natal nest sites, resulting in genetic structuring among individuals nesting in different parts of the population range. Structuring has been detected with mtDNA between females from different genetic stocks nesting 50–100 km apart in Florida (Bowen et al. 2005) and 120 km apart in Japan (Hatase et al. 2002). With the increased resolving power of microsatellites, female natal homing may be detected at an even finer scale, as demonstrated with green turtles (Lee et al. 2007). Lee et al. (2007) compared female and male microsatellite genotypes and found that females were more philopatric than males. Similarly, I predicted that females within the Mexican stock would home more precisely than males. Mexico is better suited for fine-scale structure analysis than northwestern Florida due to the higher genetic diversity, based on mtDNA, in Mexico. As an extreme example, if a population is genetically homogenous, females could exhibit natal nest site fidelity but would leave no genetic signal.

Methods

Study sites

The loggerhead rookery in Quintana Roo (QR), Mexico (20°33'N, 87°38'W) belongs to the Yucatan Peninsula genetic stock, as defined by Encalada et al. (1998). Approximately 575 females nest each season along the coast of the Yucatan Peninsula

(2300 nests/yr with an average of four nests per female per season; Ehrhart et al. 2003). I collected samples from nine nesting sites spread out along 52.4 km of coastline on the mainland as well as from Cozumel (20°40'N, 86°86'W), an island about 20 km off the coast (Figure 3.2). Seven of the mainland sites (Aventuras, Chemuyil, Xcacel, XelHa, Tankah, Kanzul, Cahpechen) are patrolled nightly, where nearly all nesting turtles are observed and tagged. This allows each female and her nests to be tracked throughout the season. At the remaining two mainland sites (Paamul and Punta Cadena) and on Cozumel females are not tagged, but nests are located and marked. I collected samples during the nesting seasons in 2006 and 2008 (Table 3.1).

The loggerhead rookery on St. George Island (SGI), Florida (29°68N, 84°80W) belongs to the northwestern Florida genetic stock, as defined by Encalada et al. (1998) (Figure 3.3). Approximately 150 females nest in northwestern Florida each season (600 nests/yr with an average of four nests per female per season; Ehrhart et al. 2003). Nests are located and marked throughout the nesting season, but females are not tagged. I collected samples in SGI during the 2007 and 2008 nesting seasons (Table 3.1). Alligator Point (29°89'N, 84°38'W), a peninsula about 35 km northeast of SGI, is within the northwestern Florida population (Figure 3.3). I collected a sample from one female I encountered nesting in 2007. For the sake of simplicity, I grouped the Alligator Point sample with the samples from SGI and refer to these two locations collectively as SGI. Because the remigration interval (the time between two consecutive nesting seasons) for female loggerheads ranges from one to nine years, with an average interval of two and a half to three years (Schroeder et al. 2003), the chances of sampling the same matrilines in SGI in 2007 and 2008 were slight.

Field methods

At sites where females were observed and tagged, I collected samples from females after nesting, using a 3 or 6 mm biopsy punch to biopsy a skin plug from the posterior edge of the female's fore flipper (Table 3.1). At sites where females were not observed during nesting or tagged, I collected samples from hatchlings that were assumed to be unrelated (i.e. no full or half siblings) by sampling one hatchling per clutch from clutches laid within a 10-day window (Table 3.1). The inter-nesting interval (the time between oviposition cycles) typically lasts 13 to 14 days (Broderick et al. 2002, Schroeder et al. 2003), but can be shorter (ca. 10 days) especially when the water is warmer (Hays et al. 2002). I therefore decided to use a conservative 10-day sampling window to avoid pseudoreplication even though 15-day windows are often used (Carreras et al. 2007, Garofalo et al. 2009). Also, I collected samples from 8–25 hatchlings per clutch from 41 females' clutches in Mexico and 22 clutches in SGI to use for the reconstruction of paternal genotypes. Dead hatchlings were sampled by cutting a small piece of tissue from the front flipper. Live hatchlings were sampled by drawing 0.1 cc blood using a ½ cc 28 gauge disposable insulin syringe (Kendall) from the dorsal cervical sinus following the method of Owens and Ruiz (1980). I released hatchlings at the nest site immediately after sampling. Skin plugs and flipper clips were stored in 1 ml DMSO buffer (20% DMSO and 6M NaCl) and blood was stored in 1 ml of Longmire's lysis buffer (Longmire et al. 1992) in 1.5-2 ml tubes.

All procedures were approved by University of Miami Institutional Animal Care and Use Committee # 07-114. Samples in Mexico were collected under permit # 07656 issued by Secretaría de Medio Ambiente y Recursos Naturales, and in Florida under

marine turtle permit # 189 issued by Florida Wildlife Conservation Commission. Mexican samples were imported into the United States under CITES permit # 124476.

DNA extraction, amplification, and genotyping

I extracted DNA from both skin plugs and blood using a standard ethanol precipitation protocol. I selected 10 microsatellite loci from the literature that had been designed for sea turtles and were polymorphic in loggerhead turtles (Moore and Ball 2002, Zbinden et al. 2007, Monzón-Argüello et al. 2008) (Table 3.2). I amplified the 10 microsatellite loci in two multiplex polymerase chain reactions (PCR) (Saiki et al. 1988), grouped by annealing temperature (T_A). I optimized annealing temperatures (T_A) on an Eppendorf Mastercycler Gradient PCR cycler (Eppendorf AG) using a temperature range that spanned published T_A for each primer. All loci amplified at either 56°C or 60°C (Table 3.2). Primer concentrations were optimized by adjusting the amount of primer added such that the electrophoretic peaks of all loci in a multiplex reaction were of similar height (Table 3.2). Each 10 µl reaction consisted of ca. 50 ng template DNA, 0.2 µM dNTP's, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.08-0.32 µM of each primer pair, and 0.5 unit Taq DNA polymerase (Promega Corporation). PCR cycling conditions on the Eppendorf Mastercycler Gradient PCR cycler (Eppendorf AG) consisted of an initial denaturation step at 95°C for 3 min followed by six cycles of denaturation at 92°C for 30 sec, annealing at 56°C or 60°C for 55 sec (see Table 3.2 for T_A), and extension at 72°C for 1 min 25 sec, followed by 30 cycles of denaturation at 89°C for 30 sec, annealing at T_A -2°C (either 54°C or 58°C) for 55 sec, extension at 72°C for 1 min 25 sec, followed by a final extension at 72°C for 10 min (Bowen et al. 2005).

Amplified fragments were resolved on an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, California). Alleles were sized using the software STRAND (Toonen and Hughes 2001), initially using the automated scoring option, and subsequently each electrophoretic pattern was visually inspected to maximize accurate allele-calling in the presence of stutter and low-height alleles, as recommended (Dewoody et al. 2006).

Characterization of microsatellite loci

Summary statistics for the microsatellite loci were based on unrelated individuals (no full or half siblings or mother-offspring samples) from QR and SGI divided into the following datasets: QR hatchlings, QR females, QR males, SGI hatchlings, SGI females, and SGI males. See below for method of reconstructing male and female genotypes. Sampling years in QR and SGI were grouped and analyzed together based on lack of structure of the mtDNA between years (see Chapter 2).

Allele frequencies, number of alleles, and allelic richness were calculated in FSTAT version 2.9.3.2 (Goudet et al. 2002). Observed and expected heterozygosity and deviations from Hardy-Weinberg equilibrium (HWE) were calculated for each dataset using ARLEQUIN version 3.5 (Excoffier and Lischer 2010). Deviations from HWE often cause an excess of homozygotes which can arise from inbreeding, selection (while microsatellite loci themselves are assumed to be neutral, they may be linked to markers that are under selection), or sub-structuring within the sampling site (Wahlund effect) (Selkoe and Toonen 2006). The presence of null alleles (non-amplified alleles), large allele drop-out, and mis-scoring of alleles due to stutter also can result in an overestimate

of homozygotes that would not be caused by a biological phenomenon (Dewoody et al. 2006). I used MICRO-CHECKER (van Oosterhout et al. 2004) to assess the presence of these three types of scoring errors. MICRO-CHECKER is able to distinguish between scoring errors and actual biological processes, because each type of scoring error leaves its own allelic "signature" at the affected loci (van Oosterhout et al. 2004, Dewoody et al. 2006).

I also tested for linkage disequilibrium (LD) in the datasets with ARLEQUIN. While microsatellite loci are assumed to be neutral and thus most likely not functionally linked, they can be clustered together, which may cause them not to assort independently (Bachtrog et al. 1999, Selkoe and Toonen 2006). For genotypic data where the phase is unknown, a likelihood ratio test is performed to test for LD (Excoffier and Slatkin 1998*)*. An expectation-maximization (EM) algorithm (Dempster et al. 1977, Excoffier and Slatkin 1995, Weir 1996) is used to estimate haplotype frequencies, followed by a permutation procedure to estimate the distribution of the likelihood ratio statistic, which follows a chi square distribution. LD was calculated over 10,000 permutations and a starting value of five random initial conditions from which the EM algorithm starts to repeatedly estimate the sample likelihood.

Reconstruction of parental genotypes

I used the program COLONY (Jones and Wang 2010a) to assess paternity and to reconstruct paternal genotypes of 41 clutches from QR and both paternal and maternal genotypes of 22 clutches from SGI. COLONY uses a group full-likelihood approach, in which the multilocus genotype of all individuals is used to determine relationships and is

more accurate than pairwise methods (Jones and Wang 2010b). The program accounts for genotyping errors (null alleles, mutations, and mis-scoring of alleles) using user-specified error rates for each locus. I estimated the error as the sum of the null allele rate, as calculated in MICRO-CHECKER, and the mutation rate, which I scored in the following way: the total number of mutations, defined as a novel allele that is not present in the maternal genotypes and only occurs at one locus in only one offspring per clutch, divided by the total number of alleles at a given locus. This classification of mutations is consistent with other authors (FitzSimmons 1998, Moore and Ball 2002, Lee and Hays 2004, Zbinden et al. 2007). Loci that had an error rate of zero were assigned an error rate of 0.0001 to allow for unidentified mutations in the offspring. Population allele frequencies, needed to run the analysis, were calculated in FSTAT. Alleles that only were present in the offspring and not in the background population were added to the population alleles, as required by the program, at a low frequency of 0.0001. Following a maximum likelihood framework, COLONY uses the population allele frequencies, the error rate, and any known relationships (eg mother-offspring, sibling groups) to determine the most likely full and half sib families and parental genotypes along with the probability associated with the alleles at each locus. I used only alleles for a locus that had a greater than 0.5 probability. If the probability was equal to or less than 0.5, I treated the alleles as unknown.

To ensure samples from SGI in 2007 and 2008 were not from the same female, I analyzed the clutches with and without entering maternal sibling group information.

Sex-biased dispersal between Quintana Roo and St. George Island

To test for sex-biased gene flow between QR and SGI, genetic distances between females from the two stocks and between males from the two stocks were calculated with Wright's fixation index (pairwise $F_{ST,}$ Wright 1951) and Slatkin's R_{ST} (Slatkin 1995). F_{ST} can be understood as the correlation between two random alleles relative to all the randomly sampled alleles from the total population, and measures the heterozygote deficiency relative to its expectation under HWE (Wright 1951, 1965, Hartl and Clark 1997). R_{ST} is based on the stepwise mutation model that microsatellites appear to follow (Slatkin 1995, Balloux and Lugon-Moulin 2002). While F_{ST} is derived from the variances of *allele frequencies*, R_{ST} is calculated from the variances of *allele sizes* (Balloux and Lugon-Moulin 2002). Values for both statistics range from 0 to 1, with 0 indicating panmixia (negative values should be considered as 0), and 1 indicating complete genetic differentiation. To help interpret F_{ST} and R_{ST} values, the following scale has been suggested as a rough guideline: 0 - 0.05 indicates little genetic differentiation; 0.05 - 0.15 moderate differentiation; 0.15 - 0.25 great differentiation; and values greater than 0.25 very great genetic differentiation (Wright 1978, Hartl and Clark 1997). Both statistics have their limitations, especially when sample sizes and the number of loci are small (Balloux and Lugon-Moulin 2002). Studies have shown that R_{ST} may be a better predictor of interspecific divergence involving longer historical separations than F_{ST}, and F_{ST} may be more sensitive to detect intraspecific differentiation (Forbes et al. 1995, Lugon-Moulin et al. 1999). F_{ST} is more sensitive than R_{ST} to the high mutation rate of microsatellites when migration rate is low (Balloux and Lugon-Moulin 2002). For these reason, F_{ST} may be more reliable in my study, which deals with

intraspecific variation, unless the migration rate is low. For both F_{ST} and R_{ST} statistical significance was obtained over a minimum of 99,999 permutations as implemented in ARLEQUIN.

Calculating F_{ST} and R_{ST} involves multiple pairwise tests at the same significance level which increases the type I error rate (Zaykin et al. 2002). Typically, this problem is circumvented by applying a sequential Bonferroni correction which maintains an experiment-wise significance level while adjusting the significance level of individual tests to reduce the probability of spurious results (Holm 1979, Rice 1989). Although its use has been debated as it increases the type II error rate (Moran 2003), I applied the sequential Bonferroni correction modified by Jaccard and Guilamo-Ramos (2002) as implemented in MACBONFERRONI (Watkins 2002) for all cases of multiple comparisons. I report results both with and without the correction. Also in ARLEQUIN, I estimated the number of female and male migrants (Nm) between QR and SGI, which is estimated from F_{ST} and assumes migration-drift equilibrium (Slatkin 1991). Male-biased gene flow will be evidenced by a shorter genetic distance and a greater number of migrants between QR and SGI males than between females from the two populations.

In another approach to investigate sex-biased dispersal, I used three different tests, two assignment tests and one based on F-statistics, as implemented by the software FSTAT. For the first two tests, an assignment index for each individual was calculated based on the multilocus genotypes of individuals in the population (Favre et al. 1997). Because populations can have different levels of gene diversity, assignment indices across populations cannot be directly compared. To control for this, the mean assignment probability of the population was subtracted from individual assignment probabilities

after log-transformation to give a corrected assignment index (AI_C). A positive AI_C value indicates a genotype that is more likely than average to originate from the population where it was sampled (likely a resident), and a negative value indicates a genotype that is less likely than average to originate from the population where it was sampled (likely an immigrant). The first test of sex-biased dispersal compared mean AI_C values of females and males based on the prediction that immigrants are more likely to have lower AI_C values compared to residents. If there is sex-biased dispersal, the dispersing sex is expected to have a lower mean AI_C value than the philopatric sex. The second test compared the variance of the AI_C values. This tests the prediction that members of the dispersing sex include both dispersed (immigrants) and philopatric (residents) individuals, which will then have a greater variance than the other sex that only includes philopatric individuals. The third test relies on the F_{IS} statistic (Weir and Cockerham 1984), sometimes known as the inbreeding coefficient, which describes how well genotype frequencies within a population fit Hardy-Weinberg expectation (Hartl and Clark 1997). If individuals from the dispersing sex, sampled from a single location, are a mixture of immigrants and residents, due to the Wahlund effect they should be deficient in heterozygotes and have a positive F_{IS} (inbred individuals also will be deficient in heterozygotes, but for a different reason). The dispersing sex is therefore expected to have a higher F_{IS} than the more philopatric sex. To test for statistical significance, a one-tailed test with females expected to be the philopatric sex and a randomization approach with 10,000 permutations were implemented in FSTAT.

Fine-scale structure

To assess fine-scale structuring among females, males, and hatchlings in QR, genetic distances were calculated between pairs of sites for each dataset in ARLEQUIN, with statistical significance obtained over a minimum of 99,999 permutations. First, all pairs of sites for each dataset were compared. Genotypes of females and males that reproduced at multiple sites were used for each site for the calculation of genetic distances. Hatchlings were counted only once for their natal site, when this site was known. Hatchlings sampled in 2008 were labeled with the mother's ID rather than the clutch ID, so if the mother nested at multiple sites, the natal site was not known, and therefore the hatchling's genotype was counted once for each of the sites at which the mother nested. For this reason, some individuals were counted more than once, resulting in an overestimation of the actual sample size (see Table 3.1). Also, I calculated genetic distances among regions in QR by grouping sites in the following way: 1) Paamul, 2) north – consisting of Aventuras, Chemuyil, Xcacel, and XelHa, 3) central – consisting of Punta Cadena and Tankah, 4) south – consisting of Kanzul and Cahpechen, and 5) Cozumel.

Genetic variance between years also was calculated for females and hatchlings (all sites combined). This was not calculated for male genotypes because they were only available for 2006.

In another approach to detect fine-scale structuring of females and males, as a result of homing to their natal region to breed, I used an assignment test to calculate individual assignment probabilities to their natal region in QR using the program GENECLASS2 (Piry et al. 2004). These assignment probabilities are calculated in the same

was as in FSTAT, except GENECLASS2 does not control for differences in gene diversity among populations. Assignment tests are less sensitive to gene flow than F_{ST} and provide results similar to mark and recapture studies (Pearse and Crandall 2004, Berry et al. 2004). I used a Bayesian analysis (Rannala and Mountain 1997) to test the hypothesis that an individual hatched in the region where it was sampled. I refer to the region in which an individual was sampled as the "home" region, because I cannot know if it actually corresponds to the natal region. Bayesian and frequency-based methods for assignment tests appear to perform better than distance-based approaches (Cornuet et al. 1999). Hatchling genotypes were used reference for each region, because the natal region of each sampled hatchling was known. From the original five regions, I excluded Paamul because no females were sampled there, and also central due to the low sample size of both hatchlings and females from this region. Assignment probabilities based on different numbers of loci, due to missing data, are not comparable (Piry et al. 2004). I opted to retain individuals with a minimum of seven amplified loci to maximize sample size while using the same loci. Thus, I excluded 15 hatchlings that had amplified at less than seven loci and used the remaining 62 ($n_{north} = 35$, $n_{south/Cozumel} = 27$) for the analysis. To determine the power of the test to correctly assign individuals, hatchling genotypes were resampled with a Monte Carlo algorithm of 10,000 simulated individuals (Paetkau et al. 2004). This simulation results in a percentage of correctly assigned individuals and a quality index which is the mean assignment probabilities of all individuals to their natal region. To compute assignment probabilities for females and males to each region, I selected the same resampling algorithm. As with hatchlings, I excluded female and male genotypes that had amplified at less than seven loci. The assignment probability for the

region where each female nested ("home") was compared to the assignment probability for the other region ("other") with the Wilcoxon matched pairs signed-rank test in SPSS version 17 (IBM, Chicago, Illinois). Male assignment probabilities were compared in the same manner, and "home" constituted the region where each male sired offspring. Female and male assignment probabilities to home regions were then compared with one another with the Mann-Whitney U test in SPSS.

I also used the three tests described above (mean AI_C, variance of AI_C, and F_{IS}) as implemented in FSTAT, to test for sex-biased dispersal within QR. Statistical significance was obtained applying a randomization approach with 10,000 permutations to a one-tailed test with females expected to be the philopatric sex.

Results

Characterization of microsatellite loci

Genetic diversity indices were based on the following three datasets from each population: 78 hatchlings, 91 females and 71 reconstructed male genotypes from QR, and 24 hatchlings, five females (four of which were reconstructed), and nine reconstructed male genotypes from SGI (see below for details on reconstruction of genotypes).

High levels of polymorphism were observed at all 10 loci (Table 3.2). The number of alleles ranged from 10 for CCM2 to 37 for Cc5F01. Allele frequencies per locus for each dataset are illustrated in Figure 3.4.

All but two loci (CC141, CC7) in QR hatchlings deviated from HWE ($p \leq 0.05$), even after Bonferroni correction (Table 3.3). Only one of these loci (Cc5F01) also tested positive for the presence of null alleles, which had an estimated frequency of 0.0560

(Table 3.3). In QR females, only one locus was in HWE (CC7, $p = 0.4046$) (Table 3.3). The remaining nine loci deviated from HWE after Bonferroni correction, and none had null alleles. In QR males, all but two loci (Cc2G10, CC117) deviated from HWE ($p \leq 0.05$). After Bonferroni correction, the deviations from HWE of three loci (Ccar176, CC141, CC7) did not remain significant. The presence of null alleles was not detected in any of the loci in QR males. Only one locus deviated from HWE (Cc5F01) in SGI hatchlings ($p = 0.0019$) and in SGI males ($p = 0.0130$) (Table 3.3). Only Cc5F01 in SGI hatchlings continued to deviate significantly from HWE after Bonferroni correction. Null alleles were detected in neither of these loci. No additional loci showed indications of mis-scoring due to stutter or large allele dropout.

In QR hatchlings, four pairs of loci were in LD (χ^2 test, $p \leq 0.05$) (Table 3.4). Each of these pairs included CC117. One of these pairs (CC117 and CC17) were in significant LD after Bonferroni correction was applied. Five pairs of loci were in LD in QR females (Table 3.5). Three of these pairs included CC117. After Bonferroni correction, none of these pairs of loci remained in significant LD. No loci were in LD in SGI nor in males from QR (χ^2 test, $p > 0.05$) (Tables 3.4, 3.5, 3.6). Because the Bonferroni correction increases the probability of a type II error and its controversial use (Moran et al. 2003), I decided to discard CC117 from further analyses as recommended for loci that are consistently in LD (Selkoe and Toonen 2006). The remainder of the loci was assumed to be independent.

Reconstruction of parental genotypes

Parentage analysis was based on 38 unrelated clutches and three females' pooled clutches (totaling 41 'families') from QR and 22 unrelated clutches from SGI. Between eight and 25 hatchlings (average = 20.2) were sampled from each clutch (average clutch size = 120.6). COLONY confirmed that each clutch from SGI was from a different mother. COLONY initially reconstructed 78 unique paternal genotypes based on the known hatchling and maternal genotypes from QR. Seven of these genotypes were discarded because all of the loci had a probability of 0.5 or less. The final number of reconstructed male genotypes from QR was 71. In SGI, initially 29 male genotypes were reconstructed as well as 22 female genotypes, based on known hatchling genotypes. In cases of single paternity and when the maternal genotype is unknown, the parental alleles cannot be distinguished and each allele had a probability of 0.5 or less. Of the six clutches in SGI with multiple paternity, COLONY only was able to reliably reconstruct four maternal genotypes and nine male paternal genotypes. The average probability of the inferred alleles at each locus for all of the reconstructed genotypes after discarding alleles with $p \leq 0.5$, was very high (QR males $p = 0.9612 \pm 0.1043$; SGI males $p = 0.9258 \pm 0.1098$; SGI females $p = 0.9345 \pm 0.1127$).

When clutches from SGI were analyzed without entering information on known maternal sibling groups, COLONY confirmed that each clutch was from a different mother.

Sex-biased dispersal between Quintana Roo and St. George Island

The first test of sex-biased dispersal between QR and SGI was based on F_{ST} and number of female and male migrants. The genetic distance between females from QR

and SGI was not significant (F_{ST} = 0.0283, p = 0.0918, R_{ST} = 0.1062, p = 0.1102), but it was greater than the genetic distance between males, which also was not significant (F_{ST} = .0178, p = 0.05, R_{ST} = 0.0102, p = 0.1348) (Table 3.7). There were almost three times the number of male migrants than female migrants between the two populations (males Nm = 24.3; females Nm = 8.6) (Table 3.7).

The tests for sex-biased dispersal between QR and SGI using both mean AI_C (mean $AI_{Cfemales}$ = 0.06226, mean AI_{Cmales} = -0.04903, p = 0.4195) and the variance of AI_C (variance of $AI_{Cfemales}$ = 9.6252, variance of AI_{Cmales} = 14.74351, p = 0.0654) were non-significant (Table 3.8). The F_{IS} test of females being more philopatric than males was significant ($F_{ISfemales}$ = -0.0347, $F_{ISmales}$ = 0.0272, p = 0.0084) (Table 3.8).

Fine-scale structure within Quintana Roo

In the analysis of structure at the finest scale of resolution, hatchlings from two sites were significantly differentiated in 2006 with F_{ST}, Xcacel and XelHa (F_{ST} = 0.0865, p = 0.0216, R_{ST} = 0.1312, p = 0.0634) (Table 3.9). With R_{ST}, hatchlings from XelHal and Aventuras were significantly differentiated (F_{ST} = 0.2199, p = 0.0572, R_{ST} = 0.4450, p = 0.0269). In 2008, hatchlings from Chemuyil and Cozumel were significantly differentiated but only with F_{ST} (F_{ST} = 0.1373, p = 0.0353, R_{ST} = -0.1550, p = 0.6953) (Table 3.9). Genetic distances between hatchlings from several sites between years also were significant, including between Aventuras from 2006 and 2008 with both F_{ST} and R_{ST} (F_{ST} = 0.2257, p = 0.0175, R_{ST} = 0.2853, p = 0.0194), Aventuras 2006 and Xcacel 2008 only with F_{ST} (F_{ST} = 0.19228, p = 0.0183, R_{ST} = 0.1029, p = 0.1421), Aventuras 2006 and Cozumel 2008 also only with F_{ST} (F_{ST} = 0.1377, p = 0.0269, R_{ST} = 0.0970, p =

0.1909), and Punta Cadena 2008 and XelHa 2006 (F_{ST} = 0.1705, p = 0.0280, R_{ST} = 0.1661, p = 0.1943). When Bonferroni correction was applied, no significant differences remained between any pair of sites with either statistic. Females sampled in 2006 and 2008 analyzed by site and year were not significantly differentiated from one another by site or year with F_{ST} (all $p > 0.05$) (Table 3.10). With R_{ST}, there were significant differences between years between eight pairs of sites ($p \leq 0.05$), but not between sites within the same year (Table 3.10). Despite the small differences between F_{ST} and R_{ST}, after Bonferroni correction no structuring among sites remained significant. No genetic distances among males by site were significant with either F_{ST} or R_{ST} ($p > 0.05$) (Table 3.11).

Regional grouping of sites greatly reduced the double-counting of individuals. Based on tracking data provided by Flora, Fauna, y Cultura de Mexico, A.C, before grouping into regions, 30 of the 91 sampled females (33%) nested at two or three sites. After regional groupings, only four females (4.4%) nested in more than one region. Two (2.2%) nested in both the northern and southern regions. The only two sampled females that nested the central region also nested in the south (6.5% of the 31 sampled females from the south). Based on nest locations, two males out of the 71 reconstructed male genotypes from 2006 (2.8%) reproduced in both the northern and the southern regions. These individuals (four females and two males) were included in all regions they nested/reproduced in to retain the signal of connectivity among the regions. Because females are not monitored or tagged on Cozumel no data is available on movement between the island and the mainland.

When grouped into regions there was no genetic differentiation between hatchlings from the north and the south in 2006 with either statistic (F_{ST} = -0.0108, p = 0.9413, R_{ST} = 0.0107 , p = 0.2942) (Table 3.12). Within 2008, both statistics detected significant differentiation between hatchlings from the north and the following three regions: central (F_{ST} = 0.2073, p = 0.0052, R_{ST} = 0.4730, p = 0.0147), south (F_{ST} = 0.1568, p = , R_{ST} = 0.4431, p =), and Cozumel (F_{ST} = 0.1742, p = , R_{ST} = 0.4871, p =). Only the differentiation between hatchlings from the north and Cozumel remained significant after Bonferroni correction. Between years, hatchlings from north 2006 consistently were significantly differentiated from hatchlings from Paamul 2008, north 2008, and Cozumel, even after Bonferroni correction (F_{ST} = 0.0368 – 0.2552, $p \leq 0.05$, R_{ST} = 0.3002 – 0.9184, $p \leq 0.05$), as were hatchlings from south 2006 and north 2008 (F_{ST} = 0.3058, p < 0. 0001, R_{ST} = 0.8155, p < 0.0001) (Table 3.12).

Females from 2006 were not significantly differentiated among regions (Table 3.13). In 2008, females from north and south were significantly differentiated with FST after Bonferroni correction, but not with RST after Bonferroni correction (F_{ST} = 0.0721 p = 0.0007, R_{ST} = 0.2154, p = 0.0104). Between years, females from the north and the south, and females between years in the north were significantly differentiated with both statistics after Bonferroni correction (F_{ST} = 0.0645 – 0.0950, p < 0.0001, R_{ST} = 0.2826 – 4863, p < 0.001).

Male genotypes were only inferred from 2006, and the genetic distance between male genotypes derived from clutches in the north and south was not significant (F_{ST} = 0.0011, p = 0.5962, R_{ST} = 0.0268, p = 0.2943) (Table 3.14).

Overall, both hatchlings and females were significantly differentiated across the two sampling years (hatchlings: $F_{ST} = 0.0839$, $p < 0.0001$, $R_{ST} = 0.4273$, $p < 0.0001$; females: $F_{ST} = 0.0665$, $p < 0.0001$, $R_{ST} = 0.3229$, $p < 0.0001$), even after Bonferroni correction was applied (Table 3.15).

For the calculation of assignment probabilities in GENECLASS2, hatchlings were grouped into regions in the north and the south, with Cozumel included in the southern region as there was no genetic structure found between hatchlings from the south and Cozumel with either F_{ST} or R_{ST} (Table 3.12). Paamul and the central region were excluded from this analysis due to low sample size. The simulation of assigning hatchlings to their natal region returned a relatively low percentage of 57.9% of hatchlings being correctly assigned to their natal region, and also a fairly low quality index of 66.1% (the mean assignment probabilities of all individuals to their home region).

The two females and two males that had reproduced in both the northern and southern regions were excluded from the calculation of assignment probabilities as they could not be assigned a home region. The two females who nested in the central and southern regions were retained and exclusively assigned to the southern region since the central region was not part of the analysis. Assignment probabilities for females and males were even lower than for hatchlings. Females had a 41% probability of being correctly assigned to their home region and males had a 46% probability (Figure 3.5). These assignment probabilities were not significantly different between the sexes (Mann-Whitney U test: $z = -0.1048$, $p = 0.2944$). Assignment probabilities to the home region were slightly higher for females and males nesting in the north (females: 44%, males:

48%) than in the south (females: 35%, males: 33%). There also were no differences in female and male assignment probabilities when the northern and southern regions were analyzed separately (north: z = - 0.763, p = 0.4456; south: z = -0.685, p = 0.6845).

For both females and males, the probability of being correctly assigned to their home region was significantly greater than the probability of being assigned to the other region (Wilcoxon matched pairs signed-rank test – females: n=69, z = -2.063, p = 0.0391; males: n = 65, z = -5.076, p < 0.0001) (Figure 3.5). When the regions were analyzed separately, females and males who reproduced in the north had a significantly greater probability of being assigned to the north than to the south (females: n = 46, z = -2.109, p = 0.035; males: n = 57, z=-5.454, p < 0.0001). But, females and males who reproduced in the south did not have a significantly greater probability of being assigned to the south (females: n = 23, z = -0.639, p = 0.5230; males: n = 8, z = -0.507, p = 0.6121).

The tests for sex-biased dispersal between the northern and southern regions for females and males indicated non-significant differences in both mean AI_C (mean $AI_{Cfemales}$ = 0.20375, mean AI_{Cmales} = -0.16241, p = 0.2781) and the variance of AI_C (variance of $AI_{Cfemales}$ = 9.14759, variance of AI_{Cmales} = 14.40299, p = 0.0587) (Table 3.16). The F_{IS} test of females being more philopatric than males was significant ($F_{ISfemales}$ = -0.0262, $F_{ISmales}$ = 0.0408, p = 0.0122) (Table 3.16).

Discussion

One of the main objectives of this project was to test the prediction that male loggerhead turtles provide an avenue for gene flow between two genetic stocks, the Yucatan Peninsula in Mexico and northwestern Florida, which show strong

differentiation between females based on mtDNA (φ_{ST} = 0.62, p <<0.0001, see Chapter 2). A second objective was to test the prediction that females nesting in different sites within Mexico are genetically structured, indicating precise female homing to their natal sites and insignificant gene flow among rookeries. To test these predictions, I compared multilocus genotypes of hatchlings, females, and males from each population. In a novel approach to assess genetic structuring in loggerhead turtles, male genotypes were reconstructed using hatchling genotypes and when available, maternal genotypes.

Sex-biased gene flow between Quintana Roo and St. George Island

In the first test for sex-biased gene flow between QR and SGI, the genetic distance between females was greater than between males. This resulted in almost three times more male migrants (24.3) than female migrants (8.6) between the two populations (Table 3.7). This supports my prediction that gene flow between QR and SGI predominantly is mediated males. Also, the results suggest that some gene flow between females may be occurring, currently or in the recent past, as indicated by a non-zero number of female migrants and the lack of significant structuring between females from QR and SGI at microsatellite loci. Alternatively, the lack of genetic structuring between females from these two stocks may be a product of the bi-parental mode of inheritance. The high amount of male-mediated gene flow will manifest itself in half a female's microsatellite alleles. The sample sizes of both females and males from SGI were very low, and it is possible that with larger sample sizes the genetic distances between QR and SGI may become significant.

The genetic distances between QR and SGI (females F_{ST} = 0.0283, males F_{ST} = 0.0102, $p > 0.05$) (Table 3.7) are an order of magnitude greater than the genetic distances found among three genetic stocks in the Mediterranean (F_{ST} = 0.006, $p < 0.001$) (Carreras et al. 2007) and among four genetic stocks in the southeastern United States (which included northwestern Florida) (F_{ST} = 0.002, $p > 0.05$) (Bowen et al. 2005). Because significant, albeit very slight, structuring was detected with microsatellite markers in the Mediterranean, larger sample sizes from SGI will likely allow us to detect significant structuring between QR and SGI. The differentiation between QR and SGI as measured by R_{ST} (females R_{ST} = 0.1062, males R_{ST} = 0.0178, $p > 0.05$) was one to two magnitudes greater than differentiation among stocks in the southeastern United States that the QR ($R_{ST} < 0.001$, $p > 0.05$) (Bowen et al. 2005). Due to the much greater genetic distance between QR and SGI than among regional populations in the two other studies, the Mexican stock and the northwestern Florida stock are likely more isolated from one another than the analogous populations in the southeastern United States and in the Mediterranean.

In another approach to test for sex-biased gene flow, the variance in AI_C was predicted to be greater for the dispersing sex, due to a mixture of individuals originating from the population sampled and dispersed individuals originating from other populations, compared to the philopatric sex which was predicted to have a low variance. Although this test was not significant ($p = 0.0654$) (Table 3.8), the variance tended to be higher among males suggesting that the male dataset may have included individuals from other populations as well. Males had a significantly lower F_{IS} than females ($p = 0.0084$) (Table 3.8) suggesting that the composition of males did indeed include individuals from

different genetically discrete populations, while females comprised a more homogeneous population and were philopatric to their natal origin. The F_{IS} test is based on detecting a heterozygote deficit due to the Wahlund effect, which describes a scenario of reduced heterozogozity caused by subpopulation structuring. A number of other historical and contemporary processes can lead to reduced heterozygosity, such as small population size, genetic drift, inbreeding, and founder and bottleneck events (Nei et al. 1975, Boileau et al. 1992, Frankham 1996, Richards and Leberg 1996). If there is no sex-biased dispersal then these processes should effect both sexes equally.

To summarize, these results indicate that males mediate gene flow between QR and SGI more so than females. Males do not need to actually disperse, but can mate with females outside of their natal population, as these methods of analysis track the movement of genes rather than individuals. The results also imply that while at least some males mate with females from other populations, other males breed with females from their natal population based on the significantly lower F_{IS} for males and the tendency for males to have a higher variance in AI_C. If the majority of males had been from other populations, the mean AI_C likely would have been significantly lower for males than females. Whether males return to their natal site or not is not discernable from this study. Regardless, the pattern appears to be consistent with the reproductive and migratory behavior of green turtles in Australia (FitzSimmons et al. 1997a, FitzSimmons et al. 1997b). Nuclear DNA indicated gene flow among regional geren turtle nesting populations, and direct sampling of males at courtship areas followed by mtDNA analysis indicated that males were just as philopatric as females. The authors postulated that genetic material was exchanged as turtles mated with individuals from

other populations during migrations from foraging areas back to their natal areas. Similarly, loggerhead turtles in the southeastern United States exhibited male-mediated gene flow based on a lack of structuring of microsatellite markers across regions that showed little to no gene flow among females based on mtDNA (Bowen et al. 2005). Because individuals are in contact with turtles from other populations in foraging areas, they may easily mate before or while migrating to their natal nesting areas. When females return to their natal region to nest, males will have accomplished gene flow through game dispersal (Hoelzel et al. 2007).

Fine-scale structure within Quintana Roo

Hatchlings, females, and males showed almost no evidence of genetic structuring between all pairs of sites within and across year with either F_{ST} or R_{ST}, contrary to my expectations. After Bonferroni correction, no significant differentiation among sites remained. Many of the sample sizes for each site were very small, which reduced the power to detect genetic differences. Because of the amount of connectivity among sites, especially within the northern and within the southern region, the lack of consistent differentiation at this scale is not surprising. Estimates of genetic distances with F_{ST} and R_{ST} were similar, although in some cases R_{ST} returned unrealistically high genetic distances, although not necessarily significant, that were not consistent with the F_{ST} estimate. This was especially true when sample sizes were low or uneven, such as the genetic distance between males from Kanzul (n = 2) and XelHa (n = 20) (F_{ST} = -0.0531, R_{ST} = 0.6957, $p > 0.05$). It has been reported that F_{ST} may be a better statistic to use than

R_{ST} when sample sizes are low (n < 10, Gaggiotti et al. 1999). Both statistics should be interpreted with extreme caution when the analysis is based on two individuals

When sites were grouped into regions, there was no evidence of structuring among hatchlings, females and males in 2006. Within 2008 the only indication of significant genetic structuring with both F_{ST} and R_{ST} after Bonferroni correction was between hatchlings from the north and from Cozumel. Also, the southern region and Cozumel were not genetically differentiated indicating greater rates of gene flow between these two regions than between the north and Cozumel. On Cozumel, females are not monitored or tagged so it is unknown whether females will nest both on the island as well as in the southern region. They could be faithful to one region throughout an entire nesting season, or they could nest in both regions within the same season. Alternatively, females born on Cozumel may faithfully nest in the southern region on the mainland throughout their reproductive years, or vice versa. Cozumel is further from the southern region (over 75 km to Kanzul and Cahpechen) than from the northern region (between 35 and 40 km) so it is surprising that the connectivity between Cozumel and the southern region is greater. Satellite tracking and/or flipper tagging of turtles on Cozumel would complement the mark-recapture program on the mainland and would be instrumental to determine patterns of breeder exchange between these two regions.

Also within 2008, genetic distances with both F_{ST} and R_{ST} were significant between hatchlings and females from the north and the south, although not after Bonferroni. For both hatchlings and females, genetic differentiation between years was significant with both F_{ST} and R_{ST} and after Bonferroni (north 2006 - north 2008, south 2006 - north 2008).

This pattern of structuring between years and in 2008, but no significant structuring within 2006 is intriguing. The inconsistency, both spatially and temporally, of the pattern raises questions about the actual nest site fidelity of females. Mark-recapture data of the sampled females indicated some, but little nesting in more than one region: 4.4% nested in more than one region (north, central, and south) and 2.2% nested in the northern and the southern region (Flora, Fauna y Cultura de Mexico, A.C. *unpublished data*). The mark-recapture data from QR spans about 20 years, and the impending analysis of female nest site fidelity (or lack thereof) will be extremely informative as to the connectivity between the northern and the southern regions. In QR, most females nest consecutively within each site or in a close the neighboring site (I. Iturbe *personal communication*) and this nest site fidelity is common throughout the species with females generally nesting within a 5-km range during a season (Schroeder et al. 2003). Because populations will not differentiate (as calculated by F_{ST}) as a result of genetic drift if there is more than one migrant per generation (Wright 1931, Wang 2002), a small amount of connectivity will homogenize genetic variance across sites.

There was some support for my prediction of female natal nest site fidelity as evidenced by the differentiation between the northern region and Cozumel. Regarding the rest of the observed patterns, I argue that they are more likely a result of a population that is not in equilibrium, than true genetic structuring resulting from female natal nest site fidelity. The deviation from HWE in most of the loci from the Mexican hatchling, female, and male datasets also suggest non-equilibrium. This genetic stock of loggerheads is likely in the process of recovering from severely low numbers. Sea turtle exploitation and nesting habitat destruction increased rapidly during the 1950's as the

population, and demand for sea turtle products, grew and outboard motors and nylon nets became available (Fuentes 1967 and Márques 1976 in Hildebrand 1987). Sea turtle exploitation was not banned in Mexico until 1990 (Garduño-Andrade et al. 1999). The period from 1987 to 2008 saw an almost doubling of the annual number of loggerhead nests, from 734 to 1403, along the coast of Quintana Roo (Flora, Fauna y Cultura de Mexico, A.C. 2008). This trend may likely reflect a partial recovery of the population, due to improved conservation measures in Mexico and in international waters. The genetic effects of this bottleneck event may be the cause of the deviations from HWE of the microsatellite loci and the erratic pattern of spatial and temporal structuring. Continued genetic analysis of structuring over a number of nesting seasons, longer than the average re-migratory period for the species, and throughout the region may provide a clearer picture of the patterns of genetic diversity in within this genetic stock.

The assignment test implemented in GENECLASS2 showed slight evidence for precise female homing, providing meager support for my prediction. The reference dataset consisting of hatchlings had a fairly low probability of just under two thirds being assigned to their home site, with a little over half of the individuals being assigned correctly. Females and males had even lower probabilities than the hatchlings of being assigned to their home region (less than 50%). Females and males sampled in the northern region both had a significantly greater probability of being assigned to the northern than to the southern region. For both sexes, individuals from the southern region had an equal probability of being assigned to the southern and the northern regions. This suggests that both females and males from the northern region are slightly,

and equally, philopatric. Individuals in the southern region do not appear to return as precisely to their natal region to reproduce.

The sample size of hatchlings from the northern region was somewhat larger than for the southern region, which may have provided a more complete genetic signature for the north than for the south. Alternatively, females and males reproducing in the north could be more philopatric than individuals from the south, perhaps due to environmental factors or changes in their natal nesting habitat. Another explanation for the overall low assignment values, including those for the hatchlings, could be the genetic structuring that was present between years and within 2008. I did not disaggregate the data into separate years, as the reference (hatchling) sample size for the south would have been too low for 2006 (n = 7) to provide a meaningful genetic profile.

The FSTAT's F_{IS} test indicated that females were significantly more philopatric than males, supporting my prediction. As further evidence for greater female philopatry than male philopatry in QR, males tended to have a greater variance of AI_C than females ($p = 0.0587$).

Based on the results from these tests, there is some evidence of precise female homing to within the northern region and possibly Cozumel (separated by 60 km), but not the central or southern regions. Otherwise, the inconsistent lack of structure between years and among regions indicates an overall tendency for genetic connectivity among rookeries throughout QR. While many females may exhibit natal nest site fidelity, this will only result in genetic patterning if their nesting sites are close to their *natal* sites. In addition, movement by just a few females will eliminate the opportunity for genetic divergence between sites.

Broader impacts

With this work I have demonstrated that while female loggerheads are more philopatric than males (facilitating the observed divergence in *maternal* lineages), males provide an avenue for gene flow. Male-mediated gene flow between stocks reduces the negative effects of genetic drift and inbreeding in small populations, which alleviates some of the concern for the conservation of loggerhead turtles. The connectivity among genetic stocks has implications for management. Nesting populations should not be managed in isolation from one another, nor should they be seen as one big panmictic population. Rather, they should be regarded as a metapopulation. Mortality of males from a single stock will affect numerous nesting populations and reduce genetic diversity on a large scale, whereas mortality of females from a single stock may have dire consequences and result in extirpations of local nesting sites.

Mark-recapture data show some restrictions in females nesting in both the northern and southern regions of the study area in Quintana Roo, Mexico. But, as evidenced with the genetic data, there is enough exchange of breeders among regions to homogenize the pattern of genetic diversity. The only exception may be Cozumel, which appears to have limited connectivity with the northern region on the mainland coast. It is critical to protect the entire range of nesting habitat within a stock in order to conserve the full range of genetic diversity. Local loss of nesting habitat may greatly affect the nesting females in that area, few of whom may be able to relocate to more distant beaches to perpetuate the lineage.

The use of various types of markers (e.g. nDNA and mtDNA) can illuminate the patterns of gene flow and natal homing at both regional and local scales. This is critical

to understand the distribution of genetic diversity and effectively manage populations at the lowest level of genetic differentiation while taking into consideration the connectivity between populations.

Table 3.1. Sample sizes for each hatchling, female, and male dataset overall and by site and year. Actual samples sizes for individual sites may be overestimated as females and males are counted once for each site at which they reproduced. The same holds for hatchlings from 2008, which were counted once for each site at which the mother nested. For sites in Quintana Roo (QR) sample sizes before the slash are from 2006 and after the slash from 2008. For St. George Island (SGI) sample sizes before the slash are from 2007 and after the slash from 2008. ¥ indicates sites with unbiased sample sizes that do not include double-counted individuals. * indicates the number of individual genotypes that were reconstructed on the basis of hatchling genotypes.

Site	Hatchlings	Females	Males
QR total¥	41 / 37	58 / 33	71*
Paamul¥	--- / 2	--- / ---	--- / ---
Aventuras	3 / 5	13 / 4	17 / ---
Chemuyil	2 / 2	9 / ---	10 / ---
Xcacel	25 / 8	28 / 15	44 / ---
XelHa	4 / 2	12 / 9	20 / ---
Punta Cadena¥	--- / 3	--- / ---	--- / ---
Tankah	--- / ---	1 / 1	--- / ---
Kanzul	2 / 3	9 / 8	2 / ---
Cahpechen	5 / 2	11 / 7	8 / ---
Cozumel¥	--- / 18	--- / ---	--- / ---
SGI total¥	11 / 13	2 (1*) / 3*	2* / 7*

Table 3.2. Microsatellite loci used for paternity analysis, type of repeat unit, primer annealing temperatures in °C (T_A) and primer concentrations (μM) for each primer pair for PCR, the total number of alleles (*k*) found in this study, primer sequences, and the source for the primer sequences.

Locus	Repeat unit	T_A	μM	*k*	Forward (F) and reverse (R) primers 5'-3'	Reference
Ccar176	di	60	0.12	25	F: GGCTGGGTGTCCATAAAAGA R: TTGATGCAGGAGTCACCAAG	Moore and Ball 2002
Cc2G10	tetra	60	0.10	28	F: CAGTCGGGCGTCATCAGTGGCAAGGTCAAATACAG R: GTTTGCCCTTATTTGGTCACAC	Shamblin et al. 2007
Cc5F01	tetra	60	0.30	37	F: GTTTAAAGGATTTGAGATGTTGTATG R: CCAGTTGTCTTTCTCCAGTG	Shamblin et al. 2007
CC117	di	60	0.20	10	F: TCTTTAACGTATCTCCTGTAGCTC R: CAGTAGTGTCAGTTCATTGTTTCA	FitzSimmons et al. 1995
CC17	di	60	0.10	11	F: CCACTGGAAGTCTAAGAAGAGTGC R: GGAATTGAAGGGATTTTGCT	Monzón-Argüello et al. 2008
DC107	di	56	0.20	12	F: GTCACGGAAAGAGTGCCTGC R: CAATTTGAGGTTATAGACC	P. Dutton in Bowen et al. 2005
CC141	di	56	0.08	18	F: CAGCAGGCTGTCAGTTCTCCAC R: TAGTACGTCTGGCCTGACTTT	N. FitzSimmons in Bowen et al. 2005
CC7	di	56	0.10	15	F: TGCATTGCTTGACCAATTAGTGAG R: ACATGTATAGTTGAGGAGCAAGTG	N. FitzSimmons in Bowen et al. 2005
Cc5C08	tetra	56	0.32	17	F: GTTTCTTTGATGGTTTTTCTGTTATC R: TCAGTCTTCAGGGTATCGTGTAAT	Shamblin et al. 2007
CCM2	di	56	0.14	10	F: GTTTTGGCACTGGTGGAAT R: TGACTCCCAAATACTGCT	Francisco 2001

Table 3.3. Allelic richness (*R*), number of alleles (*k*), observed and expected heterozygosity (H_O and H_E), *p*-values from Hardy-Weinberg (HWE) exact tests for homozygote excess, and the estimated frequency of null alleles (if present) at the 10 microsatellite loci based on the multilocus genotypes of 78 hatchlings (H), 91 females (F), and 71 males (M) from Quintana Roo (QR), and 24 H, 5 F, and 9 M from St. George Island (SGI). * indicates significant deviations from HWE at $p \leq 0.05$ and **bold** indicates significance after Bonferroni correction.

Locus	*R*			*k*			H_O			H_E			HWE			Frequency of null alleles		
	H	F	M	H	F	M	H	F	M	H	F	M	H	F	M	H	F	M
QR																		
Ccar176	5.98	5.91	5.87	18	21	23	0.68	0.71	0.75	0.86	0.86	0.83	**0.0000***	**0.0000***	0.0225*	---	---	---
Cc2G10	7.56	7.57	7.57	22	23	23	0.74	0.77	0.90	0.92	0.92	0.93	**0.0003***	**0.0000***	0.3458	---	---	---
Cc5F01	7.65	7.86	7.91	27	29	32	0.59	0.48	0.86	0.88	0.73	0.94	**0.0000***	**0.0013***	**0.0015***	0.0560	---	---
CC117	4.52	4.51	4.41	8	8	8	0.63	0.65	0.76	0.82	0.82	0.77	**0.0000***	**0.0000***	0.1630	---	---	---
CC17	3.61	3.65	3.97	10	8	11	0.50	0.57	0.55	0.76	0.76	0.73	**0.0000***	**0.0000***	**0.0000***	---	---	---
DC107	4.53	4.69	4.93	10	10	9	0.74	0.79	0.69	0.79	0.81	0.81	**0.0011***	**0.0000***	**0.0020***	---	---	---
CC141	5.92	5.87	5.45	17	13	13	0.81	0.89	0.76	0.87	0.87	0.82	0.0897	**0.0028***	0.0345*	---	---	---
CC7	5.24	5.24	5.48	14	12	13	0.76	0.85	0.75	0.82	0.82	0.84	0.0703	0.4046	0.0160*	---	---	---
Cc5C08	7.02	7.01	7.02	16	16	15	0.76	0.82	0.79	0.91	0.92	0.92	**0.0000***	**0.0000***	**0.0000***	---	---	---
CCM2	4.32	4.17	4.31	9	8	9	0.62	0.66	0.66	0.78	0.75	0.79	**0.0000***	**0.0004***	**0.0000***	---	---	---
SGI																		
Ccar176	5.82	5.93	6.00	14	6	7	0.92	1.00	0.89	0.81	0.84	0.90	0.5756	1.0000	0.1719	0.0942	---	---
Cc2G10	7.80	7.58	6.59	17	7	9	0.96	1.00	0.78	0.94	0.93	0.90	0.9754	0.3574	0.1099	---	---	---
Cc5F01	7.73	8.03	7.96	22	7	11	0.71	1.00	0.89	0.93	0.91	0.95	**0.0019***	1.0000	0.0130*	---	---	---
CC117	4.84	4.62	4.23	8	4	5	0.71	1.00	0.67	0.80	0.73	0.82	0.1413	0.3915	0.1351	---	---	---

CC17	3.76	3.52	3.11	7	3	4	0.79	0.80	0.33	0.69	0.64	0.63	0.4876	0.6190	0.0880	---	---	---
DC107	4.71	4.77	4.10	7	4	4	0.75	0.80	0.78	0.80	0.80	0.77	0.3954	1.0000	0.0558	---	---	---
CC141	6.15	6.04	4.46	12	7	6	0.96	1.00	0.89	0.87	0.91	0.84	0.9202	1.0000	0.6304	---	---	---
CC7	4.95	5.22	2.63	9	7	3	0.79	1.00	0.67	0.78	0.93	0.67	0.9655	1.0000	0.0607	---	---	---
Cc5C08	7.10	7.10	6.19	13	7	7	0.79	1.00	1.00	0.92	0.93	0.89	0.1871	1.0000	0.0680	---	---	---
CCM2	4.57	3.97	2.93	6	3	3	0.92	0.40	0.67	0.79	0.71	0.62	0.7196	0.1101	0.6057	---	---	---

Table 3.4. χ^2 test values and (degrees of freedom) for testing linkage disequilibrium between pairs of loci. Values for hatchlings from Quintana Roo (n = X) are below the diagonal and values hatchlings from St. George Island (n = 24) are above the diagonal. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction.

	Ccar176	Cc2G10	Cc5F01	CC117	CC17	DC107	CC141	CC7	Cc5C08	CCM2
Ccar176	---	108.32 (208)	113.95 (273)	59.57 (91)	52.72 (78)	56.45 (78)	82.58 (143)	66.5 (104)	88.46 (156)	48.43 (65)
Cc2G10	317.75 (396)	---	150.86 (336)	77.91 (112)	50.42 (96)	84.52 (96)	101.24 (176)	82.12 (128)	116.85 (192)	69.64 (80)
Cc5F01	270.56 (486)	351.23 (594)	---	85.67 (147)	62.94 (126)	80.29 (126)	106.16 (231)	90.16 (168)	143.99 (252)	71.6 (105)
CC117	174.96* (144)	225.61* (176)	204.77 (216)	---	25.23 (42)	30.84 (42)	54.89 (77)	38.3 (56)	65.03 (84)	34.18 (35)
CC17	175.51 (180)	223.29 (220)	222.99 (270)	**155.78*** (80)**	---	25.36 (36)	42.22 (66)	31.54 (48)	46.9 (72)	21.2 (30)
DC107	122.38 (180)	137.69 (220)	140.41 (270)	70.03 (80)	69.58 (100)	---	53.72 (66)	44.71 (48)	67.48 (72)	26.43 (30)
CC141	207.97 (306)	234.42 (374)	222.32 (459)	119.23 (136)	119.52 (170)	113.02 (170)	---	50.1 (88)	89.98 (132)	44.77 (55)
CC7	141.58 (252)	180.31 (308)	186.72 (378)	87.50 (112)	86.09 (140)	116.18 (140)	146.99 (238)	---	72.82 (96)	40.73 (40)
Cc5C08	214.52 (288)	299.2 (352)	276 (432)	170.16* (128)	155.25 (160)	143.19 (160)	206.55 (272)	169.16 (224)	---	51.03 (60)
CCM2	159.13 (162)	150.61 (198)	162.5 (243)	75.51 (72)	73.47 (90)	73.68 (90)	114.95 (153)	111.81 (126)	166.29 (144)	---

Table 3.5. χ^2 test values and (degrees of freedom) for testing linkage disequilibrium between pairs of loci. Values for females from Quintana Roo (n = 91) are below the diagonal and values females from St. George Island (n = 5) are above the diagonal. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction.

	Ccar176	Cc2G10	Cc5F01	CC117	CC17	DC107	CC141	CC7	Cc5C08	CCM2
Ccar176	---	22.48 (30)	18.67 (30)	16.94 (15)	8.82 (10)	15.55 (15)	21.44 (30)	22.48 (30)	19.71 (30)	10.01 (10)
Cc2G10	355.01 (483)	---	21.44 (36)	14.17 (18)	10.55 (12)	15.55 (18)	24.21 (36)	25.26 (36)	22.48 (36)	10.01 (12)
Cc5F01	271.52 (609)	316.31 (667)	---	13.12 (18)	13.32 (12)	14.51 (18)	23.16 (36)	24.21 (36)	24.21 (36)	14.51 (12)
CC117	205.05* (168)	235.12* (184)	154.76 (232)	---	4.32 (6)	11.05 (9)	13.12 (18)	14.17 (18)	14.17 (18)	7.24 (6)
CC17	173.55 (168)	235.31* (184)	179.31 (232)	102.64* (64)	---	6.39 (6)	13.32 (12)	10.55 (12)	10.55 (12)	3.28 (4)
DC107	119.22 (210)	196.6 (230)	153.17 (290)	83.26 (80)	71.29 (80)	---	17.28 (18)	15.55 (18)	15.55 (18)	7.24 (6)
CC141	165.95 (273)	232.44 (299)	200.04 (377)	108.65 (104)	77.38 (104)	122.66 (130)	---	24.21 (36)	24.21 (36)	14.51 (12)
CC7	147.66 (252)	192.24 (276)	161.26 (348)	78.31 (96)	71.77 (96)	128.66 (120)	123.66 (156)	---	22.48 (36)	15.55 (12)
Cc5C08	228.61 (336)	292.73 (368)	250.62 (464)	115.14 (128)	110.16 (128)	143.19 (160)	195.74 (208)	164.82 (192)	---	12.78 (12)
CCM2	99.13 (168)	154.02 (184)	119.46 (232)	63.28 (64)	70.15 (64)	87.5 (80)	122.03 (104)	81.82 (96)	168.8* (128)	---

Table 3.6. χ^2 test values and (degrees of freedom) for testing linkage disequilibrium between pairs of loci. Values for males from Quintana Roo (n = 71) are below the diagonal and values males from St. George Island (n = 9) are above the diagonal. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction.

	Ccar176	Cc2G10	Cc5F01	CC117	CC17	DC107	CC141	CC7	Cc5C08	CCM2
Ccar176	---	50.02 (63)	56.41 (77)	34.91 (35)	20.83 (21)	27 (28)	33.93 (35)	29.68 (21)	40.83 (49)	17.5 (14)
Cc2G10	252.91 (529)	---	59.53 (99)	34.21 (45)	19.78 (27)	26.29 (36)	37.04 (45)	28.83 (27)	42.89 (63)	17.83 (18)
Cc5F01	271.06 (713)	335.73 (713)	---	37.82 (55)	30.33 (33)	39.27 (44)	47.25 (55)	32.45 (33)	52.05 (77)	27 (22)
CC117	111.18 (184)	135.07 (184)	152.81 (248)	---	15.05 (15)	21.47 (20)	24.7 (25)	14.77 (15)	30.55 (35)	11.89 (10)
CC17	140.8 (253)	118.36 (253)	172.85 (341)	63.6 (88)	---	12.6 (12)	16.16 (15)	9.34 (9)	26.51 (21)	7.08 (6)
DC107	110.21 (207)	172.92 (207)	171.25 (279)	78.39 (72)	58.65 (99)	---	25.95 (20)	10.08 (12)	28.52 (28)	14.86 (8)
CC141	166.42 (276)	195.68 (276)	240.26 (372)	86.72 (96)	103.77 (132)	90.36 (108)	---	18.79 (15)	32 (35)	13.88 (10)
CC7	149.76 (299)	209.15 (299)	230.16 (403)	90.93 (104)	85.36 (143)	110.39 (117)	119.32 (156)	---	20.68 (21)	5.13 (6)
Cc5C08	202.62 (345)	227.85 (345)	261.65 (465)	99.38 (120)	107.87 (165)	140.34 (135)	151.75 (180)	146.17 (195)	---	23.18 (14)
CCM2	132.89 (184)	145.72 (184)	157.64 (248)	74.07 (64)	46.06 (88)	57.68 (72)	105.28 (96)	95.41 (104)	99.96 (120)	---

Table 3.7. Pairwise genetic distances as measured by F_{ST} (p-value) and R_{ST} (p-value) between females from Quintana Roo (QR, n = 91) and St. George Island (SGI, n = 5), and between males from QR (n = 71) and SGI (n = 9), and the number of migrants (Nm). * $p \leq 0.05$.

	F_{ST} (p)	R_{ST} (p)	Nm
Females QR - SGI	0.0283 (0.0918)	0.1062 (0.1102)	8.6
Males QR - SGI	0.0102 (0.1348)	0.0178 (0.4355)	24.3

Table 3.8. Three tests of sex-biased dispersal using mean corrected assignment indices (AI_C), variance of AI_C and F_{IS} for females and males from Quintana Roo ($n_{females} = 58$, $n_{males} = 71$) and St. George Island ($n_{females} = 5$; $n_{males} = 9$), with significance over 10,000 permutations, and one-tailed tests with females as the philopatric sex. * $p \leq 0.05$.

	Females	Males	p
mean AI_C	0.06226	-0.04903	0.4195
variance of AI_C	9.6252	14.74351	0.0654
F_{IS}	-0.0347	0.0272	0.0084*

Table 3.9. Genetic partitions among hatchlings from each site in Quintana Roo separated by year. F_{ST} values are below the diagonal and R_{ST} values are above the diagonal. Sample sizes for each site are listed in Table 3.1. AV: Aventuras, CH: Chemuyil, XC: Xcacel, XH: XelHa, KZ: Kanzul, CP: Cahpechen, PL: Paamul, PC: Punta Cadena, CZ: Cozumel. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction. Underlined values indicate consistent significance between F_{ST} and R_{ST} after Bonferroni correction.

	AV2006	CH2006	XC2006	XH2006	KZ2006	CP2006	PL2008	AV2008	CH2008	XC2008	XH2008	PC2008	KZ2008	CP2008	CZ2008
AV2006	---	0.5476	-0.0408	0.4450*	0.4017	-0.0024	-0.0866	0.2853*	-0.0543	0.1029	-0.1300	-0.0949	-0.1104	0.2207	0.0970
CH2006	0.2492	---	0.1642	0.0674	-0.1329	0.1910	0.0197	-0.0251	0.1763	0.1226	0.1821	0.2604	0.0962	0.2512	0.1838
XC2006	0.0821	-0.0164	---	0.1312	0.0931	-0.0107	-0.1723	0.0381	-0.1426	0.0071	-0.1069	-0.0979	-0.0734	0.0322	-0.0418
XH2006	0.2199	-0.0075	0.0865*	---	0.2271	0.0884	-0.1750	0.0096	0.0422	-0.0066	0.2229	0.1661	0.1604	0.0088	0.1306
KZ2006	0.1376	-0.2549	-0.0661	0.0718	---	0.1654	0.0292	-0.0062	0.1267	0.1333	-0.0234	0.1764	-0.0483	0.2868	0.1263
CP2006	0.1333	-0.0256	-0.0195	-0.0060	-0.0256	---	-0.2753	0.0900	-0.1914	-0.0463	-0.0419	-0.1195	-0.0223	-0.1623	-0.0474
PL2008	0.1651	-0.0526	-0.1064	-0.0717	-0.0526	-0.2009	---	-0.0841	-0.3234	-0.2413	-0.1883	-0.2537	-0.2063	-0.3967	-0.2922
AV2008	0.2257*	-0.0604	0.0196	0.0280	-0.0256	-0.0308	-0.1908	---	-0.0155	0.0037	0.0019	0.0681	-0.0165	0.1438	0.0257
CH2008	0.3197	0.0725	0.0559	0.1980	0.0725	0.0338	-0.0526	-0.0427	---	-0.1569	-0.1792	-0.2492	-0.1700	-0.2093	-0.1550
XC2008	0.1923*	-0.0510	0.0108	0.0131	-0.0202	-0.0466	-0.1683	-0.0722	-0.0104	---	-0.0086	-0.0623	-0.0030	-0.1123	-0.0376
XH2008	0.1795	0.0000	-0.0420	0.0571	-0.0435	-0.0811	-0.1930	-0.0846	0.0000	-0.0760	---	-0.1557	-0.2601	0.0815	-0.0641
PC2008	0.2571	0.0873	0.0371	0.1705*	0.0621	0.0099	-0.1379	0.0134	-0.0880	0.0139	-0.0686	---	-0.1358	-0.0711	-0.0726
KZ2008	0.0308	-0.0686	-0.0516	0.0166	-0.1341	-0.0673	-0.1475	-0.0029	0.0892	-0.0083	-0.1111	0.0105	---	0.0567	-0.0862
CP2008	0.1559	-0.1015	-0.0039	-0.0667	-0.0556	-0.0598	-0.1429	-0.0141	0.0617	-0.0568	-0.0370	-0.0318	-0.0727	---	0.0395
CZ2008	0.1377*	0.0457	-0.0099	0.0435	0.0183	-0.0521	-0.1235	0.0046	0.1373*	-0.0142	-0.0566	0.0823	-0.0516	0.0022	---

Table 3.10. Genetic partitions among females from each site in Quintana Roo separated by year. F_{ST} values are below the diagonal and R_{ST} values are above the diagonal. Sample sizes for each site are listed in Table 3.1. AV: Aventuras, CH: Chemuyil, XC: Xcacel, XH: XelHa, TK: Tankah, KZ: Kanzul, CP: Cahpechen. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction. Underlined values indicate consistent significance between F_{ST} and R_{ST} after Bonferroni correction.

	AV2006	CH2006	XC2006	XH2006	TK2006	KZ2006	CP2006	AV2008	XC2008	XH2008	TK2008	KZ2008	CP2008
AV2006	---	0.0136	-0.0058	0.0601	-0.0034	0.0031	-0.0357	0.0058	-0.0073	-0.0132	-0.0910	0.0300	-0.0220
CH2006	-0.0125	---	0.0807	0.0649	0.0330	-0.0608	0.0621	0.1167*	0.0800*	0.0851	-0.2680	0.2135*	0.0772
XC2006	-0.0161	0.0059	---	0.0763*	-0.0166	0.0723*	-0.0297	0.0137	-0.0217	-0.0251	-0.0089	-0.0570	-0.0351
XH2006	-0.0113	0.0014	-0.0121	---	-0.2718	0.0228	0.0960	0.2214*	0.0860*	0.1125	-0.1190	0.1374	0.0156
TK2006	-0.0901	-0.0522	-0.0648	-0.0722	---	-0.0996	0.0152	0.0521	-0.0448	-0.0306	-0.1071	0.1274	-0.1305
KZ2006	-0.0009	0.0222	0.0104	0.0161	-0.2280	---	0.0594	0.1278*	0.0768*	0.0850	-0.2784	0.1613*	0.0438
CP2006	-0.0114	0.0110	-0.0178	-0.0045	-0.0712	-0.0074	---	-0.0326	-0.0372	-0.0464	-0.0163	-0.0607	-0.0490
AV2008	-0.0094	-0.0292	-0.0141	0.0116	-0.1128	-0.0157	0.0095	---	-0.0318	-0.0660	-0.0304	-0.0550	0.0042
XC2008	0.0026	0.0175	-0.0010	0.0086	-0.0995	-0.0004	0.0048	-0.0520	---	-0.0440	-0.0355	-0.0920	-0.0415
XH2008	0.0011	0.0135	-0.0095	-0.0102	-0.0253	0.0253	0.0107	-0.0188	-0.0184	---	-0.0410	-0.1053	-0.0412
TK2008	-0.1626	-0.2781	-0.1582	-0.1908	-0.3333	-0.1338	-0.1649	-0.1707	-0.1253	-0.1560	---	0.1863	-0.0682
KZ2008	-0.0361	0.0072	-0.0291	0.0010	-0.0406	-0.0008	-0.0118	-0.0208	-0.0267	0.0020	-0.0406	---	-0.0557
CP2008	-0.0182	0.0094	-0.0007	0.0054	-0.1339	0.0091	0.0233	-0.0418	-0.0136	0.0061	-0.0842	-0.0702	---

Table 3.11. Genetic partitions among males from each site in Quintana Roo. Male genotypes were only reconstructed from 2006 data. F_{ST} values are below the diagonal and R_{ST} values are above the diagonal. Sample sizes for each site are listed in Table 3.1. AV: Aventuras, CH: Chemuyil, XC: Xcacel, XH: XelHa, KZ: Kanzul, CP: Cahpechen. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction. Underlined values indicate consistent significance between F_{ST} and R_{ST} after Bonferroni correction.

	AV	CH	XC	XH	KZ	CP
AV	---	0.0049	0.0427	-0.0092	-0.1022	0.1117
CH	0.0132	---	0.0312	0.0153	-0.0527	0.1073
XC	0.0019	0.0121	---	0.1402	0.7510	0.2183
XH	-0.0119	-0.0022	-0.0029	---	0.6957	0.1703
KZ	-0.0529	-0.0309	-0.0376	-0.0531	---	0.4679
CP	0.0038	0.0090	0.0026	0.0022	-0.0575	---

Table 3.12. Genetic partitions among hatchlings from Quintana Roo grouped into regions: Paamul (PL), north, central, south, and Cozumel), by year. F_{ST} values are below the diagonal and R_{ST} values are above. Sample sizes for each site are listed in Table 3.1. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction. Underlined values indicate consistent significance between F_{ST} and R_{ST} after Bonferroni correction.

	North 2006	South 2006	Paamul 2008	North 2008	Central 2008	South 2008	Cozumel 2008
North 2006	---	0.0107	**0.9004***	**0.9184*****	0.7433*	**0.7368****	**0.3002****
South 2006	-0.0108	---	0.6889*	**0.8155*****	0.4041	0.4194*	0.1359
Paamul 2008	**0.1031***	0.1164*	---	0.4509*	0.0178	0.0220	0.1002
North 2008	**0.2552*****	**0.3058*****	0.1109	---	0.4730*	0.4431*	**0.4781****
Central 2008	**0.0740****	0.0901*	0.0108	0.2073*	---	-0.1344	-0.0461
South 2008	0.0369*	0.0375	-0.0125	0.1568*	-0.0142	---	-0.0165
Cozumel 2008	**0.0368*****	0.0240	0.0213	**0.1742*****	0.0271	-0.0085	---

Table 3.13. Genetic partitions among females from Quintana Roo grouped into regions: north, central, and south by year. F_{ST} values are below the diagonal and R_{ST} values are above. Sample sizes for each site are listed in Table 3.1. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction. Underlined values indicate consistent significance between F_{ST} and R_{ST} after Bonferroni correction.

	North 2006	Central 2006	South 2006	North 2008	Central 2008	South 2008
North 2006	---	0.0629	0.0373	**0.4863*** **	0.6390	**0.2826** **
Central 2006	0.0611	---	-0.1167	0.2985	0.9935	-0.0792
South 2006	-0.0033	0.0233	---	**0.4126*** **	0.5833	0.1487*
North 2008	**0.0950*** **	0.1422	**0.0866** **	---	0.1013	0.2154*
Central 2008	0.0468	0.1724	0.0389	0.0548	---	0.1943
South 2008	**0.0645*** **	0.0724	**0.0434***	**0.0721** **	-0.0063	---

Table 3.14. Genetic partitions among males from Quintana Roo grouped into regions: north and south. F_{ST} values are below the diagonal and R_{ST} values are above. Sample sizes for each site are listed in Table 3.1. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction. Underlined values indicate consistent significance between F_{ST} and R_{ST} after Bonferroni correction.

	North 2006	South 2006
North 2006	---	0.0268
South 2006	0.0011	---

Table 3.15. Genetic partitions among years and datasets in Quintana Roo. F_{ST} values are below the diagonal and R_{ST} values are above the diagonal. Sample sizes for each site are listed in Table 3.1. * $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.0001$ and **bold** indicates significance after Bonferroni correction. Underlined values indicate consistent significance between F_{ST} and R_{ST} after Bonferroni correction.

	Hatchlings 2006	Females 2006	Males 2006	Hatchlings 2008	Females 2008
Hatchlings 2006	---	**0.0703***	**0.0499***	**0.4273*****	**0.4836*****
Females 2006	**0.0793***	---	0.0119	**0.2972*****	**0.3229*****
Males 2006	-0.0001	0.0026	---	**0.3121*****	**0.3529*****
Hatchlings 2008	**0.0839*****	**0.0480*****	**0.0587*****	---	0.0120
Females 2008	**0.1106*****	**0.0665*****	**0.0851*****	0.0099	---

Table 3.16. Three tests of sex-biased dispersal using mean corrected assignment indices (AI_C), variance of AI_C and F_{IS} for females and males between northern and southern regions in Quintana Roo with significance obtained over 10,000 permutations, and one-tailed tests with females as the philopatric sex. * $p \leq 0.05$.

	Females	Males	p
mean AI_C	0.20375	-0.16241	0.2781
variance of AI_C	9.14759	14.40299	0.0587
F_{IS}	-0.0262	0.0408	0.0122*

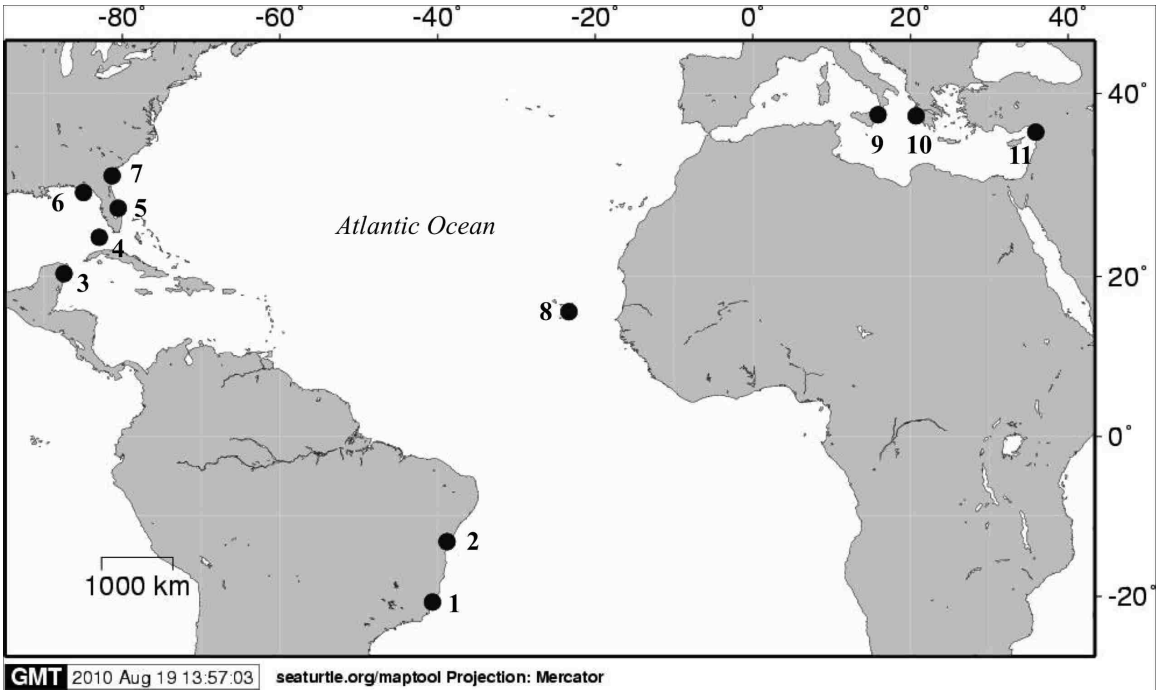


Figure 3.1. Loggerhead turtle genetic stocks identified in the Atlantic Ocean. 1) Southern Brazil, 2) northern Brazil, 3) Yucatan Peninsula, Mexico, 4) Dry Tortugas, Florida, USA, 5) southern Florida, USA, 6) northwestern Florida, USA, 7) northern Florida- North Carolina, USA, 8) Cape Verde, Portugal, 9) Italy, 10) Greece, and 11) eastern Turkey (Encalada et al. 1998, Laurent et al. 1998, Garofalo et al. 2009, Pearce 2001, Reis et al. 2010, Monzón-Argüello et al. in press).

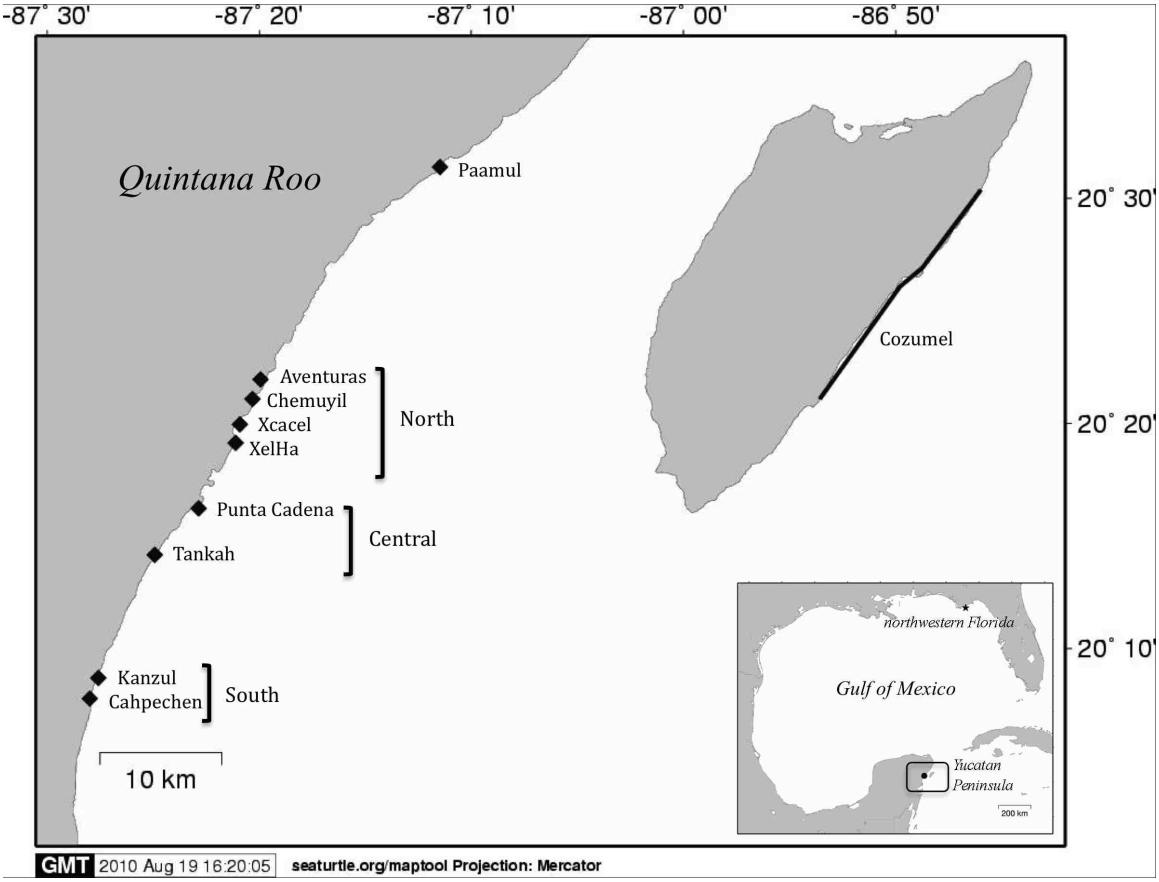
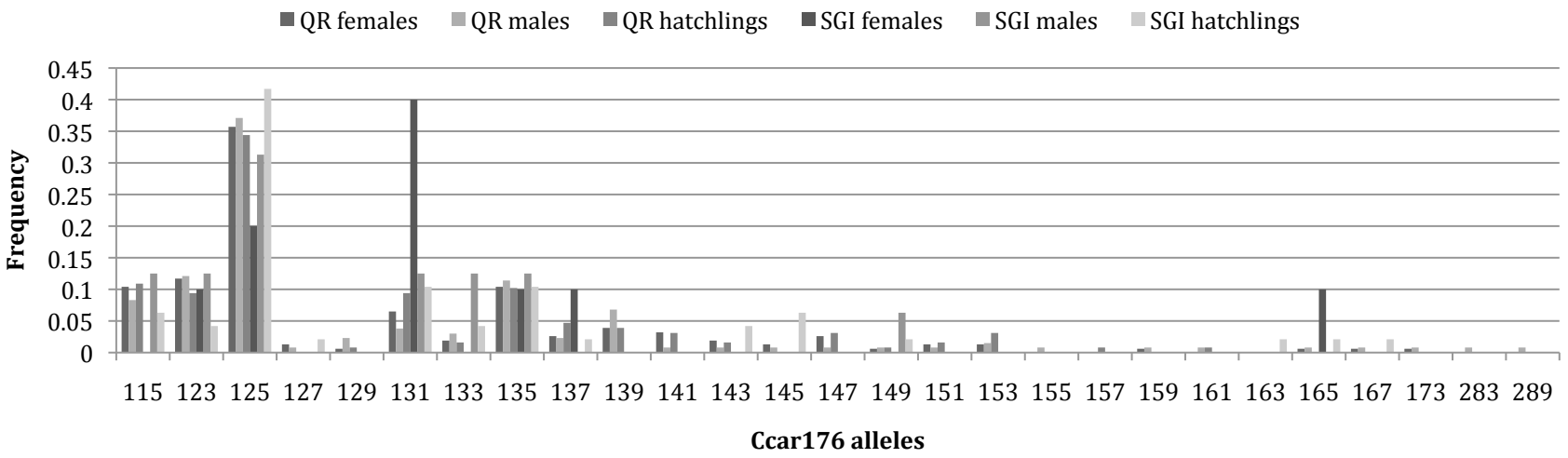


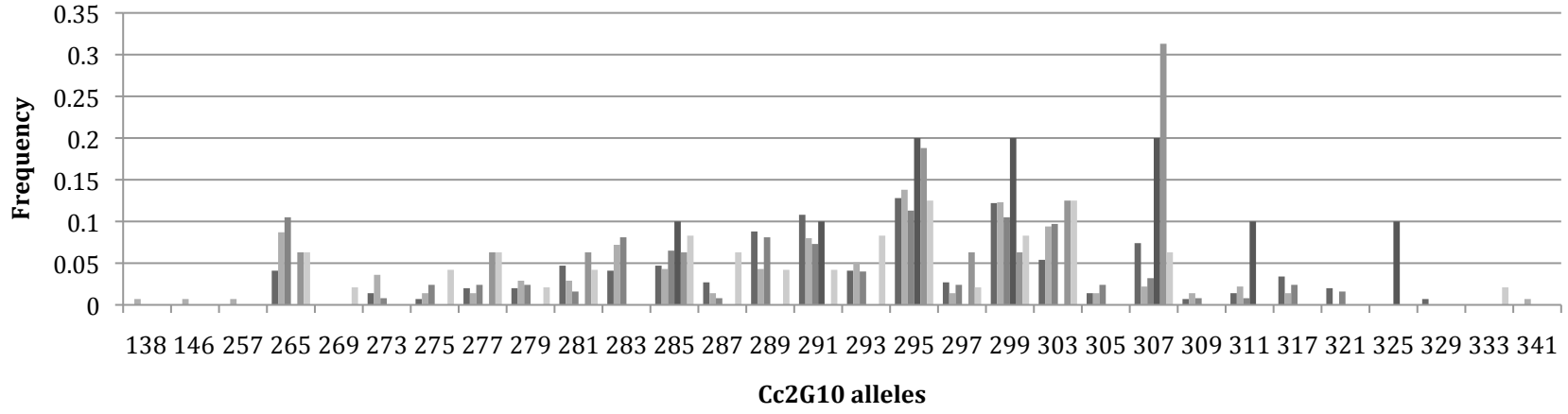
Figure 3.2. Sampling sites and regional groupings of rookeries in Quintana Roo, Mexico, which are part of the Yucatan Peninsula genetic stock.

Figure 3.3. Sampling sites from rookeries on St. George Island and Alligator Point in Florida, which belong to the northwestern Florida genetic stock.

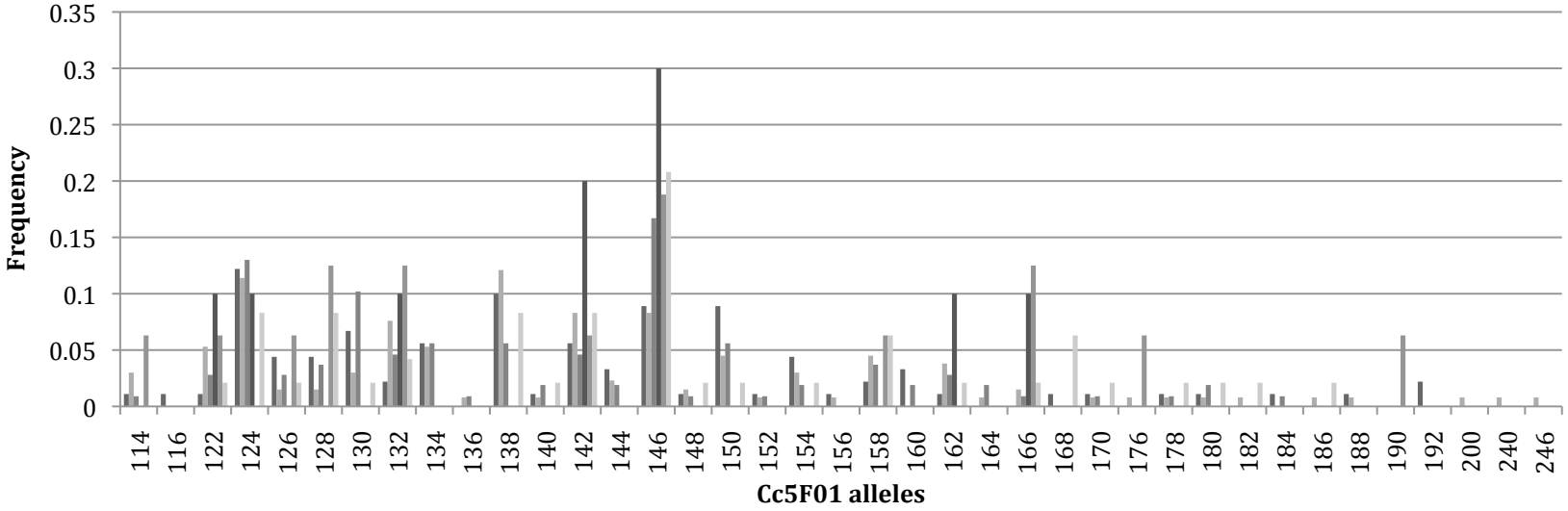
A


QR females
QR males
QR hatchlings
SGI females
SGI males
SGI hatchlings
Frequency
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
115 123 125 127 129 131 133 135 137 139 141 143 145 147 149 151 153 155 157 159 161 163 165 167 173 283 289
Ccar176 alleles

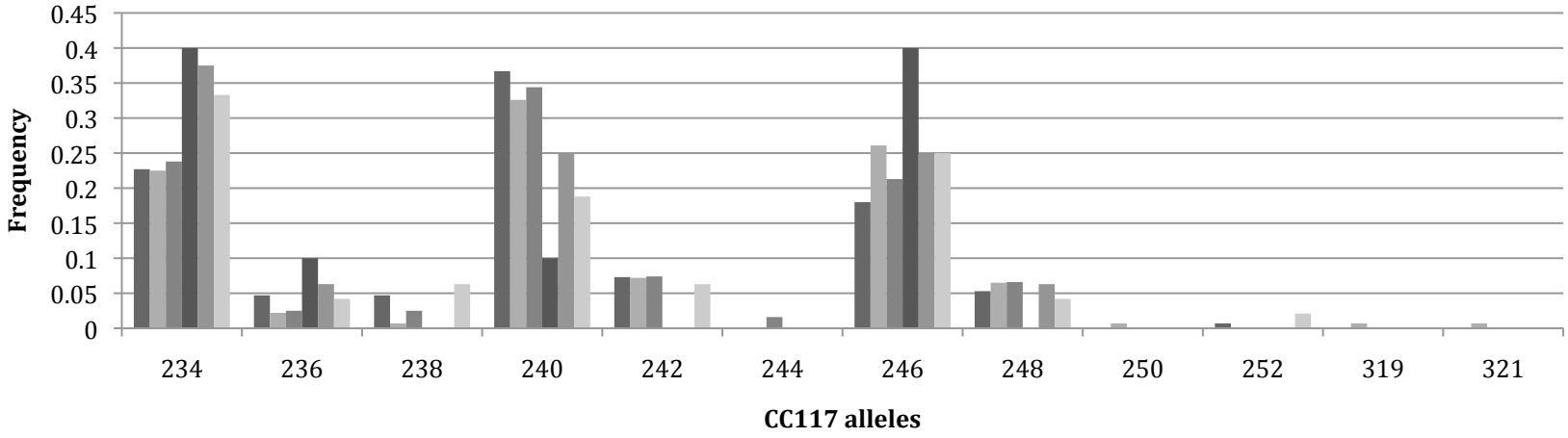
B


Frequency
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
138 146 257 265 269 273 275 277 279 281 283 285 287 289 291 293 295 297 299 303 305 307 309 311 317 321 325 329 333 341
Cc2G10 alleles

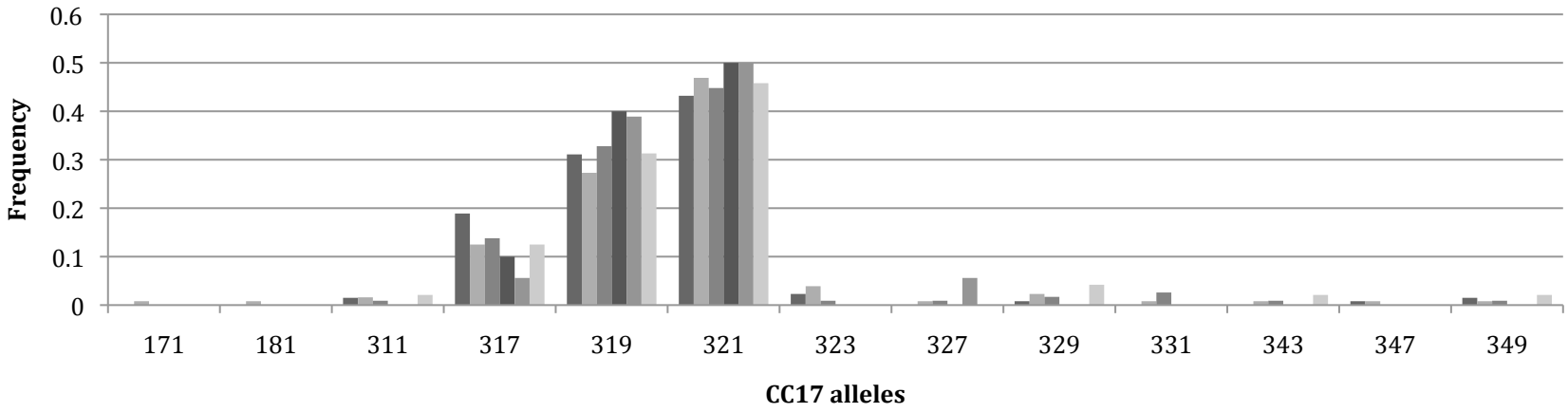
C


0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Frequency
114 116 122 124 126 128 130 132 134 136 138 140 142 144 146 148 150 152 154 156 158 160 162 164 166 168 170 176 178 180 182 184 186 188 190 192 200 240 246
Cc5F01 alleles

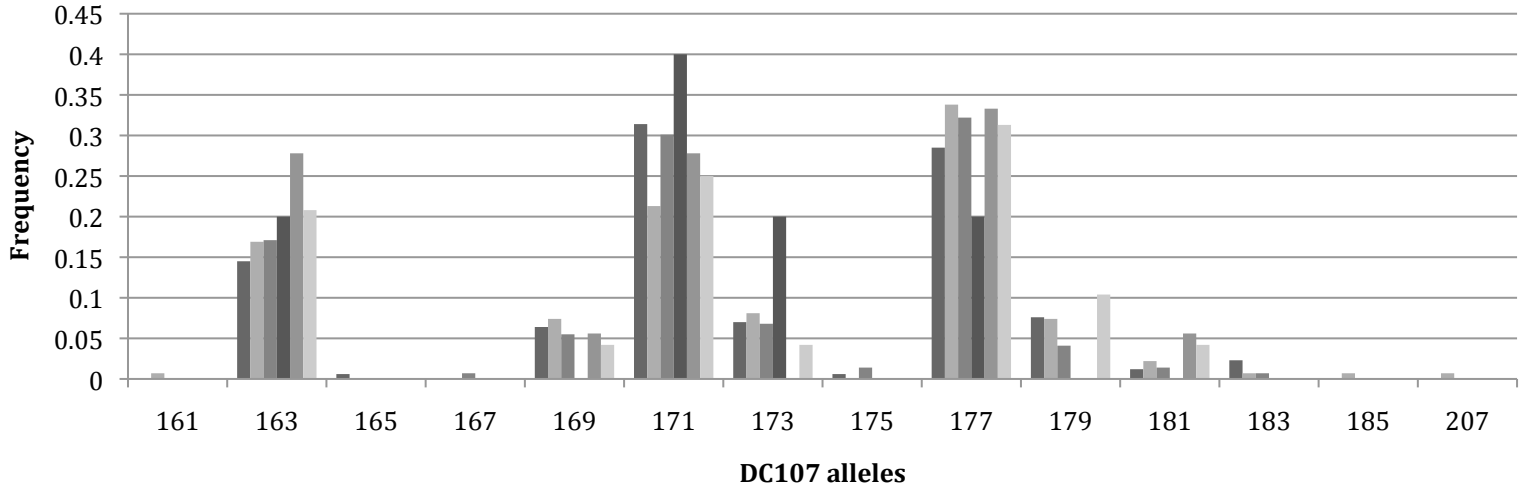
D


0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Frequency
234 236 238 240 242 244 246 248 250 252 319 321
CC117 alleles

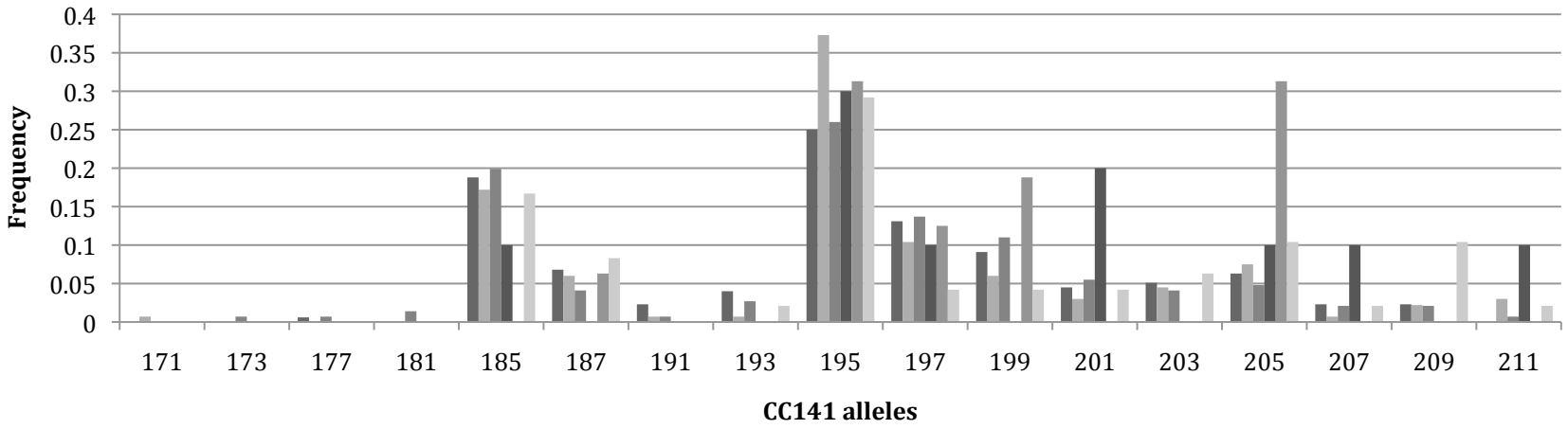
E


0.6
0.5
0.4
0.3
0.2
0.1
0
Frequency
171
181
311
317
319
321
323
327
329
331
343
347
349
CC17 alleles

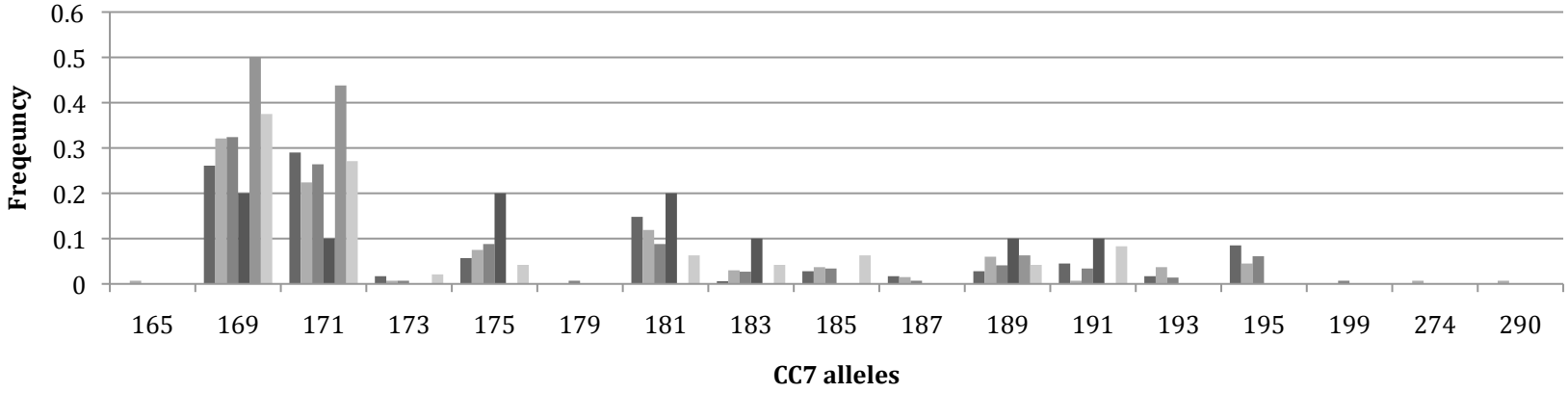
F


0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Frequency
161
163
165
167
169
171
173
175
177
179
181
183
185
207
DC107 alleles

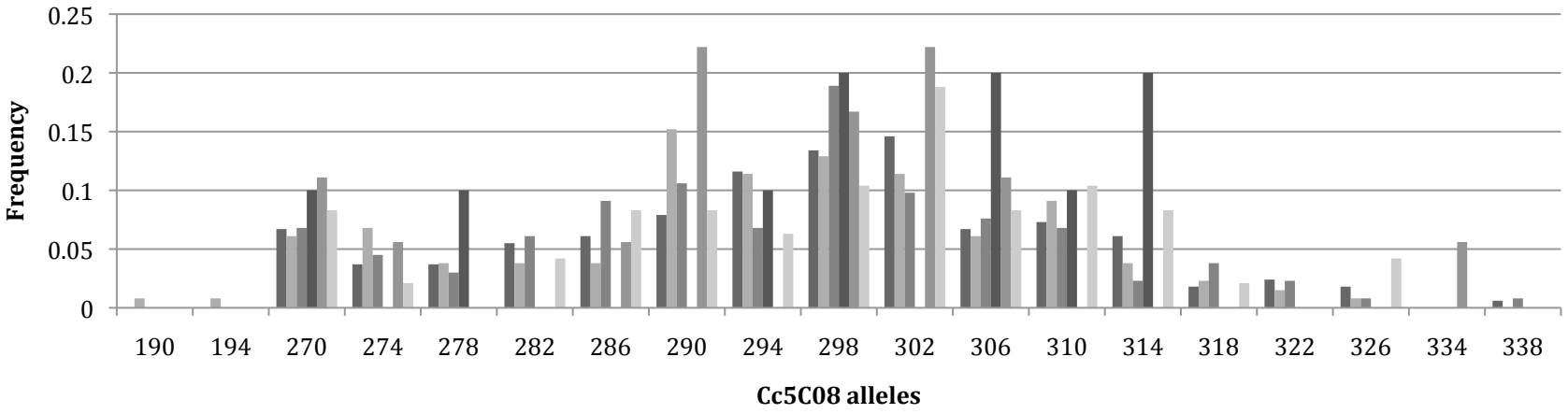
G


0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Frequency
171 173 177 181 185 187 191 193 195 197 199 201 203 205 207 209 211
CC141 alleles

H


0.6
0.5
0.4
0.3
0.2
0.1
0
Freqeuncy
165 169 171 173 175 179 181 183 185 187 189 191 193 195 199 274 290
CC7 alleles

I



J

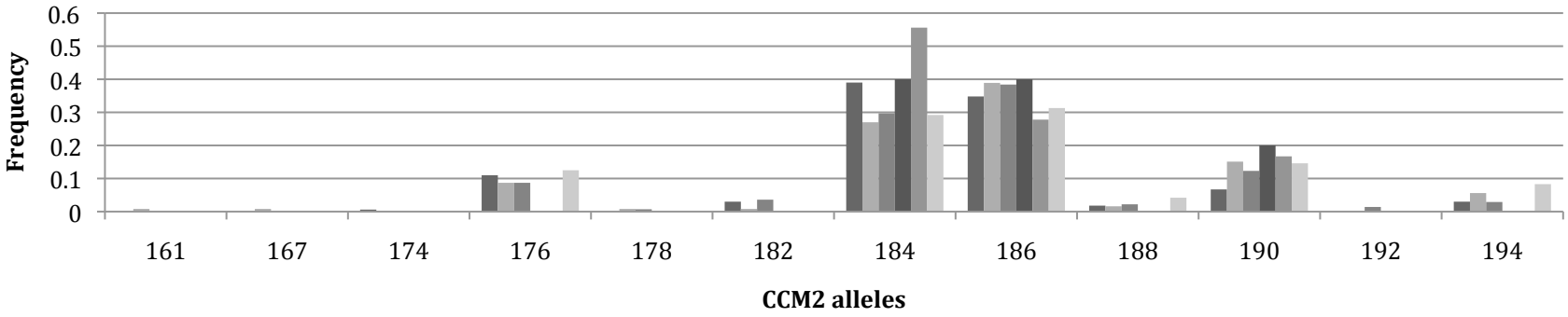


Figure 3.4. A-J: Population allele frequencies for the 10 microsatellite loci based 78 hatchlings, 91 females, and 71 males from Quintana Roo (QR) and 24 hatchlings, 5 females, and 9 males from St. George Island (SGI).

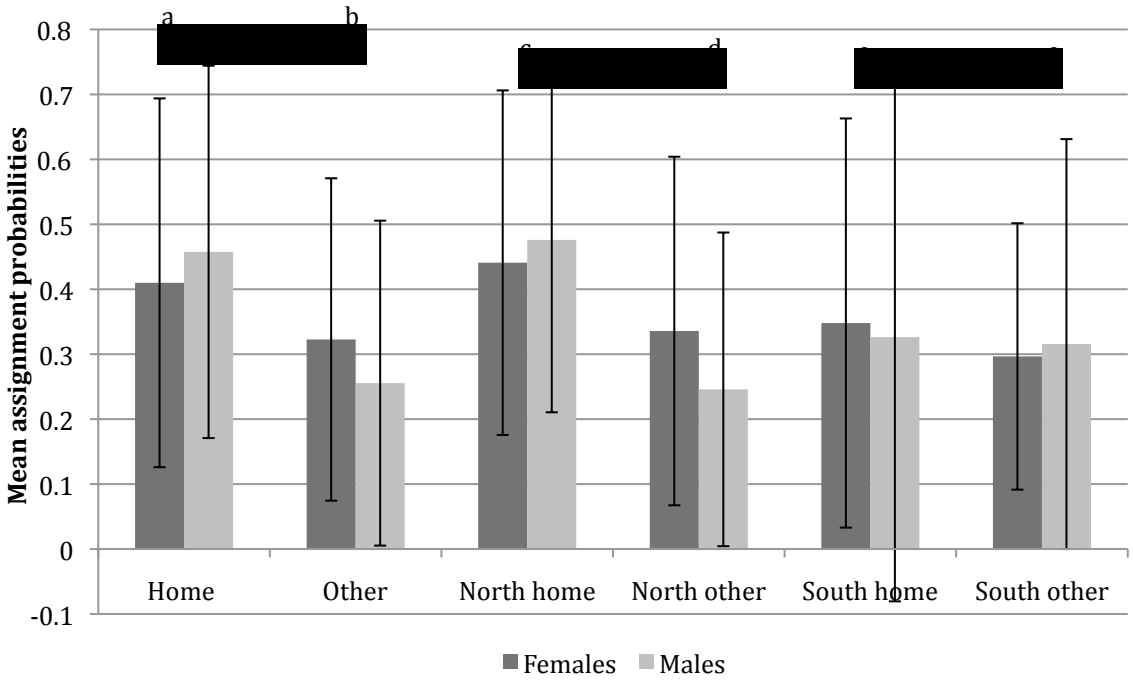


Figure 3.5. Mean assignment probabilities (± one standard deviation) for female and male genotypes for the region (north or south) in Quintana Roo where they reproduced (Home) and for the other region (Other). Within each category, there were no significant differences in female and male assignment probabilities. Brackets indicate comparisons of female-female and male-male assignment probabilities with the Wilcoxon matched pairs signed-rank test. Both females and males had on average a significantly greater probability of being assigned to their home region than to the other region (females: $n=69$, $z = -2.063$, $p = 0.0391$; males: $n = 65$, $z = -5.076$, $p < 0.0001$). When the north was analyzed separately, both females and males had a greater probability of being assigned to the north (their home region) than to the south (females: $n = 46$, $z = -2.109$, $p = 0.035$; males: $n = 57$, $z=-5.454$, $p < 0.0001$) When the south was analyzed separately, females and males were equally likely to be assigned to the south (their home region) or the north (females: $n = 23$, $z = -0.639$, $p = 0.5230$; males: $n = 8$, $z = -0.507$, $p = 0.6121$).

Chapter 4

The Mating System of Two Populations of Loggerhead Turtles.

Background

With the application of molecular techniques to study parentage in natural populations, it has become evident that females across vertebrate taxa commonly mate with multiple males and produce offspring sired by more than one male (multiple paternity) (Birkhead and Møller 1998, Birkhead 2000, Griffith et al. 2002, Avise et al. 2002, Pearse et al. 2002). Sperm storage across breeding seasons enables females to use sperm from mates from multiple seasons to fertilize offspring (Uller and Olsson 2008). Multiple paternity has been identified in all species of non-avian reptiles studied to date (Uller and Olsson 2008).

Not only is multiple paternity widespread, but it is also highly variable. Significant differences in the frequency of multiple paternity have been observed among populations within the same species, raising the question of what factors affect the frequency of multiple mating (Birkhead 2000, Pearse and Avise 2001, Ireland et al. 2003). Population parameters, climatic conditions, and seasonal variation are expected to affect mating and patterns of paternity (Uller and Olsson 2008). Population size and density have been proposed to affect mate encounter rate and the propensity for mjnultiple paternity (Ireland et al. 2003). The operational sex ratio (the ratio of breeding males to females at a given time, Emlen and Oring 1977) also has been suggested to play a role in the frequency of multiple paternity (Bollmer et al. 1999). Temperature and sunlight directly affect activity patterns, especially in reptiles, and the length of mating seasons, which further may cause variation in mating patterns (Prosser et al. 2002).

Another avenue of inquiry in mating system studies is the evolutionary significance of multiple paternity and the role of the sexes in choosing to mate more than once (Arnqvist and Nilsson 2000, Jennions and Petrie 2000). According to the theory of sexual selection, both males and females are under selection to increase their reproductive fitness and the choice to mate once or multiple times can have a significant impact on individual fitness (Birkhead 2000, Uller and Olsson 2008). Females and males may have conflicting mating strategies and a different optimal number of mates. Following Bateman's principle, males are predicted to mate as often as possible to maximize their reproductive output, due to relatively little investment in gamete production compared to females (Bateman 1948). Conversely, females are expected to mate multiple times only if the benefits outweigh the costs.

Female benefits are often divided into two groups, direct and indirect. Direct benefits refer to resources a female obtains for herself, which can include i) gifts such as food (de Waal 1995), ii) access to resources (Hunter and Davis 1998), and iii) paternal care (Darwin 1839, Faaborg et al. 1995). Indirect benefits refer to genetic benefits for the offspring, and can arise from i) genetic bet-hedging with a diversity of offspring being favored due to environmental fluctuations and changes in selection pressures (Yasui 2001), ii) 'trading up' where a female will re-mate if a subsequent male is of higher quality (Jennions and Petrie 2000), iii) sperm competition which increases the chance that good genes are passed on resulting in increased offspring viability (Fisher 1930, Madsen et al. 1992, Andersson 1994), and iv) cryptic female choice to increase genetic compatibility and inbreeding avoidance (Trivers 1972, Zeh and Zeh 1996, Tregenza and Wedell 2000). Costs of mating can include loss of time spent foraging (Rowe 1992),

transmission of disease (Loehle 1997), and physical injury inflicted by males (Miller 1997).

The reproductive biology of sea turtles, such as female promiscuity, little male investment, no parental care, and multiple seasonal clutches (Bowen and Karl 2007, Lee 2008), makes them a useful system in which to study variations in the frequency of multiple paternity and what benefits, if any, females may gain from mating multiply (Lee and Hays 2004). Both males and females are promiscuous breeders based on observations in courtship areas (Miller 1997, FitzSimmons 1998, Hamann et al. 2003) and six out of the seven species studied are genetically polygynous as evidenced by multiple paternity of clutches (Table 4.1). The frequency of multiple paternity documented in sea turtle species ranges from 0-93% of clutches that have multiple paternity. Among populations within species, the greatest range of multiple paternity is observed in the loggerhead turtle. The frequency of multiple paternity in this species ranges from 31% in Melbourne on the east coast of Florida (Moore and Ball 2002) to 93% on the Greek island of Zakynthos in the Mediterranean (Zbinden et al. 2007).

To date, there only has been two studies of multiple paternity in loggerhead turtles with a sample size greater than 10, and with high-resolution markers, such as DNA-based markers like microsatellites (Moore and Ball 2001, Zbinden et al. 2007). Two other studies have been conducted, one with a sample size of three (Bollmer et al. 1999), and one that used allozymes (Harry and Briscoe 1988). The small sample size of one and the low resolution of the marker of the other make their findings difficult to directly compare to studies with larger sample sizes and markers with higher resolution. Analyses of

additional populations are necessary in order to elucidate trends and relationships with population parameters that may be influencing the mating system.

This study assesses how several population parameters are related to multiple paternity and if they may be able to predict the frequency of multiple paternity. To accomplish this goal, I selected two genetically discrete populations, that varied in population size and density of nesting in order to provide suitable counterparts to the previous studies. Additionally, I look at multiple paternity from the female perspective in order to add to the scarce literature on what benefits female sea turtles may gain from multiple matings. Because direct observation is difficult, little is known about the timing of mating and the consequences for male migratory behavior. To the extent possible, I infer such information from the following genetic parentage analysis.

I tested four hypotheses using polymorphic microsatellite markers to characterize the pattern of multiple paternity in two genetically discrete populations (genetic stocks) of loggerhead turtles, one nesting in the Yucatan Peninsula in Mexico and the other in northwestern Florida. Yucatan Peninsula stock is an "intermediate"-sized nesting population (Ehrhart et al. 2003). Mitochondrial DNA (mtDNA) studies have shown that this population harbors the highest amount of genetic diversity of Atlantic loggerheads (Encalada et al. 1998, Bowen et al. 2005). The region's postulated history of providing refugia for loggerhead turtles during glacial maxima is thought to be behind this high level of diversity. Local communities in the region have fished for sea turtles since pre-Hispanic times, but with rapid population growth, development of the tourism industry and increased commercial demand for sea turtle products in the 1950s, the abundance of loggerhead turtles, and other sea turtles in the area, was likely dramatically reduced

(Hildebrand 1987). With increased protection and conservation since the late 1970s both in Mexico and internationally (Garduño-Andrade et al. 1999), the population of loggerheads probably has experienced a partial recovery as seen in the increase in nest numbers over the past 20 years (Flora, Fauna y Cultura de Mexico, A.C. 2008). Despite this bottleneck the population has been through, genetic diversity has remained high.

Northwestern Florida is host to a "minor" genetic stock with scarce nesting throughout the panhandle region (Ehrhart et al. 2003). This population is characterized by lower mtDNA diversity than is observed in the Mexican stock (Encalada et al. 1998, Bowen et al. 2005, also see Chapter 2), likely due to its more recent colonization history and possible founder effects (Bowen et al. 1993, Bowen and Karl 2007). Perhaps not surprisingly, the small northwestern Florida stock has been overshadowed by its massive neighbor, the southern Florida genetic stock which is one of the world's two largest nesting populations (Ehrhart et al. 2003, Baldwin et al. 2003). Monitoring programs that were implemented on beaches throughout the state in 1989 were not applied to nesting beaches in northwestern Florida until almost 10 years later (Witherington et al. 2009). The trend in nesting since standardized monitoring began has been decreasing (Florida Fish and Wildlife Conservation Commission *unpublished data*).

The mating system of loggerheads may play a role in their ability to recover from exploitation (Bell et al. 2010). Factors such as multiple paternity and male-mediated gene flow may mitigate the effects of genetic drift due to small population size. The overlap of genetic stocks in foraging areas may increase mate availability and buffer potential Allee effects, but biased sex ratios may reduce the ability to find a mate (berec et al. 2001 in bell). An analysis of the mating system of the Mexican and northwestern

Florida stocks, which differ in their (recent) evolutionary histories, genetic composition, and in their current population sizes and trajectories, will help us understand how the mating system is affected by different population parameters.

First, I tested the hypothesis that the frequency of multiple paternity is associated with the abundance of females. I predicted that the frequency of multiple paternity is positively associated with female abundance. The effect of population abundance on levels of multiple paternity has been demonstrated in various taxa, including birds (Griffith et al. 2002), small mammals (Dean et al. 2006, Bryja et al. 2008), and fish (Soucy and Travis 2003). All else being equal, in populations with high abundance, females should encounter potential mates at a higher rate than in populations with low abundance (Uller and Ollson 2008). Alternatively, in species with high territoriality or rigid social hierarchies, when abundance is high, dominant males may be able to control and prevent copulations by inferior males, thus decreasing the mate encounter rate for females and as a result the frequency of multiple paternity (Bronson 1979). The former scenario likely is more applicable to sea turtles because they do not exhibit social hierarchy nor territoriality (Pearse and Avise 2001). Female abundance (instead of overall population abundance because males are difficult to survey), was first introduced as a potential covariate of multiple paternity in sea turtles by Ireland et al. (2003), and its relationship with multiple paternity has been investigated in subsequent studies (Jensen et al. 2006, Zbinden et al. 2007). Jensen et al. (2006) found female abundance to be significantly correlated with the frequency of multiple paternity in ridley sea turtles (*Lepidochelys* spp.) based on four populations, and an increasing trend of multiple paternity with female abundance across all species of sea turtles. Zbinden et al. (2007)

found that loggerheads did not fit this pattern, but only based on data from two populations in which the studies employed microsatellite markers (Melbourne, FL: Moore and Ball 2002, Greece: Zbinden et al. 2007, and one study that used allozyme markers (Australia: Harry and Briscoe 1988). The correlation between female abundance and the frequency of multiple paternity can better be evaluated with data from additional populations.

Also, I predicted that the frequency of multiple paternity is positively associated with the density of nests. At higher female abundances, the density of the resultant nests on the beach should be higher compared to areas with a lower abundance of females. Nest density has not been analyzed in relationship to multiple paternity, and it may prove to be a good predictor.

Second, I hypothesized that the operational sex ratio is associated with the frequency of multiple paternity. Here I define operational sex ratio as the ratio of reproductively successful males to females, which is slightly different from the original definition by Emlen and Oring (1977) due to the inability to account for reproductively active but unsuccessful individuals in this system. I predicted that the more the operational sex ratio is skewed towards females, the higher the frequency of multiple paternity. The operational sex ratio also has been called upon to explain variation in multiple paternity in sea turtles (FitzSimmons 1998, Bollmer et al. 1999). However, hypotheses regarding the operational sex ratio have not been tested in any species of sea turtle because the number of breeding males is unknown. I used a novel approach to estimate the operational sex ratio from paternity analyses of clutches. I reconstructed paternal genotypes based on hatchling genotypes (and maternal genotypes when

available), and used the number of unique male genotypes identified and the number of different females' clutches analyzed to estimate the ratio of reproductively successful males to females. Males, who remain at sea throughout their lives, are much more difficult to census than females who are readily observed as they come ashore to nest (Bollmer et al. 1999, Moore and Ball 2002). When the operational sex ratio is skewed towards males, competition among males is expected to increase. This is costly in terms of time and energy and may decrease male mating success (Emlen and Oring 1977, Jirotkul 1999). Aggressive and dominant behavior has been observed in male green turtles, such as biting and ramming mounted males to dislodge them from females (Hendrickson 1958, Booth and Peters 1972, Bustard 1972). In a green turtle courtship area where males outnumber females (Booth and Peters 1972, Limpus 1993), but the actual ratio is unknown, the frequency of multiple paternity was found to be very low at only nine percent (FitzSimmons 1998). The sex ratio in an olive ridley courtship area appeared to be highly biased towards females, and almost all clutches (92%) in this nesting population had multiple paternity (Jensen 2006).

Third, I tested the hypothesis that multiple paternity of offspring confers greater fitness to mothers compared to single paternity. I predicted that females who are fertilized by multiple males will have greater reproductive success than females who mate with a single male. While female reproductive success is best measured by tracking individual females' fecundity over time as well as the long-term survival and eventual reproductive success of offspring, I used more immediate indicators of success using clutch and hatchling characteristics, such as number of clutches laid in a season by each, number of eggs per clutch, proportion of eggs hatching, and hatchling weight. Because

there is no post-hatching care, except for yolk reserves used by hatchlings after emergence, to maximize reproductive success maternal investment is limited to optimizing the number and size of eggs/neonates and the number of clutches per reproductive season (Congdon 1989).

The effects of multiple paternity on female reproductive success continue to be debated. Few studies have investigated female benefits to multiple paternity in turtles (but see Pearse et al. 2002, Lee and Hays 2004), and none have thoroughly tested it in loggerhead turtles. In this type of promiscuous mating system where males contribute little, the selection on males to mate multiply is expected to be stronger than in a mating system where males provide resources such as paternal care (Bateman 1948, Andersson 1994, FitzSimmons 1998). However, because males of fully aquatic turtle species do not appear to be able to force copulation (Berry and Shine 1980), it commonly has been hypothesized, without empirical evidence, that females benefit in some way (FitzSimmons 1998, Bollmer et al. 1999, Moore and Ball 2002). Because males do not defend territories, form social bonds, or care for their offspring (Pearse and Avise 2001), female sea turtles are not likely to profit from direct benefits. But all of the indirect benefits are likely relevant to this promiscuous breeding system. Multiply-mated females may be able to better minimize the effects of inbreeding and genetic incompatibility and may produce hatchlings with increased viability through sperm competition. Evidence across taxa suggests that post-copulatory female choice and sperm competition may operate to bias paternity to produce offspring with greater fitness (Zeh and Zeh 1996, Jennions and Petrie 2000).

Fourth, I hypothesized that females mate prior to all nesting activity and not in between nesting events. I predicted that paternity would be the same across multiple clutches laid by a single female within a season. A female loggerhead can lay up to seven clutches of eggs in a season (Schroeder et al. 2003), and all eggs can be fertilized by sperm received during mating, with one or multiple males, prior to nesting, or during mating between clutch laying. Because sea turtles do not display pair bonds (Pearse and Avise 2001), it is unlikely that a female will mate sequentially with the same male over the course of a season.

While evidence in support of female loggerheads benefiting from multiple matings is scarce, so is the evidence regarding the timing of the mating events. Harry and Briscoe (1988) found evidence from two successive clutches from a single female loggerhead that inter-nesting mating may occur. However, as the authors pointed out, the appearance of a third male siring only three offspring (out of a sample size of 20) in the latter of the two clutches could also indicate differential use of sperm to fertilize separate clutches. In addition, it is possible this third male went undetected in the earlier clutch simply due to incomplete sampling. While differential sperm usage and sampling bias cannot be ruled out in the event of disparate paternity across clutches, the same paternity across clutches nevertheless will indicate that females mated prior to nesting. By sampling at least four clutches laid by a given female (compared to only two in the study by Harry and Briscoe (1988)), I will increase the opportunity to detect the effects of differential sperm usage across multiple clutches as well as minimize sampling bias.

Examination of the paternal distribution in sequential clutches (with multiple paternity) laid by an individual female will be informative as to whether females mate

exclusively prior to nesting or in between oviposition cycles. Male green turtles have been observed to leave the breeding area prior to the onset of nesting (Limpus 1993), a behavior compatible with a first-male sperm hypothesis. Last-male sperm precedence is expected to select for males to mate with females throughout the nesting season as they continue to ovulate new eggs (FitzSimmons 1998). Thus, the timing of mating events will disclose information about the little-known migratory behavior of male loggerheads.

Methods

Study sites

The loggerhead rookeries in Quintana Roo (QR), Mexico (20°33'N, 87°38'W; Figure 4.1) belongs to the Yucatan Peninsula genetic stock, as defined by Encalada et al. (1998). Approximately 575 females nest each season along the coast of the Yucatan Peninsula (2300 nests/yr with an average of four nests per female per season; Ehrhart et al. 2003). Every nesting season, beaches where sampling took place are patrolled nightly, where nearly all nesting turtles are observed, tagged, and morhpometric data are gathered. This allows each female and her nests to be tracked throughout the season. Because tagging started in 1987, females that arrive on the beach without a tag and are tagged for the first time are presumed to be first-time nesters, or neophytes (I. Iturbe *personal communication*). Nests deemed in danger of inundation or of being dug up by other sea turtles nesting on the same beaches in high densities, are relocated to either above the high tide line in the same area of the beach, or to a nearby hatchery. The remainder of nests are left *in situ*.

During the 2006 nesting season (May – September), I collected samples from 58 females and from a single clutch from each of 37 of these females. Also, I sampled a clutch with an unknown mother. Additionally, successive clutches from three of the sampled females were sampled (four, four and six clutches, respectively). Fourteen of the sampled females were considered to be neophytes.

The loggerhead rookeries on St. George Island (SGI), Florida (29°68N, 84°80W; Figure 4.1) belong to the northwestern Florida genetic stock, as defined by Encalada et al. (1998). Approximately 150 females nest in northwestern Florida each season (600 nests/yr with an average of four nests per female per season; Ehrhart et al. 2003). Beaches on SGI are monitored each morning and new nests are marked and tracked throughout the season. Nesting females are not tagged and all nests are left *in situ*. This means individual females cannot be tracked and their respective nests cannot be differentiated from nests laid by other females. The typical inter-nesting interval lasts 13 to 14 days (Broderick et al. 2002, Schroeder et al. 2003), but it can be shorter (10 days) especially when the water is warmer (Hays et al. 2002). For this reason, I sampled nests laid within a 10-day interval to minimize pseudoreplication. I selected the 10-day window that had the highest number of nests to maximize my sample size.

I collected samples from clutches during the 2007 and 2008 nesting seasons (May – September) on SGI. During the 2007 nesting season (May – August), I collected samples from 11 clutches during a 10-day window (June 14-23). In the 2008 season, I sampled a subset of hatchlings from another 11 clutches on SGI laid within a 10-day window (June 24 - July 3). In addition, I sampled one hatchling from each of two other nests laid within the sampling window in 2008. The chances of sampling nests laid by the

same females in 2007 and 2008 were minimal as the remigration interval for female loggerheads averages two and a half to three years (Schroeder et al. 2003).

Field methods

I sampled nesting females using 3 or 6 mm sterile disposable biopsy punches and biopsying a skin plug along the posterior edge of the foreflipper immediately after the eggs had been laid. Skin plugs were stored in 1.5 ml vials with a 1-ml solution of 20% DMSO and 6M NaCl.

I tracked nests throughout the incubation period. Forty-five days after deposition, the shortest incubation time at the maximum temperature for successful embryonic development (Miller et al. 2003), I placed a mesh net around the nest to retain hatchlings upon emergence, which typically happens around 50 to 65 days after oviposition. Caged nests were monitored hourly throughout the night to minimize the duration of captivity and risk of predation by birds, crabs, raccoons, dogs, and in QR, coatis and poachers as well. In QR, caged nests were also monitored throughout the day, although day emergences were rare. In SGI, mesh nets were removed at dawn and replaced at dusk, according to Florida Fish and Wildlife guidelines. Upon emergence, I randomly selected approximately 20 hatchlings from each nest. Dead hatchlings and unhatched embryos were also sampled by cutting a small piece of tissue and storing in 1.5 ml vials with a 1-ml solution of 20% DMSO and 6M NaCl. Each nest was then evaluated for clutch size and hatching success.

I weighed live hatchlings with a spring scale (Pesola). After weighing, I drew 0.1 cc blood using a ½ cc 28-gauge disposable insulin syringe (Kendall) from the dorsal

cervical sinus following the method of Owens and Ruiz (1980). Blood was stored in 1 ml of Longmire's lysis buffer (Longmire et al. 1992). I released hatchlings at the nest site immediately after sampling, or, if hatchlings emerged at dawn, I released them the following night to prevent release during the day when predation risks were greater.

All procedures were approved by University of Miami Institutional Animal Care and Use Committee # 07-114. Samples in Mexico were collected under permit # 07656 issued by Secretaría de Medio Ambiente y Recursos Naturales, and in Florida under marine turtle permit # 189 issued by Florida Wildlife Conservation Commission. Mexican samples were imported into the United States under CITES permit # 124476.

DNA extraction, amplification, and genotyping

I extracted DNA from both skin plugs and blood using a standard ethanol precipitation protocol. I selected 10 microsatellite loci from the literature (Table 4.2) that comprised the loci used by previous loggerhead studies (Moore and Ball 2002, Zbinden et al. 2007) to facilitate a direct comparison of multiple paternity among populations I amplified the 10 microsatellite loci in two multiplex polymerase chain reactions (PCR) (Saiki et al. 1988), grouped by annealing temperature (T_A). I optimized annealing temperatures (T_A) on an Eppendorf Mastercycler Gradient PCR cycler (Eppendorf AG) using a temperature range that spanned published T_A for each primer. All loci amplified at either 56°C or 60°C (Table 4.2). Primer concentrations were optimized by adjusting the amount of primer added such that the electrophoretic peaks of all loci in a multiplex reaction were of similar height (Table 4.2). Each 10 µl reaction consisted of ca. 50 ng template DNA, 0.2 µM dNTP's, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.08-0.32 µM of each

primer pair, and 0.5 unit Taq DNA polymerase (Promega Corporation). PCR cycling conditions on the Eppendorf Mastercycler Gradient PCR cycler (Eppendorf AG) consisted of an initial denaturation step at 95°C for 3 min followed by six cycles of denaturation at 92°C for 30 sec, annealing at 56°C or 60°C for 55 sec (see Table 4.2 for T_A), and extension at 72°C for 1 min 25 sec, followed by 30 cycles of denaturation at 89°C for 30 sec, annealing at T_A -2°C (either 54°C or 58°C) for 55 sec, extension at 72°C for 1 min 25 sec, followed by a final extension at 72°C for 10 min (Bowen et al. 2005).

Amplified fragments were resolved on an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, California). Alleles were sized using the software STRAND (Toonen and Hughes 2001), initially using the automated scoring option, and subsequently each electrophoretic pattern was visually inspected to maximize accurate allele-calling in the presence of stutter and low-height alleles, as recommended (Dewoody et al. 2006).

Characterization of microsatellite loci

Population allele frequencies for QR and SGI were based on either adult females and hatchlings assumed to unrelated, i.e. no full or half siblings or mother-offspring samples. The two sampling years in SGI, 2007 and 2008, were tested for population structure by calculating F_{ST} in the program ARLEQUIN version 3.5 (Excoffier and Lischer 2010). Absence of structure would allow the two sampling years to be grouped and regarded as one population for analysis.

Allele frequencies, allelic diversity, observed and expected heterozygosity, and deviations from Hardy-Weinberg equilibrium (HWE) were calculated for each population

using ARLEQUIN. Deviations from HWE often cause an excess of homozygotes which can arise from inbreeding, selection (while microsatellite loci themselves are assumed to be neutral, they may be linked to markers that are under selection), or sub-structuring within the sampling site (Wahlund effect) (Selkoe and Toonen 2006). However, the presence of null alleles, large allele drop-out, and mis-scoring of alleles due to stutter also can result in an overestimate of homozygotes that would not be caused by a biological phenomenon (Dewoody et al. 2006). I used MICRO-CHECKER (van Oosterhout et al. 2004) to assess the presence of these three types of scoring errors. MICRO-CHECKER is able to distinguish between scoring errors and actual biological processes, because each type of scoring error leaves its own allelic "signature" at the affected loci (van Oosterhout et al. 2004, Dewoody et al. 2006).

I also tested for linkage disequilibrium (LD) in the datasets with ARLEQUIN. While microsatellite loci are assumed to be neutral and thus most likely not functionally linked, they can be clustered together, which may cause them not to assort independently (Bachtrog et al. 1999, Selkoe and Toonen 2006). Methods of parentage assignment assume independence of loci, and using linked loci to infer relationships will introduce a statistical pseudoreplication problem and inflate the confidence (Jones and Wang 2010b). For genotypic data where the phase is unknown, a likelihood ratio test is performed to test for LD (Excoffier and Slatkin 1998*)*. An expectation-maximization (EM) algorithm (Dempster et al. 1977, Excoffier and Slatkin 1995, Weir 1996) is used to estimate haplotype frequencies, followed by a permutation procedure to estimate the distribution of the likelihood ratio statistic, which follows a chi square distribution. LD was

calculated over 10,000 permutations and a starting value of five random initial conditions from which the EM algorithm starts to repeatedly estimate the sample likelihood.

The probability of identity (Paetkau et al. 1995) and probability of exclusion (Weir 1996) are measures of the efficiency and power of the marker set for paternity assignment. The probability of identity measures the probability of two individuals having identical genotypes. The probability of exclusion measures the probability of excluding an incorrect paternal genotype. Identity and exclusion probabilities were calculated for each locus individually and combined using the program IDENTITY (Wagner and Sefc 1999). I also calculated the probabilities of identity and exclusion for the marker sets used in prior loggerhead studies (Moore and Ball 2002, Zbinden et al. 2007).

The probability of detecting multiple paternity when present in the sample set was calculated in PRDM (Neff and Pritcher 2001). As the number of loci and alleles increases, so does the probability of detecting multiple paternity. However, paternal skew reduces the probability of detecting multiple paternity. I simulated various mating scenarios with two, three, and five fathers with equal and skewed contributions, for a range of sample sizes.

Paternity analyses

I used three different methods to assess paternity as implemented by the programs COLONY (Jones and Wang 2010a), DADSHARE (written by W. Amos, available at http://www.zoo.cam.ac.uk/zoostaff/amos/), and GERUD version 2.0 (Jones 2005).

COLONY uses a group-likelihood approach, in which the multilocus genotype of all individuals is used to determine relationships. This information is ignored in pairwise methods (as in DADSHARE and GERUD below). COLONY accounts for genotyping errors (null alleles, mutations, and mis-scoring of alleles) using user-specified error rates for each locus. I calculated the error as the sum of the null allele rate, as calculated in MICRO-CHECKER, and the mutation rate, which I scored in the following way: the total number of mutations defined as a novel allele at only one locus in only one offspring per clutch, divided by the total number of alleles at a given locus. This classification of mutations is consistent with other authors (FitzSimmons 1998, Moore and Ball 2002, Lee and Hays 2004, Zbinden et al. 2007). Loci that had an error rate of zero were given an error rate of 0.0001 to allow for unidentified mutations in the offspring. Population allele frequencies, needed to run the analysis, were calculated in ARLEQUIN. Alleles that only were present in the offspring, and not in the background population, were added to the population alleles, as required by the program, at a low frequency of 0.0001. Following a maximum likelihood framework, COLONY uses the population allele frequencies, the error rate, and any known relationships (e.g. mother-offspring, sibling groups) to determine the most likely full and half sib families and parental genotypes. The program reconstructs paternal genotypes and assigns individual offspring to full sib groups. To ensure samples from SGI in 2007 and 2008 were not from the same female, I analyzed the clutches with and without entering maternal sibling group information.

DADSHARE assesses the number of fathers of groups of offspring, with either known or unknown maternal genotypes. Population allele frequencies are input into the program, which uses these frequencies to account for the likelihood that a male is

heterozygous or homozygous at any given locus. After determining parental alleles, the program builds a relatedness matrix based on the paternal alleles (using the methods of Queller and Goodnight (1989)), without actually reconstructing paternal genotypes. Using the relatedness values, the program builds a dendrogram using the unweighted pair group method with arithmetic mean (UPGMA). The UPGMA algorithm identifies relationships among the operational taxonomic units (OTUs), in this case offspring, in order of similarity. First the two most similar OTUs are identified and grouped, and subsequently treated as a new OTU. Then the next pair of OTUs with the highest similarity are grouped. In this way, a dendrogram is built by sequentially clustering the most closely related offspring. DADSHARE does not search the tree exhaustively, but follows the branching order to identify clusters of offspring that are compatible with having a single father. Thus, the order in which the offspring are entered may affect the results. For this reason, I ran the analysis at least twice for each clutch, changing the order of offspring each time. If the results were different, I reordered the offspring and ran it again, and I used the consensus results for my subsequent analyses. The program does not specifically account for mutations, but because full sib groups are based on relatedness, the effect of a mutation at one locus will be averaged out across at all loci, and thus will not necessarily affect the outcome.

GERUD determines the minimum number of fathers necessary to explain a progeny array given that all offspring are full or half sibs. The program starts by determining all possible maternal genotypes, or, if provided, by checking that the maternal genotype is consistent with the offspring. The absence of a known maternal genotype has a negligible effect on the ability of the program to obtain the same solution

as when the maternal genotype is known (Jones 2005). The program then identifies all paternal alleles by subtracting the maternal alleles from the offspring genotypes. An exhaustive search then reconstructs all possible combinations of parental genotypes (or paternal if maternal genotype is known) that can explain the genotypes of the offspring and determines the minimum number of fathers (maximum six) and the distribution of offspring among the fathers. However, offspring are assigned to all fathers with which they are compatible, which means that the output may include more offspring than the original input. Parental genotype (or paternal if maternal genotype is known) combinations are ranked by likelihood based on Mendelian segregation of alleles. However, many combinations can be possible with little difference in likelihood scores. GERUD does not account for mutations. Mutations are treated as true alleles and cause the program to assign additional fathers. Thus, potential mutations were scored as described above. Mutant paternal alleles were binned with the allele closest in size so as to retain as much information as possible. A decrease in the number of fathers after re-analysis lent support to the classification of the allele as a mutation. If this procedure did not reduce the number of fathers, I assumed that the allele was not, after all, a mutation. Maternal mutations would not allow the program to run as the maternal genotype then would not be consistent with the offspring genotypes. As with paternal mutations, I grouped such mutations with the maternal allele closest to its size and reran the analysis. Missing data were not accepted so offspring with missing data were excluded. Due to memory constraints, GERUD can handle no more than five loci at a time. Thus, I divided my loci into groups and afterwards combined the results. The highest number of minimum fathers was used as the overall minimum number of fathers for a given clutch.

The use of multiple approaches to paternity assignment increases the robustness and confidence of the estimate (Lee 2008). Clutches were assigned as having multiple paternity based on consensus: at least two of the three programs agreed. In clutches with multiple paternity, if there was a consensus in the number of fathers between at least two of the programs, this consensus was used. Otherwise, the number of fathers was estimated by averaging the number indicated by each program showing multiple paternity.

For each multiply-sired clutch, the proportion of offspring sired by each father was also determined by consensus. However, if the programs detected the same number of fathers, but the assignment of individuals could not be resolved, I excluded these clutches from the analysis of paternal contributions. Primary fathers of multiply-sired clutches were divided into two groups, those that sired the majority of the clutch, and those that sired half of the clutch. Deviation from equality was evaluated with X^2 in SPSS version 17 (IBM, Chicago, Illinois).

For comparison, I used the marker sets used by published loggerhead studies (Moore and Ball 2002; Zbinden et al. 2007) to determine paternity in QR and SGI. Moore and Ball (2002) used four markers (Ccar176, CC141, CC7, and CCM2), and assigned multiple paternity to a clutch if three or more paternal alleles were at more than one locus. Zbinden et al. (2007) also used four markers (CC117, CC141, CC7 and CCM2) and assigned paternity with the program GERUD. Similar to Moore and Ball (2002), the presence of one extra (a third) paternal allele at only one locus in one hatchling was classified as a mutation prior to analysis in GERUD. These two methods of assigning paternity are thus very similar when it comes to assigning either single or

multiple paternity. I ran my data for these two marker sets in GERUD to facilitate a direct comparison of the frequency of multiple paternity of loggerheads in Melbourne, Florida (Moore and Ball 2002) and Zakynthos, Greece (Zbinden et al. 2007).

The proportion of clutches with single and multiple paternity in each sampling year in SGI were tested for equality with Fisher's exact test in SPSS to determine if the years could be grouped.

Abundance and density

I ran bivariate correlations in SPSS to test the hypotheses that female abundance or nest density was associated with the frequency of multiple paternity. In addition to the results of this study from QR and SGI, I used data from published studies to run the correlations with a total of five data points: Mon Repos, Australia (Harry and Briscoe 1988); Melbourne, FL (Moore and Ball 2002); and Zakynthos, Greece (Zbinden et al. 2007). I also ran correlations using the frequency of multiple paternity for QR and SGI obtained with the marker sets used by Moore and Ball (2002) and Zbinden et al. (2007) for comparison.

Female abundance was determined in two ways. Both methods rely on nest counts which are divided by four, the average number of nests per season for female loggerheads (Ehrhart et al. 2003). In the first method, the number of females was based on nest numbers in a geographic area within the larger area covered by the genetic stock. In some cases, the extent of this area was delineated by county lines (e.g. in Florida), which is not necessarily a biologically meaningful division of the nesting habitat. This method has been used previously to relate the frequency of multiple paternity to female

abundance (Ireland et al. 2003, Jensen et al. 2006). I will refer to this as the rookery size. Despite disagreeing with the use of this delineation for female abundance because it is not based on any biologically meaningful division, but an arbitrary one, I used this method for consistency to compare the multiple paternity results from QR and SGI to published results from other localities.

To calculate female rookery size in QR, I used 2006 nest counts from the beaches where my sampling took place (Flora, Fauna y Cultura de Mexico A.C. 2006). To calculate the female rookery size in SGI, I used nest counts averaged over 2007 and 2008 from Franklin County, Florida (Florida Fish and Wildlife Conservation Commission *unpublished data*). Female rookery size for Mon Repos, Australia was based on Mon Repos nest counts (Limpus and Limpus 2003), Melbourne, FL rookery size was based on Brevard County, Florida nest counts (Bagley et al. 1996), and Zakynthos, Greece rookery size was based on nests counts from Zakynthos Island (Margaritoulis et al. 2003).

In the second method of calculating female abundance, I used nest numbers from the entire genetic stock, which seems more biologically meaningful than using only an arbitrary subset of the region used by the genetic stock. I will refer to this as the genetic stock size. Female genetic stock sizes for the Yucatan Peninsula stock (QR), and southern Florida (Melbourne) were obtained from Ehrhart et al. (2003), and northwestern Florida (SGI) from Florida Fish and Wildlife Conservation Commission. The eastern Australia (Mon Repos) genetic stock size was obtained from Limpus and Limpus (2003), and the Mediterranean genetic stock size (Zakynthos) from Margaritoulis et al. (2003).

Nest density for each of the five locations was calculated by using nest count data available for sampling beaches within each study area and dividing by the combined

length of beaches. Nest counts and beach length data were obtained from the same sources used for rookery size determination.

Operational sex ratio

I determined the operational sex ratios in QR and SGI as the number of males detected in each population by the program COLONY to the number of females whose clutches were analyzed. To test the hypothesis that the operational sex ratio is associated with the frequency of multiple paternity, first I used Fisher's exact test to determine if the operational sex ratios in QR and SGI were different from one another. Then I determined if the frequency of multiple paternity was different in the two populations, also using Fisher's exact test. My prediction would be supported if the population with a greater proportion of females also had a higher frequency of multiple paternity.

Reproductive success

In testing the hypothesis of increased reproductive success in clutches with multiple paternity compared to clutches with single paternity, I used the following variables as indicators of reproductive success: clutch size (number of eggs in a clutch), hatching success (proportion of eggs that successfully hatched a live hatchling, whether or not the hatchling emerged from the nest successfully), hatchling weight (the average weight of hatchlings in a clutch), and clutch frequency (the number of clutches laid by a female throughout one season). Because hatching success and hatchling weight could be affected by nest relocation, I tested for differences in these variables between *in situ* and relocated nests with the Mann-Whitney *U* test in SPPS. Clutch frequency data were only

available for QR. I assumed that each of these variables is positively correlated with reproductive success.

I used the Mann-Whitney *U* test to test for differences in the indicators of reproductive success between years in SGI to ensure the sampling years could be grouped. I also tested for differences in these reproductive success variables between SGI and QR to determine if any of the variables could be grouped across populations.

Using the Mann-Whitney *U* test, I tested the effects of potentially confounding variables on paternity. These included the experience of the female (neophyte or remigrant), maternal traits such as the size of the female (curved carapace length and curved carapace width), the date of a female's first clutch (grouped into one-week intervals starting with May 7), as well as effects of the sampling scheme which included the number of offspring analyzed (which directly affects the ability to detect multiple paternity) and the clutch number sampled (e.g. a female's first clutch or third clutch). Data pertaining to females are available for QR only.

Finally, I tested whether there were differences in reproductive success between clutches with single and multiple paternity with the Mann-Whitney *U* test to test the hypothesis that multiple paternity conferred reproductive benefits to females. I also tested whether there were differences in reproductive success of multiple paternity clutches between the two types of primary father to determine if the type of primary father had an effect on reproductive success.

Results

Characterization of microsatellite loci

Population structure and genetic diversity indices were based on 58 females from QR and 25 individuals assumed to be unrelated from SGI (one hatchling per clutch as well as the female from Alligator Point).

I found no structure between the sampling years 2007 and 2008 in SGI (F_{ST} = -.00186, p = 0.59) and therefore I grouped the two years and regarded them as a single population for subsequent analyses. QR and SGI were significantly structured (F_{ST} = 0.00745, p = 0.027).

All 10 microsatellite markers were highly polymorphic with between seven and 28 alleles in QR (mean = 13.9) and between six and 22 alleles in SGI (mean = 11.5) (Table 4.3). In both populations CCM2 was the least polymorphic and Cc5F01 was the most polymorphic. Allele frequencies for each locus were skewed and varied between the populations (Figure 4.2).

Six loci (Ccar176, Cc2G10, Cc5F01, CC117, CC17, and DC107) deviated from HWE in QR and only one (Cc5F01) in SGI, indicating a homozygote excess at these loci (Table 4.3). After Bonferroni correction was applied, two loci from QR (Ccar176 and DC107) were no longer considered to significantly deviate from HWE. Cc5F01 in SGI was the only locus that test positive for the presence of null alleles, which can lead to a homozygote excess (Table 4.4). Loci that deviate from HWE do not seem to have a significant effect on accuracy of relationship assignments, especially for full-likelihood methods like COLONY (Jones and Wang 2010a) and it is not usually necessary to discard

these loci (Selkoe and Toonen 2006). No linkage disequilibrium between any pairs of loci was detected in QR and SGI (Table 4.5). All loci were assumed to be independent.

The probability of detecting multiple paternity under all simulated mating scenarios (two, three, and five fathers with equal and skewed paternal contributions) and sample sizes (eight and 25 which were the minimum and maximum number of offspring sampled) in PRDM was greater than 0.96, indicating an extremely high probability of detecting multiple paternity if present in the samples (Table 4.6).

As calculated in IDENTITY, the probability of identity for the different loci was slightly different between QR and SGI due to different allele frequencies (Figure 4.3). The probability of identity for each individual locus ranged from 0.01 (Cc5F01) to 0.29 (CC17) for QR and 0.02 (Cc2G10) and 0.27 (CC17) for SGI. The combined probability of identity for loci in linkage equilibrium was extremely low for both populations, despite different numbers of loci, indicating a very high chance of obtaining unique genotypes (QR: $p = 7.93 \times 10^{-12}$, SGI: $p = 1.59 \times 10^{-11}$). The marker sets used by Moore and Ball (2002) and Zbinden et al. (2007) also had very low probabilities of identity (QR: $p_{Moore's} = 1.37 \times 10^{-4}$, $p_{Zbinden's} = 2.84 \times 10^{-4}$, SGI: $p_{Moore's} = 1.28 \times 10^{-4}$, $p = 1.99 \times 10^{-04}$), although they were several orders of magnitude greater than the corresponding probabilities for QR and SGI.

There also were slight differences in the exclusion probabilities between populations, due to differences in allele frequencies (Figure 4.4). The exclusion probability for individual loci ranged from 0.41 (CC17) to 0.87 (Cc5F01) for QR and 0.42 (CC17) to 0.84 (Cc2G10) for SGI. The marker sets used by Moore and Ball (2002) and Zbinden et al. (2007) both had an exclusion probability of 0.98 for both populations,

while all 10 loci used in this study achieved a combined exclusion probability of 1.00 for each population, suggesting that incorrect paternal assignments were not likely.

Ten maternal and two paternal mutations were detected in QR, and three mutations were detected in SGI. Maternal and paternal mutations could not be differentiated in SGI since maternal genotypes were unknown.

Paternity analyses

Paternity analyses were based on 36 unrelated clutches and three females' pooled clutches from QR and 22 unrelated clutches from SGI. In QR, female tagging data was used to verify the relatedness of sampled clutches. COLONY confirmed that each clutch from SGI was from a different mother. Between 8 and 25 hatchlings (average = 20.2) were sampled from each clutch (average clutch size = 120.6), constituting a sampling effort of 8.1% to 34.4% (average = 17.7%) per clutch for QR and SGI combined.

The number of clutches detected with multiple paternity differed slightly between the three programs. In the 41 clutches from QR, COLONY detected 24 DADSHARE 27, and GERUD 27 clutches with multiple paternity (Table 4.7). Five clutches had to be assigned single or multiple paternity based on a majority consensus among the programs. The consensus on the number of clutches with multiple paternity was 27 out of 41 (66%).

Of the 22 clutches from SGI, COLONY detected four with multiple paternity while DADSHARE detected two and GERUD 11. The programs differed in the assignment of single or multiple fathers in 10 clutches. The consensus on multiple paternity was five out of 22 clutches (23%). Two of these were from 2007 and three were from 2008. No difference between the years was observed (Fisher's exact test, $p=0.999$).

In clutches with multiple paternity, DADSHARE tended to assign the highest number of fathers (10 out of 16 cases where one program assigned more fathers than the other(s)). Of clutches where two of the three programs assigned single paternity, GERUD tended to be the program to assign multiple paternity (six out of seven cases).

I detected multiple paternity in 59% of clutches in QR with both the marker sets of Moore and Ball (2002) and Zbinden et al. (2007). In SGI, 27% and 32% of clutches were assigned multiple paternity with these marker sets, respectively.

Paternal contributions could be resolved for all fathers in 17 out of 27 clutches with multiple paternity in QR. Of these 17, three clutches had three or more fathers. The contribution of the primary father was in consensus among at least two of the paternity analysis programs in an additional seven clutches. These clutches all had three or more fathers. The total number of clutches from QR for which the contribution of the primary father could be resolved was 24. Paternal contributions only could be resolved in two out of the six clutches with multiple paternity in SGI. These two clutches had two and three fathers, respectively. The contribution of all fathers from the clutch with only two fathers could be resolved, whereas only the primary father's contribution was in consensus among the program for the clutch with three fathers. The paternal contribution of each primary father is depicted in Figure 4.5.

I observed both types of primary fathers; one type sired the majority of the offspring (more than half; X^2, $p \leq 0.05$), and the other type sired half of the offspring (X^2, $p > 0.05$) (Figure 4.5). Eight out of the 24 clutches in QR with known paternal contributions of the primary father, had primary fathers that sired the majority of the

offspring, while in SGI, one out of the two clutches had this type of primary father. Either type of father was equally likely ($X^2 = 1.96$, $p = 0.16$; Figure 4.6).

Female abundance

No correlation was found between either method of estimating female abundance (data were log transformed as they did not fit a normal distribution) and the percent of clutches with multiple paternity (rookery abundance: Pearson's correlation coefficient = -0.128, $p = 0.837$; genetic stock abundance: Pearson's correlation coefficient = 0.007, $p = 0.991$). Using the frequency of multiple paternity calculated with the loci from the prior studies (Moore and Ball 2002; Zbinden et al. 2007) did not improve the correlation between either method of female abundance and multiple paternity (Pearson's correlation coefficient = 0 to -.196, $p > 0.752$).

Nest density

Density of nests (data were log transformed as they did not fit a normal distribution) showed a stronger correlation with multiple paternity than did female abundance, although still not significant, and explained nearly 15% of the variation (Pearson's correlation coefficient = 0.418, $p = 0.484$; Figure 4.7).

Using the frequency of multiple paternity for QR and SGI obtained with the loci of Moore and Ball (2002) and Zbinden et al. (2007) did not greatly alter the strength of the correlation between nest density and multiple paternity (Pearson's correlation coefficient = 0.394, $p = 0.511$; and Pearson's correlation coefficient = 0.330, $p = 0.588$, respectively).

Operational sex ratio

A total of 78 unique paternal genotypes were reconstructed (in COLONY), equaling an operational sex ratio of 78 males to 41 females (1.9), which is significantly different from equal (X^2= 11.504, p=0.0007). One each of eight paternal genotypes were shared among two or three clutches, suggesting that in eight incidences a male mated with two or three females. Twenty-nine unique paternal genotypes reconstructed from SGI clutches, resulting in and an operational sex ratio of 29 males to 22 females (1.3), which is not significantly different from equal (X^2= 0.961, p= 0.3270). Two reconstructed paternal genotypes were shared between two clutches each, suggesting that in two occurrences two females mated with the same male.

The estimated operational sex ratios for QR and SGI are not significantly different from one another (Fisher's exact test, p=0.3; Figure 4.8). Multiple paternity was detected in a significantly greater proportion of clutches from QR (27 out of 41) than from SGI (2 out of 27) (Fisher's exact test, p=0.004) (Figure 4.9).

Reproductive success

Neither hatching success nor hatchling weight were significantly different between *in situ* and relocated nests (Table 4.8). Therefore, all nests, regardless of treatment, were used in subsequent analyses of hatching success and hatchling weight.

There was no difference in reproductive success (clutch size, hatching success, and hatchling weight) between years in SGI (Table 4.8), so the two years were pooled. There was a significant difference in clutch size between QR and SGI (Mann-Whitney *U*: z = -3.296, p=0.001; Figure 4.10), but no difference in hatching success and hatchling

weight (Mann-Whitney *U*: z = -0.552, p = 0.581; z = -1.082, p = 0.279, respectively) (clutch frequency could not be compared between populations because these data were only available for QR) (Table 4.8). Clutch size was therefore analyzed separately for QR and SGI.

Regarding the potentially confounding factor of female experience, I observed a significant difference in date of first clutch, hatchling weight, and clutch frequency between neophytes and remigrants (Mann-Whitney *U*: z = -3.183, p=0.001; z = -2.175, p = 0.049; z = -3.566, p < 0.001, respectively), but not for female length or width, clutch size, hatching success, or paternity (Table 4.8). Remigrants nested on average over two weeks earlier than neophytes (Figure 4.11), hatchlings were heavier (18.62 g compared to 17.70 g; Figure 4.12), and laid almost twice as many clutches (3.96 clutches compared to 2.21 clutches; Figure 4.13). When neophytes and remigrants were tested separately for associations of paternity with date of first clutch, hatchling weight, and clutch frequency, none of the associations were significant (Table 4.8).

There were no significant associations between paternity and maternal traits or sampling effects (Table 4.8). Also, no measures of reproductive success (clutch size tested separately for QR and SGI) were significantly associated with single or multiple paternity (Table 4.8). There were no differences in the measures of reproductive success between the two types of primary father (Table 4.8).

Inter- nesting mating

The first six out of seven clutches from one female (XC657), and the first four clutches out of five and six clutches from two other females (XM916 and XM942, respectively), were analyzed for paternity.

XC657 – COLONY grouped the 117 sampled offspring into four full sib groups, with 62, 53, one, and one offspring in each group. GERUD grouped the offspring into three full sib groups with 60, 45, and six offspring in each (six offspring were excluded due to missing data). DADSHARE grouped the offspring into five full sib groups. The primary (most offspring overall) full sib group from COLONY match the primary full sib group from DADSHARE. The second full sib group from COLONY was split into four full sibs groups by DADSHARE, except one offspring (from clutch XC011), which was unresolved. The two lone offspring (both from clutch XC024) from COLONY also were unresolved by DADSHARE. GERUD's two primary full sib groups were consisted with COLONY's. A third full sib group consisting of offspring from clutch XC024 was determined by GERUD. Comparing the results of the three programs, there was uncertainty in the assignment of three individuals, two from XC024 and one from XC011. Excluding these three offspring, the two fathers sired offspring in both groups of full sibs in all six clutches, with equal paternal contribution overall and in each clutch except for the third clutch where paternal contribution was skewed ($X^2 = 6.37$, $p = 0.0116$; Table 4.9). This pattern of paternity suggests that XC657 mated prior to laying her first clutch of eggs, unless she remated with the same males in between nesting events.

XM916 – All three programs attributed two fathers to the 82 sampled offspring. The primary father sired significantly more offspring than the secondary father overall and in each clutch ($X^2 \geq 7.2$, $p \leq 0.0073$; Table 4.9). Both fathers sired offspring in all four sampled clutches, thus indicating that XM916 did not mate in between nesting events, assuming she did not remate with the same males.

XM942 – COLONY divided the offspring among three fathers, a primary father with 40 offspring, a secondary with 36 offspring, and a tertiary father with a single offspring in the fourth clutch (XC136). GERUD indicated paternal contributions from two fathers in all four clutches. The distribution of offspring between the two fathers was compatible with COLONY's primary and secondary full sib groups. However, it is not clear to which of the two fathers GERUD assigned the offspring that COLONY had assigned to a tertiary father. Like Gerud, DADSHARE divided the offspring into two full sub groups, of 40 and 37 offspring. Based on the distribution of offspring by each of the three programs, the primary and secondary fathers sired offspring in all four clutches with skewed paternal contribution in the first clutch ($X^2 \geq 5.00$, $p \leq 0.0253$), but equal paternal contribution overall (Table 4.9). Evidence from all programs suggests that XM942 mated with two males prior to nesting, and then did not mate again unless she re-mated with the same males.

Discussion

Paternity

Multiple paternity was evident in 66% of clutches sampled from QR and in 23% of clutches sampled from SGI, which is near or within the range of other loggerhead

populations (31-93%). This study demonstrates the tremendous variation in multiple paternity in loggerhead populations that is possible on such a relatively small spatial scale as observed between QR and SGI, considering the species is circumglobally distributed.

There was some variation in the number of fathers detected by each of the three programs, with GERUD tending to assign multiple fathers when the two others agreed on single paternity. This is likely due to the inability of GERUD to account for mutations and scoring errors, despite my efforts to identify potential mutations prior to analysis. There also was some discrepancy in the distribution of offspring among fathers. This was especially evident when three or more fathers were detected and the maternal genotype was unknown. The use of multiple robust parentage assignment program can provide a higher level of confidence in the results especially when there is concordance across programs (Lee et al. 2008).

The frequency of multiple paternity I obtained for QR and SGI using the marker sets of this study was very similar to the frequency obtained for the same two populations using the marker sets employed by two other loggerhead studies (Moore and Ball 2002; Zbinden et al. 2007). While there was some discrepancy in the frequency of multiple paternity, the results for QR and SGI were within 7% one another. Thus, comparing results of multiple paternity from different studies may be valid and general trends will likely hold, assuming probabilities of identity and exclusion provide comparable levels of confidence.

Abundance and density

A great deal of variation in multiple paternity was evident, but the abundance of females, whether in an arbitrary rookery or within a genetic stock, and the density of nests were not correlated with the frequency of multiple paternity in loggerheads. This contradicts findings from the sea turtle genus *Lepidochelys* where female abundance plotted against the frequency of multiple paternity in various rookeries shows a significant fit to an exponential regression (Jensen et al. 2006). The difference may lie in the mass nesting behavior which is observed in some populations of *Lepidochelys* and is unique to the genus. During such a mass nesting event, termed arribada, 100 to 40,000 or more females emerge synchronously to lay eggs over the course of one to three days (Miller 1997, Hamann et al. 2003, Fonseca et al. 2009). This vast number of females may trigger a mating frenzy, where potential mates are in such close proximity that males have optimal mating opportunities (Jensen et al. 2006). The sheer number of individuals involved is at a level not comparable to populations that do not exhibit arribadas. The enormous difference in female abundance and nest density between populations of *Lepidochelys* that exhibit arribadas and those that do not may be great enough show an effect on the level of multiple paternity. In comparison, the relatively minor differences observed among rookeries of loggerhead turtles may not be large enough to show an effect on multiple paternity.

However, additional studies will be useful to corroborate the lack of a correlation between female abundance or nest density and the frequency of multiple paternity. As this study shows, drawing a strong conclusion based on five data points can be misleading. For example, if one point were removed, the trend will change. Remove the

Mon Repos, Australia data point, and the correlation of nest density with multiple paternity increases almost twofold. Remove Zakynthos, Greece, and the correlation is reduced to zero.

Operational sex ratio

I predicted that a female-biased operational sex ratio would decrease competition among males and thereby increase male mating success for many males resulting in more multiple paternity compared to a situation where males compete and many do not mate successfully (Emlen and Oring 1977). The operational sex ratio in QR (1.9 males to 1 female) was not significantly different from the operational sex ratio in SGI (1.3 males to 1 female). However, the direction of the trend is opposite of what I had predicted. While neither population's operational sex ratio was skewed towards females, QR had a smaller proportion of females than SGI (although not significantly so), and also the greater frequency of multiple paternity.

Contrary to my hypothesis, a male-biased operational sex ratio may increase the availability and encounter rate of male mates to females, increasing the opportunity for multiple mating and multiple paternity (Zbinden et al. 2007). While evidence from green and olive ridley turtle populations suggests high female bias to be associated with high multiple paternity (olive ridley; Jensen et al. 2006), and high male bias to be associated with low multiple paternity (green; FitzSimmons 1998), loggerheads may have a different mating strategy. Male loggerheads may be less aggressive than male green turtles. Female green turtles engaged in copulation are often accompanied by up to five males, who will display aggressive behavior and try to dislodge the mounted male (Booth and

Peters 1972, Limpus 1993). In contrast, mating pairs of loggerheads are often solitary and no such attendant males have been observed (Frick 2000), suggesting that male competition and aggression towards one another may play less of a role than for green turtles. Under this scenario, a male-skewed operational sex ratio would enable female loggerheads to mate with many males, thus facilitating multiple paternity. In addition, lack of male territoriality, which is presumably the case for loggerheads, should increase mate encounters for females compared to mating systems where territory plays a strong role (Uller and Ollson 2008).

Because directly measuring of the operational sex ratio was not feasible, I used the ratio of the number of unique male genotypes detected in clutches to the number females sampled, which represents the sex ratio of those individuals who actually reproduced. Obviously this does not account for individuals who were reproductively active but were unsuccessful at reproducing. While this method of estimating the operational sex ratio is largely a function of the frequency and pattern of multiple paternity, any polygyny will reduce the proportion of males and no polygyny will maximize the proportion of males. Polygyny may be present when suitable males are limited, the weaker ones outcompeted, or when there are not a suffificient number of males to females. This estimate of the operational sex ratio compared to all reproductively active individuals can be considered more valuable in terms of who is actually contributing to the population and the genetic diversity of future generations. The operational sex ratio in QR was significantly skewed towards males and in SGI the ratio was not significantly different from equal. Thus, the heavily female-biased hatchling sex ratios like those found on Florida beaches (one male to nine females;

Mrosovsky and Provancha 1992) most likely do not dominate the adult sex ratios, at least not at the level of the operational sex ratios. Cooler nesting beaches produce comparably more males, and females may encounter these males during migrations, and also males may be less philopatric than females, thereby increasing the availability of mates for females from the southern reaches of the range. In addition to increasing mate encounters, this may provide an avenue for gene flow and minimize inbreeding among genetically distinct populations. The low level of polygyny detected in both QR and SGI suggests that males likely are not limiting. In the future, however, predicted temperature increases may increase incubation temperatures and alter hatchling sex ratios (Mrosovsky et al. 1984, Janzen 1994, Hawkes et al. 2007), and eventually affect the survivorship of clutches (Broderick et al. 2001, Godley et al. 2001, Hawkes et al. 2007). Ultimately, if nothing is done to counteract the feminization of hatchling sex ratios, either naturally by female turtles regulating when and where they nest or by human intervention, male sea turtles will become limiting which will have definite repercussions on reproduction.

Reproductive success

I predicted that females who mated multiply would have greater reproductive success through larger clutch sizes, greater hatching success and hatchling weight, and a higher clutch frequency. In this study, I found no association between multiple paternity and reproductive success. Evidence of reproductive benefits in reptiles has been scarce (Uller and Ollson 2008) and not always conclusive. In the aquatic painted turtle, *Chrysemis picta*, multiple paternity was associated with larger clutch sizes, although females who laid clutches with multiple paternity were also larger (Pearse et al. 2002).

Greek loggerheads demonstrated greater hatching success as the number of fathers per clutch increased, however, female body size also increased with number of sires (Zbinden et al. 2007). These females may be more sought after as mates, because larger turtles tend to be more fecund (McTaggart 2000). On the other hand, female green turtles in Ascension Island did not benefit from multiple paternity, nor was female size related to paternity or any measure of reproductive success (Lee and Hays 2004). Lack of support for females benefitting suggests that female promiscuity may have evolved through strong selection on males to mate as often as possible.

An alternative, but not mutually exclusive to the female benefit hypothesis, posits that females may mate multiply to avoid male harassment (Watson et al. 1998, Lee and Hays 2004). Courting loggerhead males have been observed to vigorously bite females on the flippers (Frick 2000). A female sea turtle may be able to reduce the cost associated with male harassment during courtship in two ways. She can give in to a courting male if the harassment becomes too costly (Lee and Hays 2004), and also she may be able to reduce the cost of harassment from other courting males during the duration of a mating event (Jablonski and Vepsäläinen 1995).

Inter-nesting mating

In support of my prediction, based on successive clutches of three females, I found no evidence of mating in between nesting events, assuming females did not remate with the same males. Prior evidence of inter-nesting mating by loggerheads is inconclusive (Harry and Briscoe 1988, Zbinden et al. 2007), and green, Kemp's ridely, and flatback turtles show no evidence of inter-nesting mating (FitzSimmons 1998,

Kichler et al. 1999, Theissinger et al. 2009, respectively). Thus, it may be common among sea turtles, that mating takes place prior to nesting, leaving the males free to depart the breeding area before nesting begins, as observed in green males (FitzSimmons 1998).

Broader impacts

With this study, I have demonstrated the there is significantly more multiple paternity in QR compared to SGI. However, neither female abundance nor nest density is a good predictor of the frequency of multiple paternity. Future studies should consider other ways of approximating the density of turtles in breeding aggregations. The operational sex ratio was not significantly different in the two populations, although in QR there were significantly more males and in SGI there were equal numbers of males and females reproducing. The direction of the trend, the more males to females, the more multiple paternity, suggests a mating system where mating success may be associated with mate encounter rate, rather than male to male competition limiting their mating success. Females did not appear to benefit from multiple paternity through increased reproductive success, suggesting that multiple mating is driven by male propensity to mate. The pattern of paternity across multiple clutches laid by the same females provides indirect evidence that females did not mate in between nesting, allowing males to leave the breeding area before nesting begins to return to the foraging areas.

Loggerheads have been listed as threatened since 1978 under the U.S. Endangered Species Act of 1973, endangered since 1996 on the IUCN Red List of Threatened Species (IUCN 2010), and are restricted from international trade by Appendix I of the Convention

on International Trade in Endangered Species. Despite these measures of protection, loggerhead nesting has declined significantly in the southeastern United States and Caribbean Mexico over the past 18 years, mostly likely due to population decline as a result of incidental capture in commercial and artisanal fisheries (Witherington et al. 2009). Effects of pollution on mortality has not been quantified, but presumably it has increased over the course of the 18-year decline Florida loggerheads (Witherington et al. 2009). The vast quantities of oil released with Deepwater Horizon oil spill in the Gulf of Mexico has definitely had and will continue to have an effect on loggerheads and other sea turtles for years to come (Florida Fish and Wildlife Conservation Commission). The more we know about the reproductive biology and migratory behavior of loggerhead sea turtles, the better we can understand the impacts of threats such as climate change, pollution, and mortality in fisheries on population parameters that are critical to effective management of this imperiled species.

Table 4.1. Studies of multiple paternity in six of the seven species of sea turtle. Only studies with a minimum sample size of 10 females are included. ¥ indicates use of allozymes instead of microsatellite markers. * indicates arribada mass nesters as opposed to solitary nesters. MP: multiple paternity; N: sample size in terms of number of females or unrelated clutches; A: Atlantic; P: Pacific; M: Mediterranean.

Species	Region	% MP (N)	Source
Loggerhead	Australia (P)	33% (24) ¥	Harry and Briscoe 1988
Caretta caretta	Florida (A)	31% (70)	Moore and Ball 2002
	Greece (M)	93% (15)	Zbinden et al. 2007
Kemp's ridley	Mexico (A)	58% (26)	Kichler et al. 1999
Lepidochelys kempi			
Olive ridley	Surinam (A)	20% (10)	Hoekert et al. 1999
L. oliviacea	Costa Rica (P)	92% (13)*	Jensen et al. 2006
	Costa Rica (P)	30% (13)	Jensen et al. 2006
Green	Costa Rica (A)	50% (18)	Peare and Parker 1996
Chelonia mydas	Australia (P)	9% (13)	FitzSimmons 1998
	Ascension Island (A)	62% (18)	Lee and Hays 2004
Flatback	Australia (P)	67% (9)	Theissinger et al. 2009
Natator depressus			
Leatherback	Costa Rica (P)	10% (20)	Crim et al. 2002
Dermochelys coriacea			

Table 4.2. Microsatellite loci used for paternity analysis, type of repeat unit, primer sequences, primer annealing temperatures in °C (T_A) and primer concentrations (μM) for each primer pair for PCR, and the reference for the primer sequences. Loci that were used in other loggerhead paternity studies are indicated.

Locus	Repeat unit	T_A	μM	Forward (F) and reverse (R) primers 5'-3'	Reference	Other loggerhead paternity studies
Ccar176	di	60	0.12	F: GGCTGGGTGTCCATAAAAGA R: TTGATGCAGGAGTCACCAAG	Moore and Ball 2002	Moore and Ball 2002
Cc2G10	tetra	60	0.10	F: CAGTCGGGCGTCATCAGTGGCAAGGTCAAATACAG R: GTTTGCCCTTATTTGGTCACAC	Shamblin et al. 2007	
Cc5F01	tetra	60	0.30	F: GTTTAAAGGATTTGAGATGTTGTATG R: CCAGTTGTCTTTCTCCAGTG	Shamblin et al. 2007	
CC117	di	60	0.20	F: TCTTTAACGTATCTCCTGTAGCTC R: CAGTAGTGTCAGTTCATTGTTTCA	FitzSimmons et al. 1995	Zbinden et al. 2007
CC17	di	60	0.10	F: CCACTGGAAGTCTAAGAAGAGTGC R: GGAATTGAAGGGATTTTGCT	Monzón-Argüello et al. 2008	
DC107	di	56	0.20	F: GTCACGGAAAGAGTGCCTGC R: CAATTTGAGGTTATAGACC	P. Dutton in Bowen et al. 2005	

CC141	di	56	0.08	F: CAGCAGGCTGTCAGTTCTCCAC R: TAGTACGTCTGGCCTGACTTT	N. FitzSimmons in Bowen et al. 2005	Moore and Ball 2002 Zbinden et al. 2007
CC7	di	56	0.10	F: TGCATTGCTTGACCAATTAGTGAG R: ACATGTATAGTTGAGGAGCAAGTG	N. FitzSimmons in Bowen et al. 2005	Moore and Ball 2002 Zbinden et al. 2007
Cc5C08	tetra	56	0.32	F: GTTTCTTTGATGGTTTTTCTGTTATC R: TCAGTCTTCAGGGTATCGTGTAAT	Shamblin et al. 2007	
CCM2	di	56	0.14	F: GTTTTGGCACTGGTGGAAT R: TGACTCCCAAATACTGCT	Francisco 2001	Moore and Ball 2002 Zbinden et al. 2007

Table 4.3. Allelic diversity (*k*), observed and expected heterozygosity (Het_{obs} and Het_{exp}), *p*-values from Hardy-Weinberg (HWE) exact tests for homozygote excess at 10 microsatellite loci based on the multilocus genotypes of 58 loggerheads from Quintana Roo (QR) and 24 loggerheads from St. George Island (SGI). * indicates significant deviations from HWE at $p \leq 0.05$ and **bold** indicates significance after Bonferroni correction.

	k		Het_{obs}		Het_{exp}		HWE	
Locus	QR	SGI	QR	SGI	QR	SGI	QR	SGI
Ccar176	19	14	0.79	0.92	0.86	0.80	0.0261*	0.5640
Cc2G10	22	17	0.91	0.96	0.94	0.94	**0.0001***	0.9734
Cc5F01	28	22	0.72	0.72	0.91	0.93	**0.0000***	**0.0004***
CC117	8	8	0.74	0.72	0.79	0.79	**0.0004***	0.1403
CC17	8	7	0.67	0.80	0.73	0.68	**0.0007***	0.4904
DC107	8	7	0.79	0.72	0.80	0.79	0.0262*	0.4015
CC141	12	12	0.91	0.96	0.86	0.86	0.1368	0.9123
CC7	12	9	0.84	0.80	0.81	0.80	0.9584	0.9638
Cc5C08	15	13	0.86	0.80	0.92	0.92	0.1589	0.1924
CCM2	7	6	0.72	0.88	0.72	0.78	0.2851	0.7226

Table 4.4. Presence of null alleles and their estimated frequency, as well as indications of stutter and large allele dropout for each locus in Quintana Roo (QR) and St. George Island (SGI).

	Null Present		Null Frequency		Stutter		Large Allele Dropout	
Locus	QR	SGI	QR	SGI	QR	SGI	QR	SGI
Ccar176	no	no	-0.0084	-0.0474	no	no	no	no
Cc2G10	no	no	0.0056	-0.0037	no	no	no	no
Cc5F01	no	**yes**	0.0045	**0.0464**	no	maybe	no	no
CC117	no	no	-0.0175	0.0175	no	no	no	no
CC17	no	no	0.0143	-0.0992	no	no	no	no
DC107	no	no	-0.0103	0.0058	no	no	no	no
CC141	no	no	0.0157	-0.0307	no	no	no	no
CC7	no	**yes**	-0.0028	**0.0622**	no	maybe	no	no
Cc5C08	no	no	-0.0153	0.007	no	no	no	no
CCM2	no	**yes**	0.0120	**0.0338**	no	no	no	no

Table 4.5. χ^2 test values and (degrees of freedom) for testing linkage disequilibrium between pairs of loci. Results from Quintana Roo are below the diagonal and results from St. George Island are above the diagonal. * $p \leq 0.05$. No pairs of loci were in significant linkage disequilibrium.

	Ccar176	Cc2G10	Cc5F01	CC117	CC17	DC107	CC141	CC7	Cc5C08	CCM2
Ccar176	---	108.32 (208)	113.95 (273)	59.57 (91)	52.72 (78)	56.45 (78)	82.58 (143)	82.12 (128)	88.46 (156)	48.43 (65)
Cc2G10	219.99 (418)	---	150.86 (336)	77.91 (112)	50.42 (96)	84.52 (96)	101.24 (176)	90.16 (168)	116.85 (192)	69.64 (80)
Cc5F01	223.11 (532)	273.5 (616)	---	85.67 (147)	62.94 (126)	80.29 (126)	106.16 (231)	38.3 (56)	143.99 (252)	71.6 (105)
CC117	127.3 (152)	130.41 (176)	136.75 (224)	---	25.23 (42)	30.84 (42)	54.89 (77)	31.54 (48)	65.03 (84)	34.18 (35)
CC17	102.4 (152)	131.98 (176)	135.28 (224)	53.32 (64)	---	25.36 (36)	42.22 (66)	44.71 (48)	46.9 (72)	21.2 (30)
DC107	85.59 (152)	149.97 (176)	140 (224)	65.15 (64)	50.77 (64)	---	53.72 (66)	44.71 (48)	67.48 (72)	26.43 (30)
CC141	135.29 (228)	188.74 (264)	185.87 (336)	83.91 (96)	69.16 (96)	89.5 (96)	---	50.1 (88)	89.98 (132)	44.77 (55)
CC7	125.06 (228)	150.32 (264)	161.71 (336)	68.55 (96)	49.55 (96)	79.79 (96)	100.14 (144)	---	72.82 (96)	40.73 (40)
Cc5C08	188.1 (285)	239.97 (330)	227.93 (420)	111.8 (120)	84.25 (120)	102.21 (120)	150.07 (180)	126.15 (180)	---	51.03 (60)
CCM2	79.92 (133)	104.72 (154)	97.3 (196)	54.77 (56)	52.33 (56)	55.14 (56)	83.56 (84)	59.27 (84)	81.54 (105)	---

Table 4.6. Probability values for detecting multiple paternity under six mating scenarios for two different sample sizes with the marker sets used for Quintana Roo (in front of slash) and for St. George Island (after slash).

Mating scenario	Number of offspring	
(hypothesized paternal skew)	8	25
2 males (0.5/0.5)	0.99 / 0.99	1.00 / 1.00
2 males (0.667/0.333)	0.96 / 0.96	1.00 / 1.00
3 males (0.33/0.33/0.33)	1.00 / 1.00	1.00 / 1.00
3 males (0.57/0.285/0.145)	0.99 / 1.00	1.00 / 1.00
5 males (0.2/0.2/0.2/0.2/0.2)	1.00 / 1.00	1.00 / 1.00
5 males (0.52/0.26/0.13/0.06/0.03)	0.99 / 1.00	1.00 / 1.00

Table 4.7. Number of fathers detected by each of three programs and the consensus number of fathers. If there was an agreement in the number of fathers between at least two of the programs, this consensus was used. Otherwise, the number of fathers was estimated by averaging the number indicated by each program showing multiple paternity.

Identifier	COLONY	DADSHARE	GERUD	Consensus
Quintana Roo				
AB362	3	3	3	3
AH675	1	1	1	1
AH793	2	2	2	2
AM035	1	1	1	1
AM064	1	1	1	1
AM371	2	2	2	2
BB287	1	1	1	1
EB742	2	4	3	3
EK609	1	1	1	1
EK616	2	5	2	2
EK661	1	1	1	1
J4688	6	4	5	5
J5643	2	2	2	2
KJ128	1	1	1	1
XA505	3	4	3	3
XA610	2	5	2	2
XC052	4	3	3	3
XC657 – six clutches	4	5	3	4
XE207	4	3	3	3
XE208	3	3	4	3
XE212	3	3	3	3
XE261	2	2	2	2
XE270	1	5	2	3.5
XH730	2	8	3	4.3
XH775	2	4	3	3
XM718	1	1	1	1
XM730	1	1	1	1
XM753	2	2	2	2
XM916 – four clutches	2	2	2	2

XM917	2	2	2	2
XM942 – four clutches	3	2	2	2
XM945	1	3	2	2.5
XN041	1	3	1	1
XN043	2	2	2	2
XN049	1	1	1	1
XN235	2	2	2	2
XN244	2	1	2	2
XP301	1	1	1	1
XP946	1	1	1	1
XP949	1	2	2	2
XP966	1	1	1	1
St. George Island				
614G1BD	1	1	1	1
614G2BD	1	1	1	1
614G3BD	1	1	1	1
615G1BD	1	1	1	1
618G1BD	1	1	1	1
618G1SB	1	3	3	3
618G3BD	1	1	2	1
620G2BD	1	1	2	1
621G2BD	2	1	2	2
621G3BD	1	1	1	1
623G2BD	1	1	2	1
624G1SB	1	1	2	1
626G1BD	1	1	1	1
627G1BD	1	1	1	1
627G3SB	1	1	2	1
628G2BD	2	1	3	2.5
629G2BD	1	1	2	1
630G4BD	1	1	1	1
630G5BD	4	1	4	4
703G6BD	3	6	3	3
703G7BD	1	1	1	1
703G8BD	1	1	1	1

Table 4.8. Mann-Whitney *U* (MWU) test of the hypothesis that the distribution of each dependent (test) variable is the same across categories of the independent (grouping) variable, and Fisher's exact test testing the equality of the two variables. * indicates significance at $p \leq 0.05$. N = sample size.

Independent Variable	Dependent Variable	N	Test	Z	p
Nest treatment – (*in situ* / relocated)					
Reproductive success	hatching success	62	MWU	-1.869	0.062
	hatchling weight	59	MWU	-0.313	0.754
SGI – (2007 / 2008)					
Reproductive success	clutch size	22	MWU	-1.347	0.178
	hatching success	22	MWU	-.099	0.922
	hatchling weight	22	MWU	-.854	0.393
Paternity	single or multiple paternity	22	Fisher's	---	1.000
Population – (QR / SGI)					
Reproductive success	clutch size	62	MWU	-3.296	**0.001***
	hatching success	62	MWU	-.552	0.581
	hatchling weight	60	MWU	-1.082	0.279
Paternity	single or multiple paternity	63	Fisher's	**---**	**0.002***
Experience – (neophyte / remigrant)					
Maternal traits	date of first clutch	40	MWU	-3.183	**0.001***
	female length	40	MWU	-1.744	0.081
	female width	40	MWU	-1.021	0. 307
Reproductive success	clutch size	40	MWU	-1.092	0. 275
	hatching success	40	MWU	-0.397	0. 691
	hatchling weight	38	MWU	-2.175	**0.049***
	clutch frequency	40	MWU	-3.566	**0. 000***
Paternity	single or multiple paternity	40	Fisher's	**---**	0.501
Neophytes: Paternity – (single / multiple)					
Maternal traits	date of first clutch	14	MWU	-1.371	0.170
Reproductive success	hatchling weight	13	MWU	-0.293	0.770
	clutch frequency	14	MWU	-1.739	0.082
Remigrants: Paternity – (single / multiple)					
Maternal traits	date of first clutch	26	MWU	-0.117	0.907
Reproductive success	hatchling weight	24	MWU	-0.367	0.713
	clutch frequency	26	MWU	-0.511	0.610

Paternity – (single / multiple)					
Maternal traits	date of first clutch	40	MWU	-1.215	0.224
	female length	40	MWU	-0.695	0.487
	female width	40	MWU	-0.567	0.571
Sampling effects	offspring analyzed	63	MWU	-1.171	0.241
	clutch number analyzed	37	MWU	-1.312	0.189
Reproductive success	clutch size SGI	22	MWU	-1.646	0.100
	clutch size QR	40	MWU	-0.099	0.921
	hatching success	62	MWU	-0.971	0.331
	hatchling weight	60	MWU	-0.865	0.387
	clutch frequency	40	MWU	-0.996	0.319
Primary father type – (sired majority / half)					
Reproductive success	clutch size SGI	2	MWU	-1.000	0.317
	clutch size QR	23	MWU	-0.872	0.382
	hatching success	26	MWU	-1.415	0.157
	hatchling weight	26	MWU	-0.849	0.396
	clutch frequency	26	MWU	-0.657	0.511

Table 4.9. Numbers of offspring from successive clutches of three females assigned to a primary (1°) or secondary father (2°). Consensus results from COLONY, GERUD, and DADSHARE are shown. The consensus number of fathers for Xc657 is four (Table 4.7), but upon analysis of the distribution of offspring by each program, the offspring can be consolidated into two groups of full sibs. Offspring were excluded when no consensus in their assignment could be reached. * indicates paternal contributions that deviate significantly from equality.

Mother	Clutch	1° father	2° father	Offspring excluded	X^2	p
XC657						
	XC011	9	10	1	0.05	0.8185
	XC024	8	9	2	0.06	0.8084
	XC038	15	4		6.37	0.0116*
	XC096	14	6		3.20	0.0736
	XC207	8	11		0.47	0.4913
	XC345	8	12		0.80	0.3711
	Total	62	52		0.88	0.3490
XM916						
	XC007	20	2		14.73	0.0001*
	XC021	17	3		9.80	0.0017*
	XC034	17	3		9.80	0.0017*
	XC102	16	4		7.20	0.0073*
	Total	70	12		41.02	.0001*
XM942						
	XC019	15	5		5.00	0.0253*
	XC032	9	10		0.05	0.8185
	XC049	11	8		0.47	0.4913
	XC136	5	13	1	3.56	0.0593
	Total	40	36		0.21	0.6464

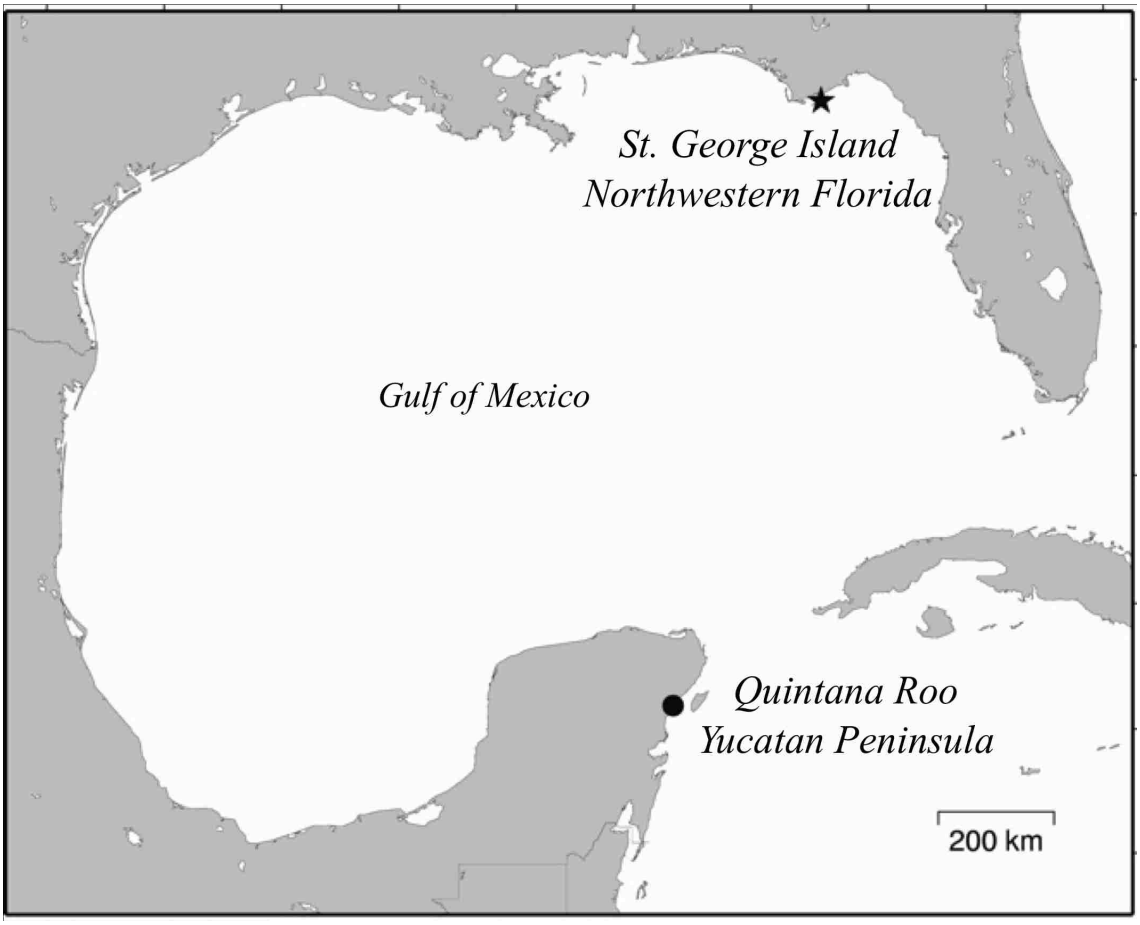
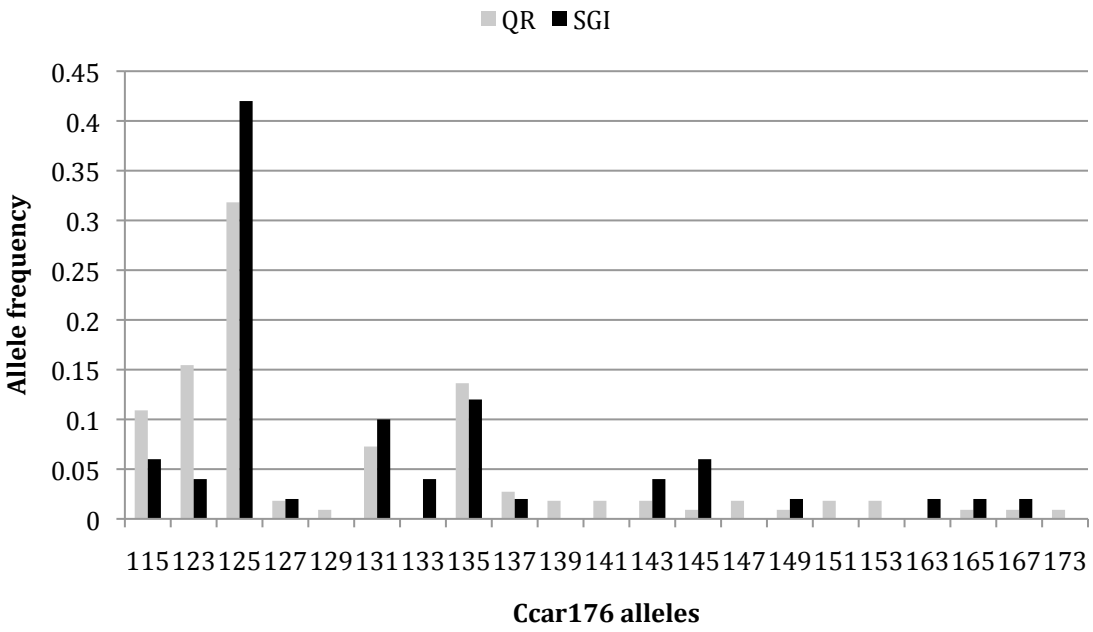
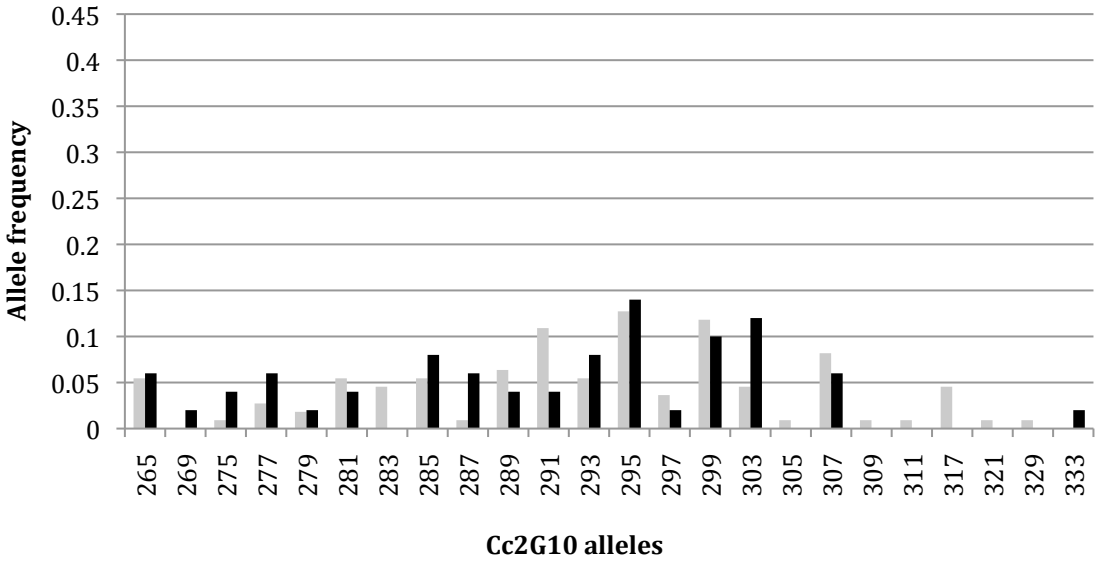


Figure 4.1. Locations of the sampling sites on St. George Island in the northwestern Florida genetic stock (star) and in Quintana Roo in the Yucatan Peninsula genetic stock (circle).

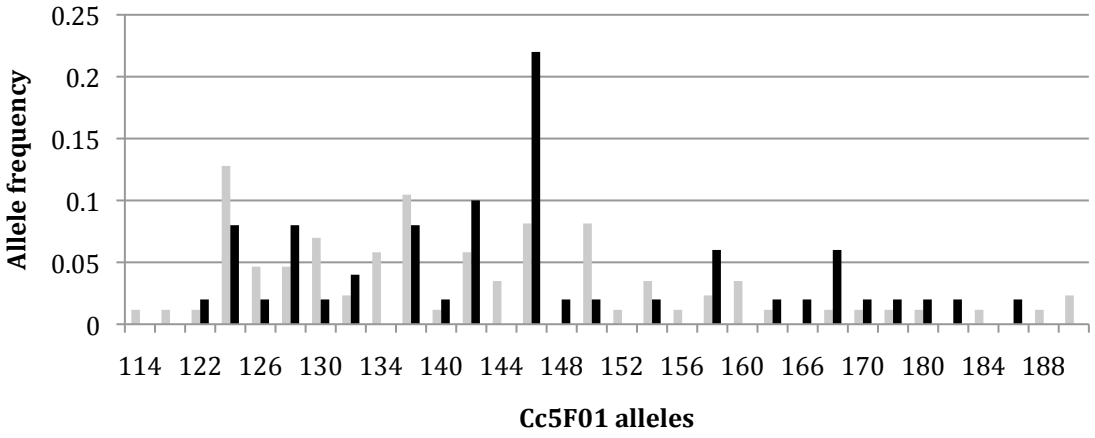
A


QR
SGI
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Allele frequency
115 123 125 127 129 131 133 135 137 139 141 143 145 147 149 151 153 163 165 167 173
Ccar176 alleles

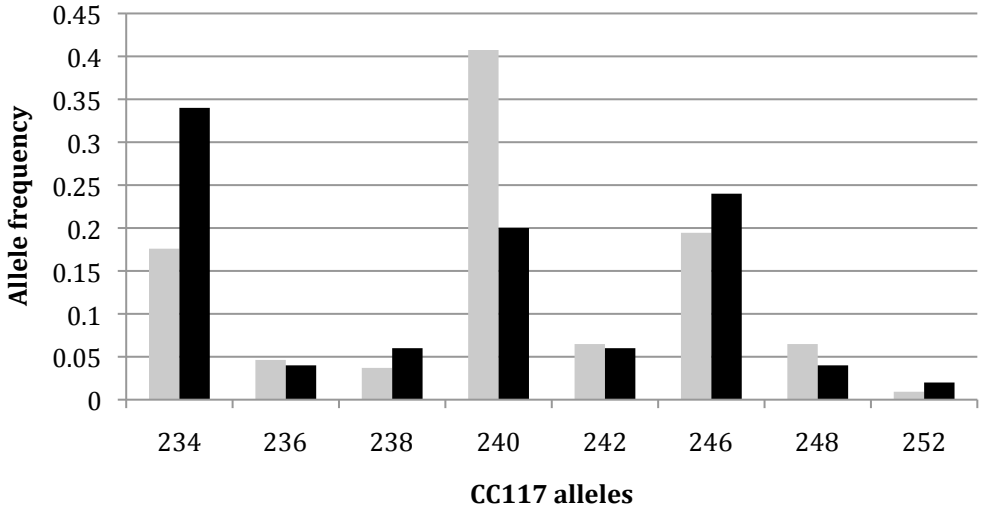
B


0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Allele frequency
265 269 275 277 279 281 283 285 287 289 291 293 295 297 299 303 305 307 309 311 317 321 329 333
Cc2G10 alleles

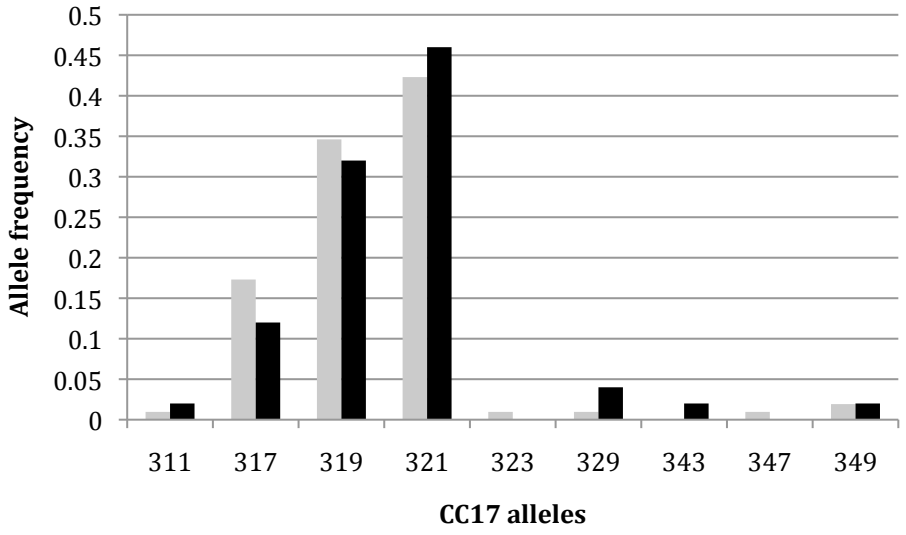
C


0.25
0.2
0.15
0.1
0.05
0
Allele frequency
114 122 126 130 134 140 144 148 152 156 160 166 170 180 184 188
Cc5F01 alleles

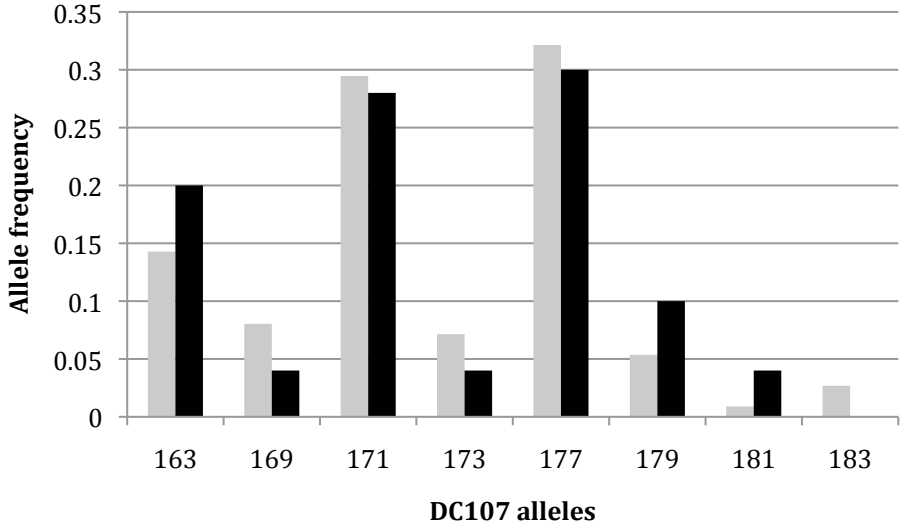
D


0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Allele frequency
234 236 238 240 242 246 248 252
CC117 alleles

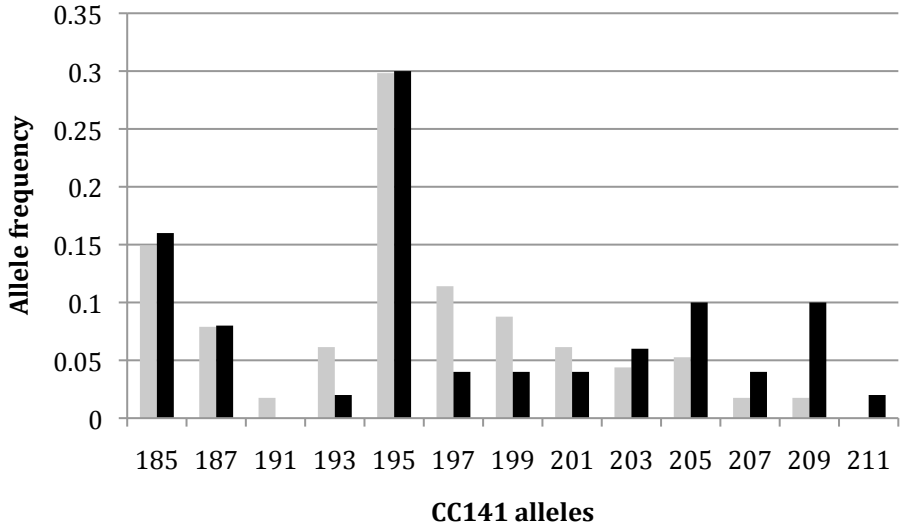
E


0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Allele frequency
311
317
319
321
323
329
343
347
349
CC17 alleles

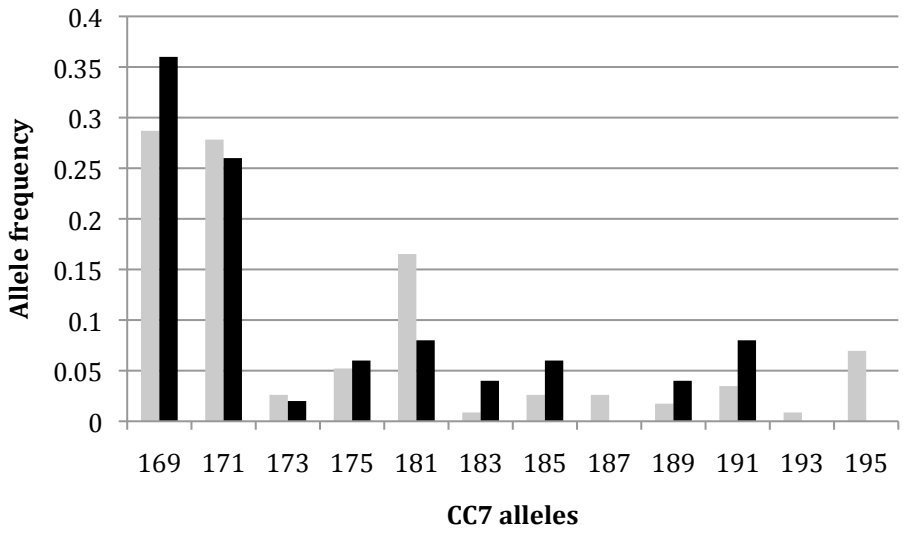
F


0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Allele frequency
163
169
171
173
177
179
181
183
DC107 alleles

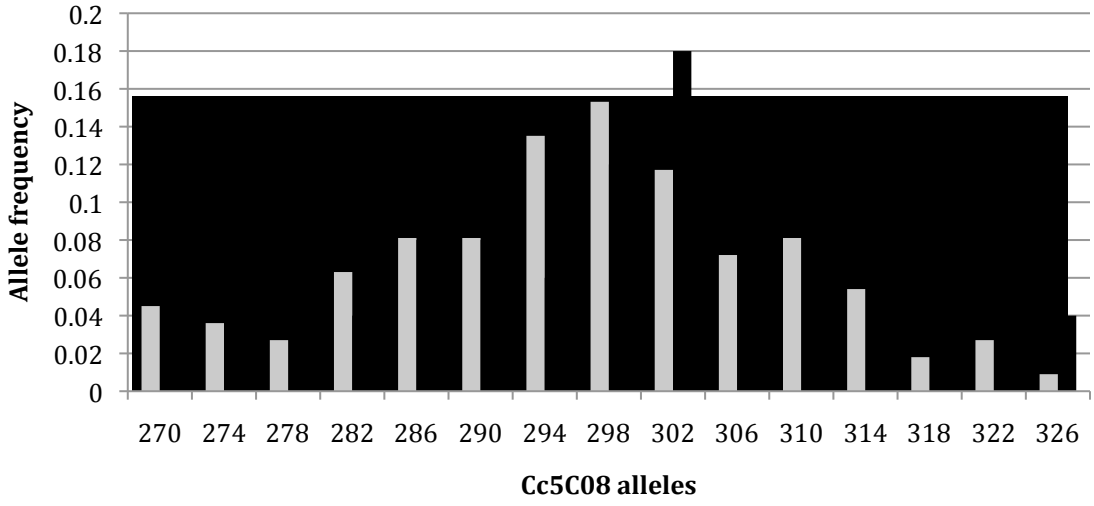
G


0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Allele frequency
185 187 191 193 195 197 199 201 203 205 207 209 211
CC141 alleles

H


0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Allele frequency
169 171 173 175 181 183 185 187 189 191 193 195
CC7 alleles

I



J

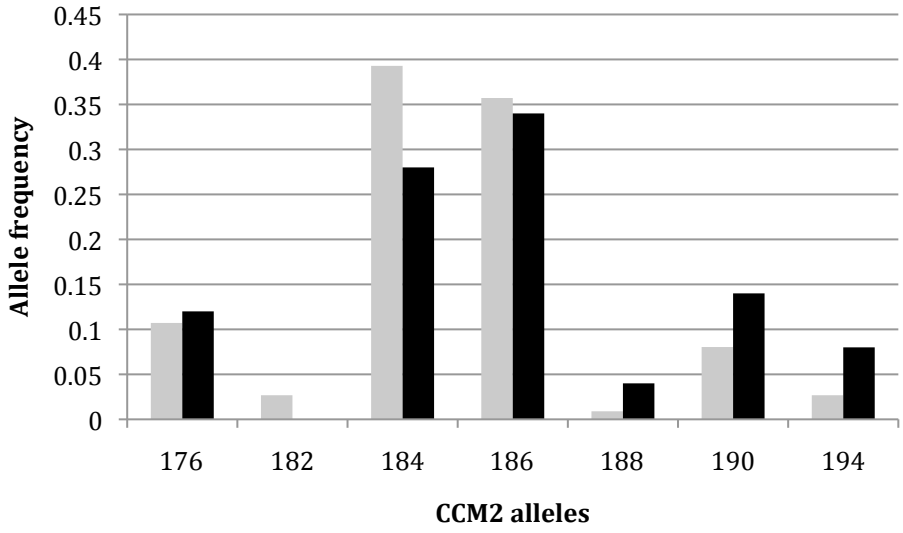


Figure 4.2. A-J: Population allele frequencies for the 10 microsatellite loci based on 58 individuals from Quintana Roo (QR) and 25 individuals from St. George Island (SGI).

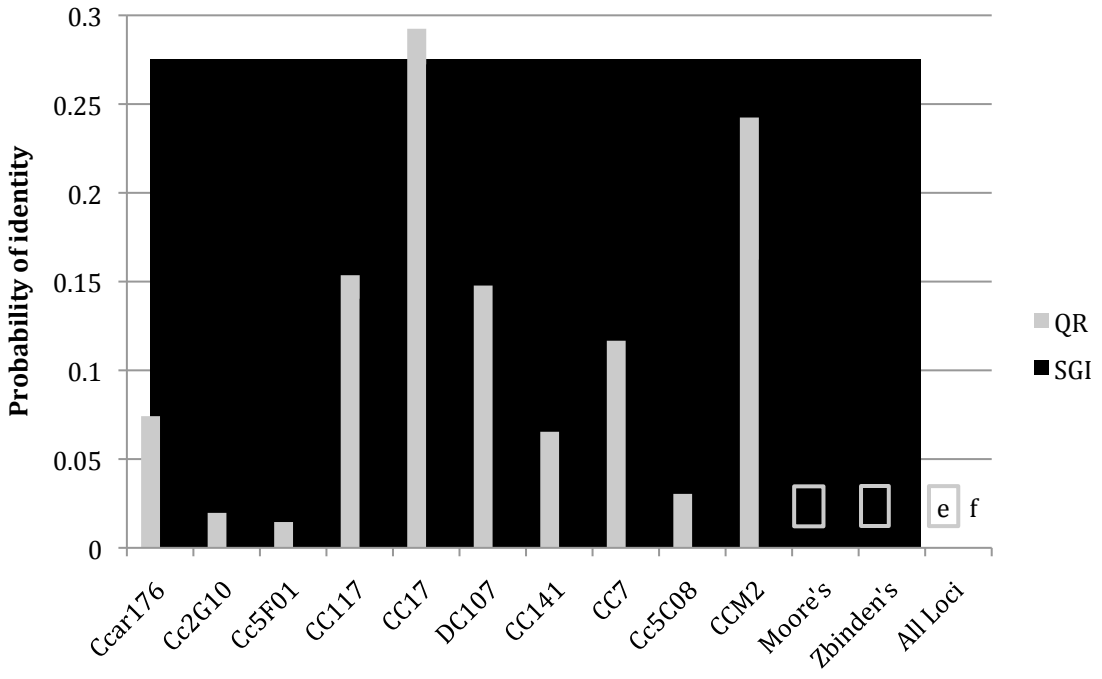


Figure 4.3. The probability of identity for each marker individually and combinations of markers calculated for Quintana Roo (QR) and St. George Island (SGI). Marker combinations: markers used by Moore and Ball (2002) (Moore's: Ccar176, CC141, CC7, CCM2), markers used by Zbinden et al. (2007) (Zbinden's: CC117, CC141, CC7, CCM2), and all 10 loci from this study. a: 1.37×10^{-4} b: 1.28×10^{-4} c: 2.84×10^{-4} d: 1.99×10^{-4} e: 7.93×10^{-12} f: 1.59×10^{-11}.

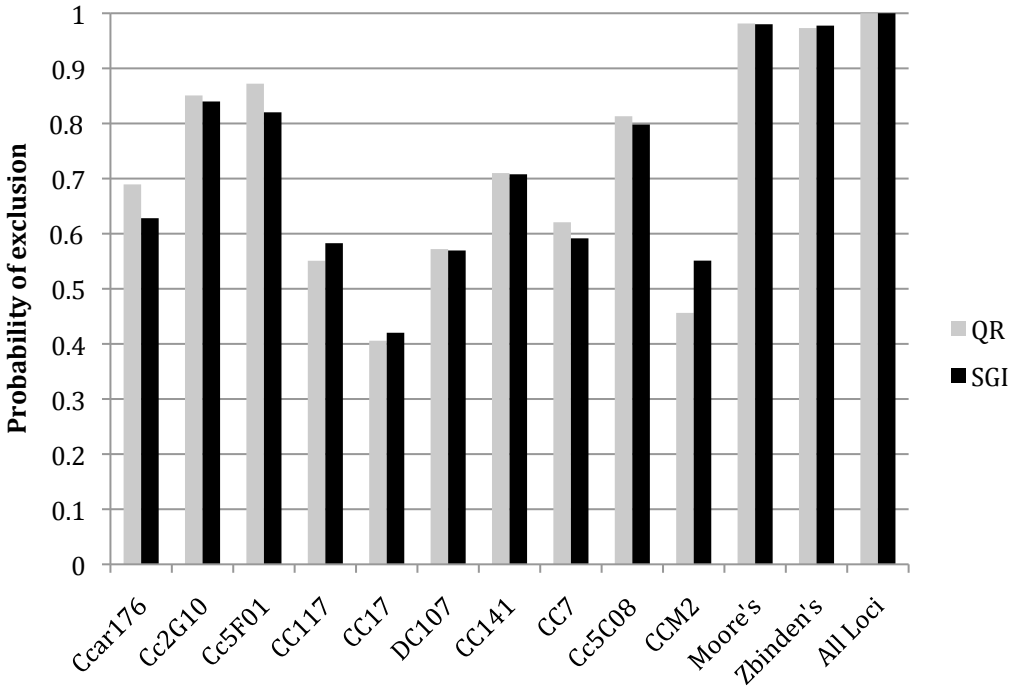


Figure 4.4. The probability of exclusion for each marker individually and combinations of markers calculated for Quintana Roo (QR) and St. George Island (SGI). Marker combinations: markers used by Moore and Ball (2002) (Moore's: Ccar176, CC141, CC7, CCM2), markers used by Zbinden et al. (2007) (Zbinden's: CC117, CC141, CC7, CCM2), and all 10 loci from this study.

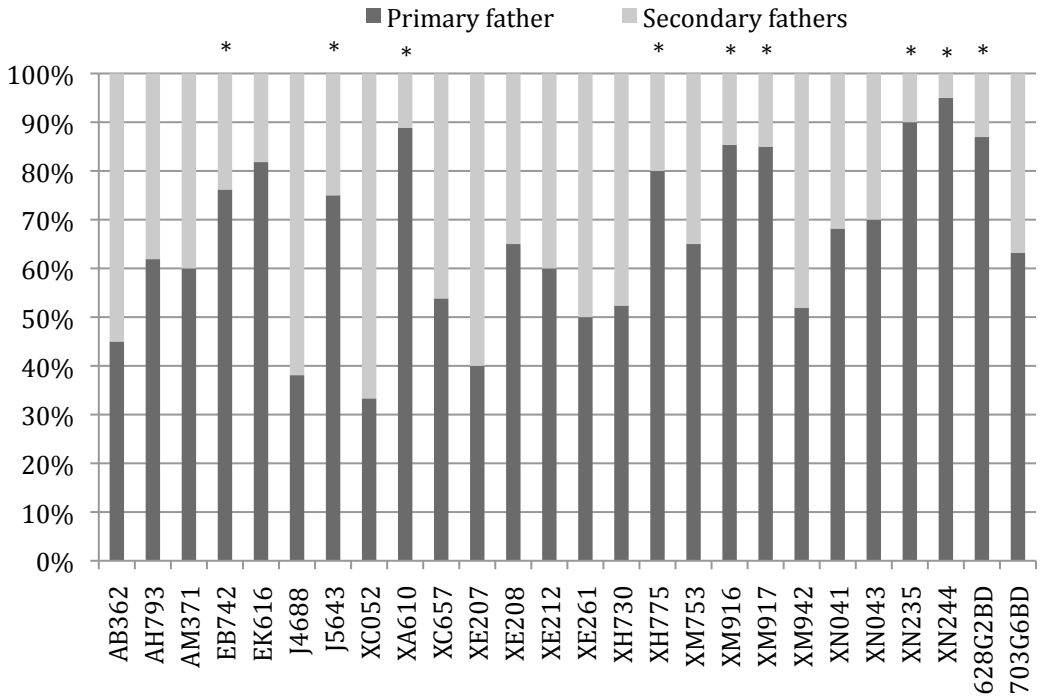


Figure 4.5. Contributions of the primary father and all other fathers combined (secondary fathers) for multiply-sired clutches in Quintana Roo and St. George Island. The contribution of the primary father in seven clutches could not be resolved and are not included in the figure. * indicates that the primary father sired more than half of the offspring (X^2, $p \leq 0.05$).

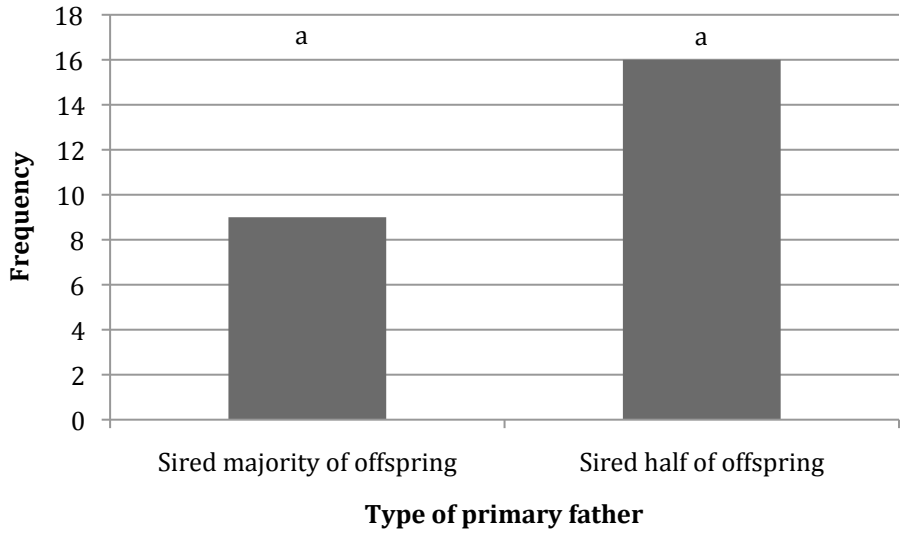


Figure 4.6. The two types of primary fathers (Quintana Roo and St. George Island combined) were equally likely ($X^2 = 1.96$, $p = 0.16$).

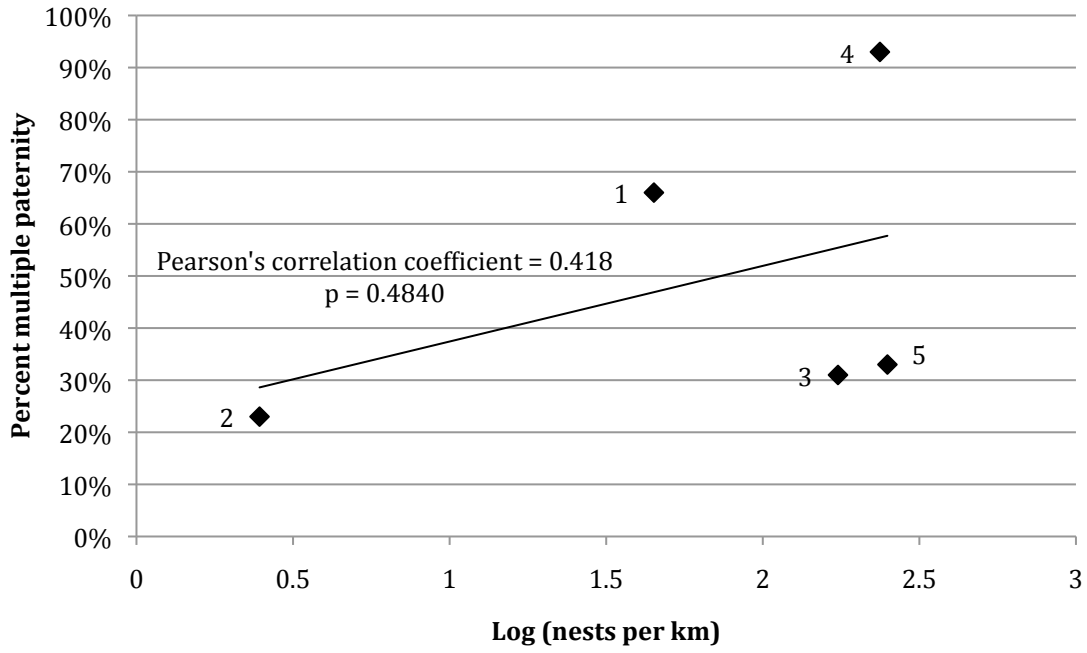


Figure 4.7. Multiple paternity plotted against the log of the nest density for all studies on loggerheads listed in Table 4.1 as well as the current study. Nest densities were calculated by dividing the seasonal nest numbers in each rookery by the length of the beach. Nest densities were derived using data from 1) Quintana Roo, Mexico (Flora, Fauna y Cultura de Mexico, A.C. 2006); 2) St. George Island, FL (Florida Fish and Wildlife Conservation Commission *unpublished data*); 3) Melbourne, FL (Bagley et al. 1996); 4) Zakynthos, Greece (Margaritoulis et al. 2003); and 5) Mon Repos, Australia (Limpus and Limpus 2003).

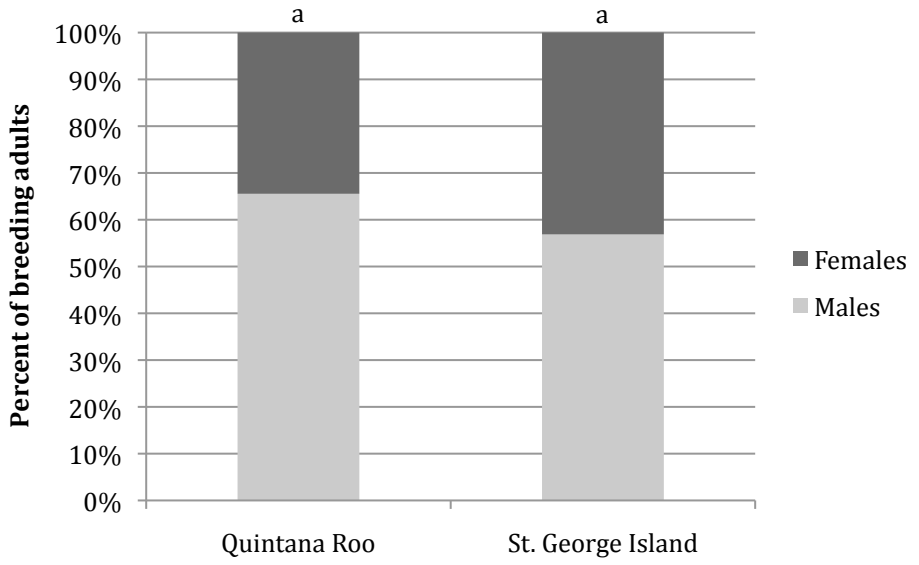


Figure 4.8. The proportion of males and females in each population that reproduced based on paternity analysis in COLONY. The operational sex ratio in Quintana Roo (78 males, 41 females) at 1.9 was not significantly different from the operational sex ratio in St. George Island (29 males, 22 females) at 1.3 (Fisher's exact test, $p = 0.3$).

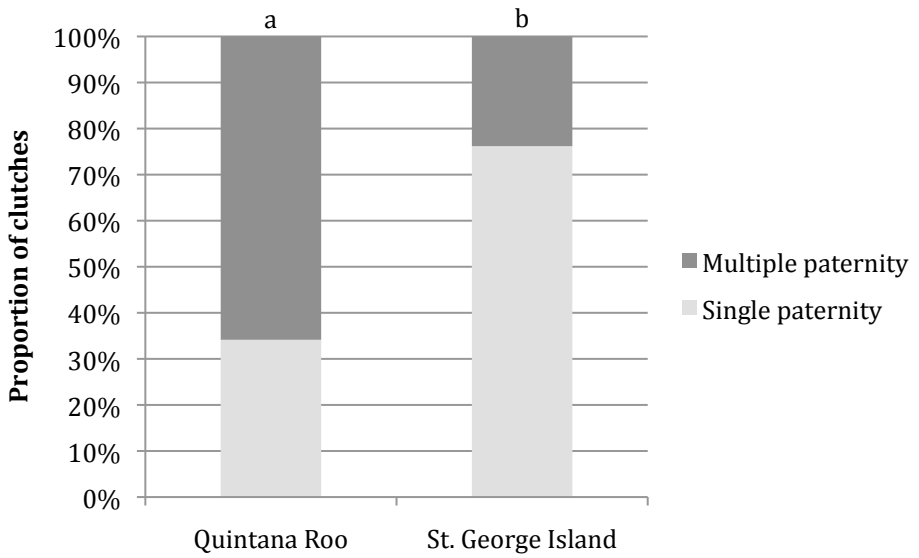


Figure 4.9. Proportion of clutches in each population with single or multiple paternity. Quintana Roo had significantly more clutches with multiple paternity (27 out of 41 clutches) than St. George Island (5 out of 22 clutches) (Fisher's exact test, p=0.002).

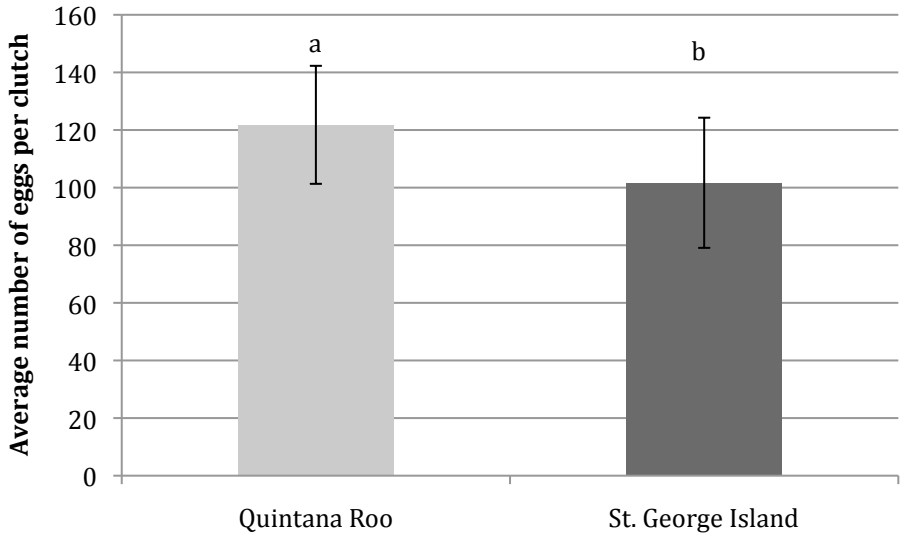


Figure 4.10. Average clutch sizes in Quintana Roo (n = 41) and St. George Island (n = 22). Clutch size in Quintana Roo is significantly greater than in St. George Island (Mann-Whitney *U*: z = -3.296, p=0.001). Error bars indicate one standard deviation.

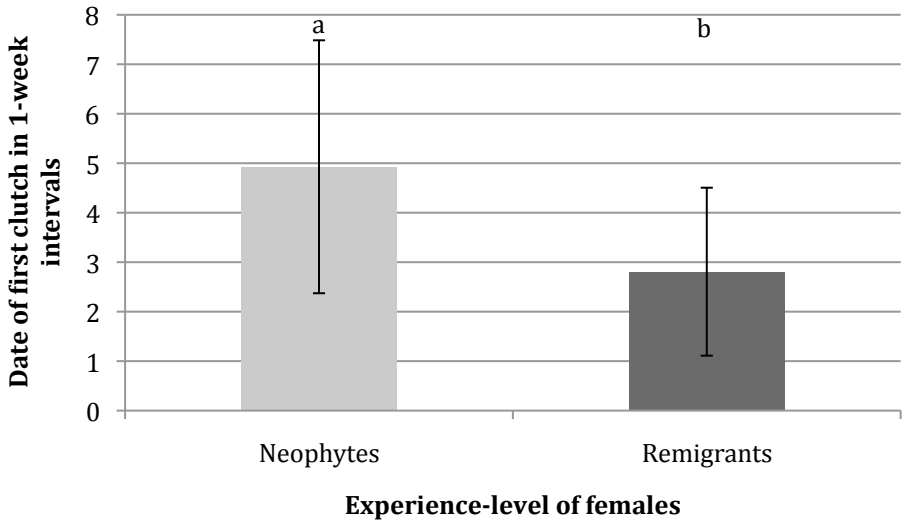


Figure 4.11. Average date of first clutch in one-week intervals starting May 7 for neophytes (n = 14) and remigrants (n = 27) in Quintana Roo. Neophytes started laying clutches significantly later in the season than remigrants (Mann-Whitney *U*: z = -3.183, p = 0.001). Error bars indicate one standard deviation.

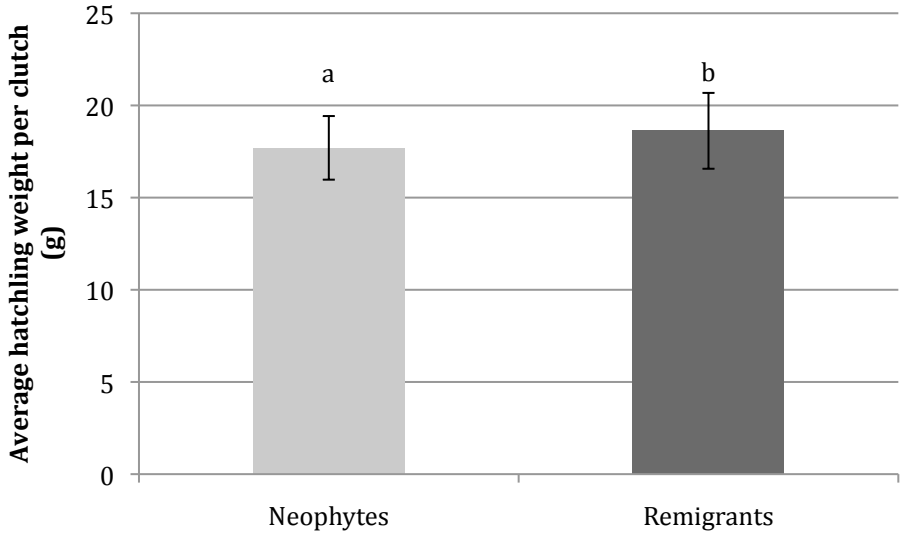


Figure 4.12. Average weight of hatchlings per clutch for neophytes (n = 14) was significantly less than for remigrants (n = 24) in Quintana Roo (Mann-Whitney *U*: z = -1.972, p = 0.049). Error bars indicate one standard deviation.

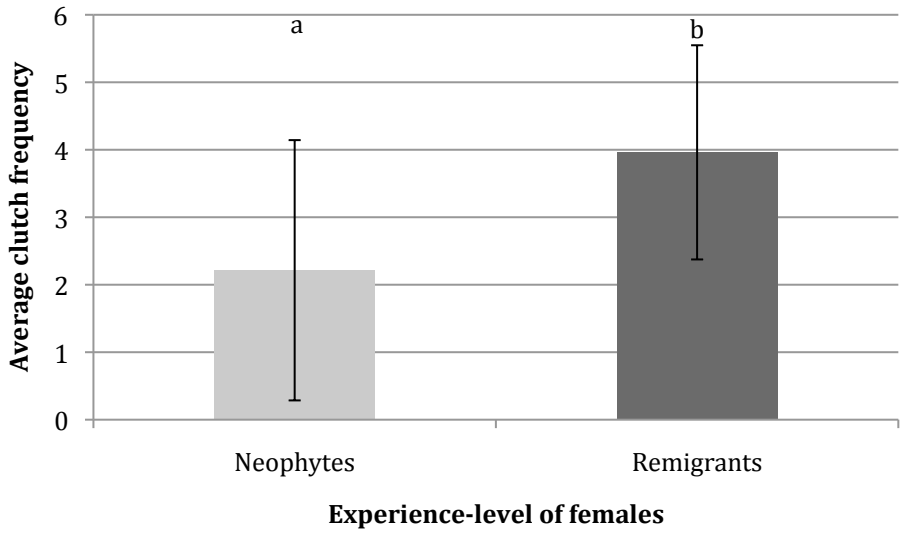


Figure 4.13. Neophytes (n = 14) laid on average significantly fewer number of clutches than remigrants (n = 27) in Quintana Roo (Mann-Whitney *U*: $z = -3.566$, $p < 0.001$). Error bars indicate one standard deviation.

Chapter 5

Conclusions

Substantial mtDNA evidence exists, complementing and adding to findings from mark-recapture studies, that support the philopatric behavior of female loggerhead turtles. This results in genetic stocks that are composed of genetically differentiated maternal lineages characterized by distinct frequencies of mtDNA haplotypes. Haplotype sharing among stocks and incomplete sampling of stocks continue to hinder stock resolution. This is especially of concern in areas where stocks converge, such as in foraging areas or migratory corridors.

With this work, I have increased sampling of two genetics stocks of loggerhead turtles, one in the Yucatan Peninsula in Mexico and one in northwestern Florida, to provide a more complete genetic profile of these stocks. In Mexico, I confirmed the presence of a common Atlantic haplotype (CC-A1) that previously had gone unreported in this stock. I uncovered additional haplotypes in both the Mexican and in the northwestern Florida stocks, including novel haplotypes that describe additional genetic diversity of the species.

To address the issue of sharing common haplotypes among stocks, such as the CC-A1 haplotype, primers were developed to analyze a longer segment of the mtDNA control region (Abreu-Grobois et al. 2006). When I applied these primers to samples from the Mexican and northwestern Florida stocks, several noteworthy haplotype divergences emerged. The most consequential may be the splitting of the ubiquitous CC-A1 haplotype into three long variants, one unique to the southeastern United States (CC-A1.1; present in northwestern Florida (this study) and Georgia (Monzón-Argüello et al. in

press)). The other two long variants (CC-A1.3 and CC-A1.4) are shared by the Mexican (this study) and Cape Verde (Monzón-Argüello et al. in press) stocks, although in very different frequencies. with CC-A1.4 being more common than CC-A1.3 in Mexico and vice versa in Cape Verde. As the long sequence analysis is applied to other regional stocks, the current geographic distribution of the longer haplotypes will change. But, the specificity of these longer haplotypes that I describe holds much promise for improving the resolution and accuracy of mixed stock analyses.

Due to the inherent difficulties of observing and directly sample males who spend their lives at sea, male migratory behavior remains elusive (Bowen and Karl 2007). A discordance in genetic patterns detected by mtDNA and nDNA can reveal insights into sex-biased dispersal. Also, a direct comparison of female and male genetic data can allow detection of sex-biased dispersal (Favre et al. 1997, Goudet et al. 2002). I circumvented the difficulty of directly sampling males by inferring paternal microsatellite genotypes from directly observed hatchling genotypes. With this approach, I demonstrated more male-mediated gene flow than female-mediated gene between the Mexican and the northwestern Florida stocks. This is consistent with a regional microsatellite study across the four genetic stocks in the southeastern United States (Bowen et al. 2005). Even though stocks are fairly isolated from one another in terms of the female lineage, influx of new genetic material is mediated by males, alleviating the potentially negative effects of inbreeding and genetic drift, especially in small populations such as the northwestern Florida stock. Male-mediated gene flow can be accomplished in two ways. Males can disperse, or they can home to their natal regions but mate with females from other stocks encountered in foraging areas or migratory

corridors. The evidence I found suggests that males that reproduced in Mexico likely consisted of both Mexican males and males from elsewhere. This is consistent with a scenario where males are philopatric but mate with females from other populations when the opportunity arises.

Precise natal site fidelity describes a situation where females not only return to their natal *region* to breed, but return precisely to their *natal* site within the region. This will results in genetically structured lineages within a region. Evidence of natal site fidelity in sea turtles has been mixed, for one of two reasons. Females may not be returning very precisely to their natal site, or the genetic markers and/or that statistics used to interpret the genetic signals may not be adequate to resolve fine-scale structuring (Lee 2008). Using my female and male microsatellite data, I applied both traditional F_{ST} and R_{ST} statistics as well as more recently developed individual-based assignment tests (e.g. Paetkau et al. 2004). There was little evidence of fine-scale genetic structuring within the Mexican stock. Mark-recapture data from females sampled in this study indicated that females mostly nested within a range of 5 km but also at distances of over 20 km, thereby connecting the northern and southern regions in Mexico. Also, the high amount of gene flow I detected between Cozumel and the southern region indicates high rates of breeder exchange. I found less connectivity between Cozumel and the northern region with F_{ST} and R_{ST}. Natal nest site fidelity within these to regions may be specific enough to result in slight but significant differentiation. The assignment test suggested that females in the northern region exhibited more natal site fidelity than in the southern region, although overall assignment probabilities were extremely low. The general tendency was for genetic cohesiveness of rookeries throughout Mexico.

The mating system of a species can play a role in its ability to recover from population declines and to adapt to environmental change and anthropogenic disturbances (Hutchings 2001, Bell et al. 2010). Many aspects of the mating system remain poorly understood, such as how it is affected by various population parameters. I characterized the patterns of multiple paternity in the Mexican and in the northwestern stocks to infer the relationships of various variables with the frequency of multiple paternity. I detected a significantly greater proportion of cutches with multiple paternity in Mexico than in northwestern Florida. None of the variables tested – female abundance, nest density, and operational sex ratio – were good predictors of the frequency of multiple paternity. In the ridley sea turtle genus, female abundance was a good predictor, with increased abundance associated with a higher frequency of multiple paternity (Jensen et al. 2006). This genus of sea turtle exhibits a unique mass nesting phenomenon called an "arribada," which consists of large numbers of females (100s to over 100,000) nesting en masse within a few days (Miller 1997, Hamann et al. 2003, Fonseca et al. 2009). The abundance of female nesters observed in other loggerheads and other sea turtle species pales in comparison. Ridley sea turtles also exhibit solitary nesting, which is the only form of nesting seen in other sea turtles species. The vast number of arribada females may trigger a mating frenzy, where potential mates are in such close proximity that males have optimal mating opportunities resulting in high rates of multiple paternity (92%), compared to solitary nesting females with a much lower frequency of multiple paternity (30%, Jensen et al. 2006). Abundance may not be a unique predictor of multiple paternity in ridley sea turtles, but the absence of arribadas in loggerheads precludes the observation of this potential correlation. In my study, the operational sex ratio was not

significantly different between Mexico and northwestern Florida, but I attribute this to the extremely small sample size from northwestern Florida. This decreased the power to detect any differences. Mate availability is paramount to reproduction and also to multiple paternity. If a female only encounters one mate, multiple paternity cannot happen. I argue that with a larger sample size the operational sex ratio may be associated with the frequency of multiple paternity. The method of estimating the operational sex ratio (of individuals who actually reproduced) that I used in this study may be especially useful to estimate the effective number of males in the population, i.e. the males who contribute to reproduction regardless of their natal origin. Climate change likely will effect primary sex ratios, resulting in an increased proportion of females (Hawkes et al. 2007). Because males are so much more difficult to survey than females, this indirect method of assessing the number of reproductively active males may be a viable option to detect trends in the sex ratio of reproductively active (and successful) individuals.

Finally, I found no support for the hypothesis that females mate multiply in order to acquire genetic benefits for their offspring and thereby increase their reproductive fitness, through such mechanisms as sperm competition (Fisher 1930, Madsen et al. 1992, Andersson 1994) and cryptic female choice (Trivers 1972, Zeh and Zeh 1996, Tregenza and Wedell 2000). Likely, the mating system is driven by males under selection to maximize their own reproductive output. I found that the experience-level of the female (first-time nester or experienced nester) was more significant than the number of mates in terms of her reproductive success, with reproductive success increasing with experience. The analysis of successive clutches laid by four females indicated that paternity of clutches remained constant throughout the season. This suggests that mating likely takes

place prior to nesting, which allows males to leave the breeding areas when females start to nest. The greatest source of sea turtle mortality is as bycatch in commercial fisheries (Witherington et al. 2009). Knowledge of migratory patterns may help guide commercial fishing regulations to minimize this industry's negative impact on sea turtle populations.

Historical exploitation of sea turtles worldwide has caused substantial population declines (McClenachan et al. 2006). While some populations have seen an increase in numbers due to legislative protection and implementation of conservation programs, the ability to recover is influenced by the species' fundamental biology (Rowe & Hutchings 2003). To ensure the persistence of the threatened loggerhead turtles (IUCN 2010), we need to continue to develop methods of stock identification to accurately assess the use of foraging areas and to protect populations at the lowest level of genetic differentiation. We need to better understand of the patterns of multiple paternity, which can help maintain genetic diversity, increase effective male population size, and increase the chances of passing on male genes to the next generation in case of nest or individual female reproductive failure (Galbraith 1993). Understanding and recognizing the interactions of population dynamics and mating behavior will become increasingly relevant to the conservation of this species in the face of climate change.

References

Abreu-Grobois FA, Horrocks J, Formia A, LeRoux R, Velez-Zuazo X, Dutton P, Soares L, Meylan P, Browne D (2006) New mtDNA Dloop primers which work for a variety of marine turtle species may increase the resolution capacity of mixed stock analysis. In: Book of abstracts of the 26th annual symposium on sea turtle biology and conservation (Eds. Frick M, Panagopoulou A, Rees AF, Williams K). Crete, Greece, 3–8 April 2006, p. 179.

Allendorf FW, Seeb LW (2000) Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. Evolution 54: 640-651.

Andersson M (1994) Sexual Selection. Princeton: Princeton University Press.

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Animal Behaviour 60: 145-164.

Avise JC (2000) Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA.

Avise JC, Jones AG, Walker DE, DeWoody JA (2002) Genetic mating systems and reproductive natural histories of fishes: Lessons for ecology and evolution. Annual Review of Genetics 36:19-45.

Bachtrog D, Weiss S, Zangerl B, Brem G, Scholotterer C (1999) Distribution of dinucleotide microsatellites in the Drosophila melanogaster genome. Molecular Biology and Evolution 16:601-610.

Bagley DA, Uong LT, Ehrhart LM (1998) Marine turtle nesting in the Archie Carr National Wildlife Refuge in 1996: a new record for leatherbacks. In: Proceedings of the Seventeenth International Sea Turtle Symposium (eds Epperly SP, Braun J), pp 130-131. US Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-415.

Baldwin R, Hughes GR, Prince RIT (2003) Loggerhead turtles in the Indian Ocean. In: Loggerhead Sea Turtles (eds. Bolten AB, Witherington BE). Smithsonian Books, Washington. Pp. 218-232.

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Molecular Ecology 11: 155-165.

Bashalkhanov S, Pandey M, Rajora OP (2009) A simple method for estimating genetic diversity in large populations from finite sample sizes. BMC Genetics 10: 84.

Bass AL, Epperly SP, Braun-McNeill J (2004) Multi-year analysis of stock composition of a loggerhead turtle (Caretta caretta) foraging habitat using maximum likelihood and Bayesian methods. Conservation Genetics 5: 783-796.

Bass AL, Good DA, Bjorndal KA, Richardson JI, Hillis Z-M, Horrocks JA, Bowen BW (1996) Testing models of female migratory beavior and population structure in the Caribbean hawksbill turtle, Eretmochelys imbricata, with mtDNA control region sequences. Molecular Ecology 5: 321-328.

Bateman AJ (1948) Intra-sexual selection in Drosophila. Heredity 2: 349-368.

Bell CD, Blumenthal JM, Broderick AC, Godley BJ (2010) Investigating potential for depensation in marine turtles: how low can you go? Conservation Biology 24: 226-235.

Berec L, Angulo E, Courchamp F (2007) Multiple Allee effects and population management. Trends in Ecology and Evolution 22: 185-191.

Berry JF, Shine R (1980) Sexual size dimorphism and sexual selection in turtles (Order Chelonia). Oecologia 44:185-191.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? Molecular Ecology 13: 551-561.

Birkhead TR (2000) Promiscuity. Harvard University Press, Cambridge, MA.

Birkhead TR, Møller AP (1998) Sperm competition, sexual selection and different routes to fitness. In Sperm Competition and Sexual Selection (eds. Birkhead TR, Møller AP). Academic Press. Pp. 757-781.

Birky CWJ, Marayama T, Fuerst P (1983) An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103:513-527.

Bjorndal KA, Meylan AB, Turner BJ (1983) Sea turtle nesting at Melbourne Beach, Florida, I. Size, growth, and reproductive biology. Biological Conservation 26: 65-77.

Boileau MG, Hebert PDN, Schwartz SS (1992) Non-equilibrium gene frequency divergence: persistent founder effects in natural populations. Journal of Evolutionary Biology 5: 25-39.

Bolker BM, Okuyama T, Bjorndal KA, Bolten AB (2003) Sea turtle stock estimation using genetic markers: accounting for sampling error of rare genotypes. Ecological Applications 13: 763-775.

Bolker BM, Okuyama T, Bjorndal KA, Bolten AB (2007) Incorporating multiple mixed stocks in mixed stock analysis: ‘‘many-to-many’’ analyses. Molecular Ecology 16: 685-695.

Bollmer JL, Irwin ME, Rieder JP, Parker PG (1999) Multiple paternity in loggerhead turtle clutches. Copeia 2: 475-478.

Bolten AB, Bjorndal KA, Martins HR, Dellinger T, Biscoito MJ, Encalada S, Bowen B (1998) Trans-Atlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analyses. Ecological Applications 8: 1-7.

Bonatto SL, Salzano FM (1997) A single and early migration for the peopling of the Americas supported by mitochondrial DNA sequence data. Proceedings of the National Academy of Sciences, USA, 94: 1866-1871.

Booth J, Peters JA (1972) Behavioural studies on the green turtle (Chelonia mydas) in the sea. Animal Behaviour 20:808-812.

Bowen BW, Abreu-Grobois FA, Balazs GH, Kamezaki N, Limpus CJ, Ferl RJ (1995) Trans-pacific migrations of the loggerhead turtle (Caretta caretta) demonstrated with mitochondrial DNA markers. Proceedings of the National Academy of Sciences, USA 92: 3731-3734.

Bowen BW, Avise JC, Richardson JI, Meylan AB, Margaritoulis D, Hopkins-Murphy SR (1993) Population structure of the loggerhead turtle (Caretta caretta) in the northwest Atlantic Ocean and Mediterranean Sea. Conservation Biology 7: 834-844.

Bowen BW, Bass AL, Garcia-Rodriguez A, Diez CE, Van Dam R, Bolten A, Bjorndal KA, Miyamoto MM, Ferl RJ (1996) The origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. Ecological Applications 6: 566-572.

Bowen BW, Bass AL, Soares L, Toonen RJ (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (Caretta caretta). Molecular Ecology 14: 2389-2402.

Bowen BW, Grant WS (1997) Phylogeography of the sardines (Sardinops spp.): assessing biogeographic models and population hisotires in temperate upwelling zones. Evolution 51: 1601-1610.

Bowen BW, Kamezaki N, Limpus CJ, Hughes JR, Meylan AB, Avice JC (1994) Global phylogeography of the loggerhead turtle (Caretta caretta) as indicated by mitochondrial DNA haplotypes. Evolution 48: 1820-1828.

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. Molecular Ecology 16: 234886-4907.

Broderick AC, Glen F, Godley BJ, Hays GC (2002) Estimating the number of green and loggerhead turtles nesting annually in the Mediterranean. Oryx 36: 227-235.

Broderick AC, Godley BJ, Hays GC (2001) Metabolic heating and the prediction of sex ratios for green turtles (Chelonia mydas). Physiological and Biochemical Zoology 74: 161-170.

Bronson FH (1979) The reproductive ecology of the house mouse. Quarterly Review of Biology 54: 265-299.

Brunelli JP, Steele CA, Thorgaard GH (2010) Deep divergence and apparent sex-biased dispersal revealed by a Y-linked marker in rainbow trout. Molecular Phylogenetics and Evolution 56: 983-990.

Bryja J, Patzenhauerová H, Albrecht T, Mošanský L, Stanko M, Stopka P (2008) Varying levels of female promiscuity in four Apodemus mice species. Behavioral Ecology and Sociobiology 63: 251-260.

Buckley HA, Johnson LR, Shackleton NJ, Blow RA (1982) Late glacial to recent cores from the eastern Mediterranean. Deep Sea Research 29: 739-766.

Buonaccorsi VP, McDowell JR, Graves JE (2001) Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (Makaira nigricans). Molecular Ecology 10: 1179-1196.

Buonaccorsi VP, Reece KS, Morgan LW, Graves JE (1999) Geographic distribution of molecular variance within the blue marlin (Makaira nigricans): a hierarchical analysis of allozyme, single-copy nuclear DNA, and mitochondrial DNA markers. Evolution 53: 568-579.

Bustard R (1972) Sea Turtles: Natural History and Conservation. Collins, London.

Carr AF, Carr MH, Meylan AB (1978) The ecology and migration of sea turtles, 7. The West Caribbean green turtle colony. Bulletin of the American Museum of Natural History, 162, 1–46.

Carreras C, Pascual M, Cardona L, Aguilar A, Margaritoulis D, Rees A, Turkozan O, Levy Y, Gasith A, Aureggi M, Khalil M (2007) The genetic structure of the loggerhead sea turtle (Caretta caretta) in the Mediterranean as revealed by nuclear and mitochondrial DNA and its conservation implications. Conservation Genetics 8: 761-775.

Casale P, Freggi D, Gratton P, Argano R, Oliviero M (2008). Mitochondrial DNA reveals regional and interregional importance of the central Mediterranean African shelf for loggerhead turtles (Caretta caretta). Scientia Marina 72: 541-548.

Casale P, Laurent L, Gerosa G, Argano R (2002) Molecular evidence of male-biased dispersal in loggerhead turtle juveniles. Journal of Experimental Marine Biology and Ecology 267: 139-145.

Chapman RW (1996) A mixed stock analysis of the green turtle: the need for null hypotheses. In: Proceedings of the international symposium on sea turtle conservation genetics (Eds. Bowen BW, Witzell WN). NOAA Technical Memorandum NMFSSEFSC- 396, pp 137–146.

Chávez G, Candela J, Ochoa J (2003) Subinertial flows and transports in Cozumel Channel. Journal of Geophysical Research 108(C2), 3037, doi: 10.1029/2002JC001456.

Clarke AB (1978) Sex ratio and local resource competition in a prosimian primate. Science, NY, 201: 163-165.

Clarke AL, Sæther B-E, Røskaft E (1997) Sex biases in avian dispersal: a reappraisal. Oikos 79: 429-438.

Clarke KE, Rinderer TE, Franck P, Quezada-Euán JG, Oldroyd BP (2002) The Africanization of honeybees (Apis mellifera) of the Yucatan: A study of a massive hybridization event across time. Evolution 56: 1462-1474.

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657-1660.

Clutton-Brock TH (1989) Mammalian mating systems. Proceedings of the Royal Society of London B 236: 339-372.

Congdon JD (1989) Proximate and evolutionary constraints on energy relations of reptiles. Physiological Zoology 62: 356-373.

Cornuet JM, Piry S, Luikart G, Estoup A, and Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000.

Crim JL, Spotila LD, Spotila JR et al. (2002) The leatherback turtle, Dermochelys coriacea, exhibits both polyandry and polygyny. Molecular Ecology, 11:2097–2106.

Darwin C (1839) Journal of Researches into the Geology and Natural History of the Various Countries Visited by H.M.S. Beagle (1832-1836). London: H. Colburn.

de Waal FBM (1995) Bonobo sex and society. Scientific American 58-64.

Dean MD, Ardlie KG, Nachman MW (2006) The frequency of multiple paternity suggests that sperm competition is common in house mice (Mus domesticus). Molecular Ecology 15: 4141-4151.

Dempster A, Laird N, Rubin D (1977) Maximum likelihood estimation from incomplete data via the EM algorithm. Journal of the Royal Statistical Society 39: 1-38.

Dewoody J, Nason JD, Hipkins VD (2006) Mitigating scoring errors in microsatellite data from wild populations. Molecular Ecology Notes 6: 951-957.

Dodd CK (1988) Synopsis of the biological data on the loggerhead sea turtle Caretta caretta (Linnaeus 1758). US Fish and Wildlife Service, Washington, DC.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. Molecular Ecology 11: 2571-81.

Ehrhart LM, Bagley DA, Redfoot WE (2003) Loggerhead turtles in the Atlantic Ocean: geographic distribution, abundance and population status. In: Loggerhead Sea Turtles (eds. Bolten AB, Witherington BE). Smithsonian Books, Washington. Pp. 157-174.

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. Science, New Series 197: 215-223.

Encalada SE, Bjorndal KA, Bolten AB, Zurita JC, Schroeder B, Possardt E, Sears CJ, Bowen BW (1998) Population structure of loggerhead turtle (Caretta caretta) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences. Marine Biology 130: 567-575.

Engelhaupt D, Hoelzel AR, Nicholson C, Frantzis A, Mesnick S, Gero S, Whitehead H, Rendell L, Miller P, De Stafanis R, Cañadas A, Airoldi S, Mignucci-Giannoni AA (2009) Female philopatry in coastal basins and male dispersion across the North Atlantic in a highly mobile marine species, the sperm whale (Physeter macrocephalus). Molecular Ecology 18: 4193-4205.

Engstrom TN, Meylan PA, Meylan AB (2002) Origin of juvenile loggerhead turtles (Caretta caretta) in a tropical developmental habitat in Caribbean Panamá. Animal Conservation 5: 125-133.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetic analyses under Linux and Windows. Molecular Ecology Resources. In press.

Excoffier L, Slatkin M (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Molecular Biology and Evolution 12: 921-927.

Excoffier L, Slatkin M (1998) Incorporating genotypes of relatives into a test of linkage disequilibrium. American Journal of Human Genetics 171-180.

Faaborg J, Parker PG, Delay L, de Vries TJ, Bernardz JC, Maria Paz S, Naranjo J, Waite TA (1995) Confirmation of co-operative polyandry in the Galapagos hawk (Buteo galapagoensis). Behavioural Ecology and Sociobiology 36: 83-90.

Fabiani A, Hoelzel AR, Galimberti F, Muelbert MMC (2003) Long-range paternal gene flow in the southern elephant seal. Science, New Series 299: 676.

Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal Crocidura russula: evidence from field data and microsatellite patterns. Proceedings of the Royal Society of London. Series B, Biological Sciences 264: 127-132.

Fisher RA (1930) The Genetical Theory of Natural Selection. Oxford: Clarendon Press.

Fitze PS, Le Galliard JF, Federici P, Richard M, Clobert J (2005) Conflict over multiple-partner mating between males and females of the polygyandrous common lizards. Evolution 59:2451-2459.

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

FitzSimmons NN, Limpus CJ, Norman JA et al. (1997b) Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proceedings of the National Academy of Sciences, USA 94: 8912-8917.

FitzSimmons NN, Moritz C, Limpus CJ , Pope L, Prince R (1997a) Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Genetics 147: 1843-1854.

FitzSimmons NN, Moritz C, Moore S (1995) Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Molecular Biology and Evolution 12: 432-440.

Flora, Fauna y Cultura de México, A.C. (2006) Programa de Protección y Conservación de Tortugas Marinas en el Litoral Central del Estado de Quintana Roo, Informe Final Temporada 2006.

Flora, Fauna y Cultura de México, A.C. (2008) Programa de Protección y Conservación de Tortugas Marinas en el Litoral Central del Estado de Quintana Roo, Informe de Trabajo y Logros Temporada 2008.

Fonseca LG, Murillo GA, Guadamúz L, Spínola RM, Valverde R (2009) Downward but stable trend in the abundance of arribada olive ridley sea turtles (Lepidochelys olivacea) at Nancite Beach, Costa Rica (1971–2007). Chelonian Conservation and Biology 8: 19-27.

Forbes SH, Hogg JT, Buchanan FC, Crawford AM, Allendorf FW (1995) Microsatellite evolution in congeneric mammals – domestic and bighorn sheep. Molecular Biology and Evolution 12: 1106-1113.

Francisco A (2001) Population genetics of the loggerhead turtle (Caretta caretta) in the Northwest Atlantic. MS Thesis, University of Florida.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conservation Biology 10: 1500-1508.

Frick MG, Slay CK, Quinn CA, Windham-Reid A, Duley PA, Ryder CM, Morse LJ (2000) Aerial observations of courtship behavior in loggerhead sea turtles (Caretta caretta) from southeastern Georgia and northeastern Florida. Journal of Herpetology 34: 153-158.

Gaggiotti OE, Lange O, Rassmann K, Gliddons C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Molecular Ecology 8: 1513-1520.

Garduno-Andrade M, Guzman V, Miranda E, Briseno-Duenas R, Abreu-Grobois A (1999) Increases in Hawksbill turtle (Eretmochelys imbricata) nestings in the Yucatán Peninsula, Mexico, 1977–1996: data in support of successful conservation. Chelonian Conservation Biology 3: 286-295.

Garofalo L, Mingozzi T, Micò A, Novelletto A (2009) Loggerhead turtle (Caretta caretta) matrilines in the Mediterranean: further evidence of genetic diversity and connectivity. Marine Biology 156: 2085–2095.

Godley BJ, Broderick AC, Downie JR, Glen F and others (2001) Thermal conditions in nests of loggerhead turtles: further evidence suggesting female skewed sex ratios of hatchling production in the Mediterranean. Journal of Experimental Marine Biology and Ecology 263: 45-63.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. Molecular Ecology 11: 1103-1114.

Goudet J, Raymond M, de Meeus T, Rousset F (1996) Testing differentiation in diploid populations. Genetics 139: 463-471.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28: 1140-1162.

Griffith S, Owens I, Thuman K (2002) Extrapair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11: 2195-2212.

Hamann M, Colin JL, Owens DW (2003) Reproductive cycles of male and females. In: The Biology of Sea Turtles (eds. Lutz PL, Musick JA, Wyneken J). CRC Press, Boca Raton, Florida. Pp 135-161.

Hamilton WD (1967) Extraordinary sex ratios. Science156: 477-488.

Harry JL, Briscoe DA (1988) Multiple paternity in the loggerhead turtle (Caretta caretta). Journal of Heredity 79: 96-99.

Hartl DL, Clark GC (1997) Principles of Population Genetics. 3rd edition. Sinauer Associates, Sunderland, MA.

Hatase H, Kinoshita M, Bando T, Kamezaki N, Sato K, Matsuzawa Y, Goto K, Omuta K, Nakashima Y, Takeshita H, Sakamoto W (2002) Population structure of loggerhead turtles, Caretta caretta, nesting in Japan: bottlenecks on the Pacific population. Marine Biology 141: 299–305.

Hawkes LA, Broderick AC, Godfrey MH, Godley BJ (2007) Investigating the potential impacts of climate change on a marine turtle population. Global Change Biology 13: 923-932.

Hays GC, Broderick AC, Glen F, Godley BJ, Houghton JDR, Metcalfe JD (2002) Water temperature and internesting intervals for loggerhead (Caretta caretta) and green (Chelonia mydas) sea turtles. Journal of Thermal Biology 27: 429-432.

Hedgpeth JW (1954) An introduction to the zoogeography of the northern Gulf of Mexico with reference to the invertebrate fauna. Pages 111-211 in Publication 3. Institute of Marine Science, University of Texas, Port Aransas, Texas.

Hendrickson JR (1958) The green sea turtle, Chelonia mydas (Linn.) in Malaya and Sarawak. Proceedings of the Zoological Society of London 130: 455-535.

Hildebrand H(1987) A reconnaissance of beaches and coastal waters from the border of Belize to the Mississippi River as habitats for marine turtles. National Oceanographic and Atmospheric Administration, National Marine Fisheries Service SEFC Panama City, FL.

Hoekert WEJ, Neufeglise H, Schouten AD, Menken SBJ (2002) Multiple paternity and female-biased mutation at a microsatellite locus in the olive ridley sea turtle (Lepidochelys olivacea). Heredity 89: 107-113.

Hoelzel AR, Hey J, Dahlheim ME, Nicholson C, Burkanov V, Black N (2007) Evolution of population structure in a highly social top predator, the killer whale. Molecular Biology and Evolution 24: 1407-1415.

Hoelzel AR, Potter CW, Best PB (1998) Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. Proceedings of the Royal Society of London 265: 1177-1183.

Holm S (1979) A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6: 65-70.

Hunter FM, Davis LS (1998) Female Adelie penguins acquire nest material from extrapair males after engaging in extrapair copulations. Auk 115: 526-528.

Ireland JS, Broderick AC, Glen F, Godley BJ, Hays GC, Lee PLM, Skibinksi DOF (2003) Mulitple 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.

IUCN (2010) IUCN Red List of Threatened Species. Version 2010.3.

Jablonski P, Vepsäläinen K (1995) Conflict between sexes in the water strider Gerris lacustris: a test of two hypotheses for male guarding behavior. Behavioral Ecology 6:388-392.

Jaccard J, Guilamo-Ramos V (2002) Analysis of variance frameworks in clinical child and adolescent psychology: issues and recommendations. Journal of Clinical Child and Adolescent Psychology 31: 130-146.

Janzen FJ (1994) Climate change and temperature-dependent sex determination in reptiles. Proceedings of the National Academy of Sciences USA 91: 7487-7490.

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biological Reviews of the Cambridge Philosophical Society 75: 21-64.

Jensen MP, Abreu-Grobois FA, Frydenberg J and Loescke V (2006) Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. Molecular Ecology 15:2567-2575.

Jirotkul M (1999) Population density influences male-to-male competition in guppies. Animal Behaviour 58: 1169-1175.

Jones AG (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.

Jones AG, Rosenqvist G, Berglund A, Avise JC (2005) The measurement of sexual selection using Bateman's principles: an experimental test in the sex-role-reversed pipefish Syngnathus typhle. Integrative and Comparative Biology 45:874–884.

Jones OR, Wang J (2010a) COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10: 551–555.

Jones OR, Wang J (2010b) Molecular marker-based pedigrees for animal conservation biologists. Animal Conservation 13:26-34.

Kichler K, Holder M, Davis S, Marquez-M R & Owens D (1999) Detection of multiple paternity in the Kemp's ridely sea turtle with limited sampling. Mol Ecol 8: 819-830.

Koch AL, Carr AF, Ehrenfeld DW (1969) The problem of open-sea navigation: the migration of the green turtle to Ascension Island. Journal of Theoretical Biology 22: 163-179.

Laurent L, Casale P, Bradai MN, Godley BJ, Gerosa G, Broderick AC, Schroth W, Schierwater B, Levy AM, Freggi D, Abd El-Mawla EM, Hadoud DA, Gomati HE, Domingo M, Hadjichristophorou M, Kornaraky L, Demirayak F, Gautier CH (1998) Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. Molecular Ecology 7: 1529-1542.

Laurent L, Lescure J, Excoffier L, Bowen B, Domingo M, Hadjichristophorou M, Kornaraky L, Trabuchet G (1993) Genetic studies of relationships between Mediterranean and Atlantic populations of loggerhead Caretta caretta with a mitochondrial marker. Comptes Rendus de l'Académie Des Sciences, Paris 316: 1233-1239.

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

Lee P, Hays G (2004) Polyandry in a marine turtle: females make the best of a bad job. Proc Natl Acad Sci 101: 6530-6535.

Lee P, Luschi P, Hays GC (2007) Detecting female precise natal philopatry in green turtles using assignment methods. Molecular Ecology 16: 61-74.

Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451-1452.

Liermann K, Hilborn R(2001) Depensation: evidence, models and implications. Fish and Fisheries 2: 33-58.

Limpus CJ (1985) A study of the loggerhead sea turtle, Caretta caretta, in eastern Australia. University of Queensland, PhD thesis.

Limpus CJ (1993) The green turtle, Chelonia mydas, in Queensland: breeding males in the southern Great Barrier Reef. Wildlife Research 20: 513-523.

Limpus CJ, Limpus DJ (2003) Loggerhead turtles in the equatorial and southern Pacific Ocean: a species in decline. In In: Loggerhead Sea Turtles (eds. Bolten AB, Witherington BE). Smithsonian Books, Washington. Pp. 199-209.

Limpus CJ, Miller JD, Parmenter CJ, Reimer D, McLachlan N, Webb R (1992) Migration of green (Chelonia mydas) and loggerhead (Caretta caretta) turtles to and form eastern Australian rookeries. Wildlife Research 19: 347-358.

Loehle C (1997) The pathogen transmission avoidance theory of sexual selection. Ecological Modelling 103: 231-250.

Longmire JL, Gee GF, Hardekopf CL, Mark GA (1992) Establishing paternity in whooping cranes (Grus americana) by DNA analysis. Auk 109:522-529.

Lu G, Basley DJ, Bernatchez L (2001) Contrasting patterns of mitochondrial DNA and microsatellite introgressive hybridization between lineages of lake whitefish (Coregonus clupeaformis); relevance for speciation. Molecular Ecology 10: 965-985.

Lugon-Moulin N, Brünner H, Wyttenbach A, Hausser J, Goudet J (1999) Hierarchical analysis of genetic differentiation in a hybrid zone of Sorex araneus (Insectivora, Soricidae). Molecular Ecology 8: 419-431.

Lund F (1986) Nest production and nesting-site tenacity of the loggerhead turtle, Caretta caretta, on Jupiter Island, Florida. Master's Thesis, University of Florida, Gainesville.

Madsen T, Shine R, Loman J & Haakansson T (1992) Why do female adders copulate so frequently? Nature 355: 440-441.

Margaritoulis D, Argano R, Baran I, Bentivegna F, Bradai MN, Camiñas JA, Casale P, De Metrio G, Demetropoulos A, Gerosa G, Godley BJ, Haddoud DA, Houghton J, Laurent L, Lazar B (2003) Loggerhead turtles in the Mediterranean Sea: present knowledge and conservation perspectives. In: In: Loggerhead Sea Turtles (eds. Bolten AB, Witherington BE). Smithsonian Books, Washington. Pp. 175-198.

McClenachan L, Jackson JBC, Newman MJH (2006) Conservation implications of historic sea turtle nesting beach loss. Frontiers in Ecology and the Environment 6: 290-296.

McTaggart SJ (2000) Good genes or sexy sons? Testing the benefits of female choice in the painted turtle, Chrysemys picta. MS Thesis, University of Guelph.

Meylan AB, Bowen BW, Avise JC (1990) A genetic test of the natal homing versus social facilitation models for green turtle migration. Science 248: 724-727.

Miller JD (1997) Reproduction in sea turtles. In: The Biology of Sea Turtles (eds Lutz PL, Musick JA) pp 51-81. CRC Press: Boca Raton, Florida.

Miller JD, Limpus CJ, Godfrey MH (2003) Nest site selection, oviposition, eggs, development, hatching, and emergence of loggerhead turtles. In: Loggerhead Sea Turtles (eds. Bolten AB, Witherington BE). Smithsonian Books, Washington. Pp. 125-143.

Monzón-Argüello C, Muñoz J, Marco A, López-Jurado LF, Rico C (2008) Twelve new polymorphic microsatellite markers from the loggerhead sea turtle (Caretta caretta) and cross-species amplification on other marine turtle species. Conservation Genetics 9:1045–1049

Monzón-Argüello C, Rico C, Naro-Maciel E, Varo-Cruz N, López P, Marco A, López-Jurado LF (in press) Population structure and conservation implications for the loggerhead sea turtle of the Cape Verde Islands. Conservation Genetics.

Moore MK and Ball M Jr (2002) Multiple paternity in loggerhead turtle (Caretta caretta) nests on Melbourne Beach, Florida: a microsatellite analysis. Molecular Ecology 11:281-288.

Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100: 403-405.

Mrosovsky N, Dutton PH, Whitmore CP (1984) Sex ratios of two species of sea turtle nesting in Suriname. Canadian Journal of Zoology 62: 2227-2239.

Mrosovsky N, Provancha J (1992) Sex ratio of hatchling loggerhead sea turtles: Data and estimates from a 5-year study. Canadian Journal of Zoology 70: 530-538.

Neff B, Pitcher T (2002) Assessing the statistical power of genetic analyses to detect multiple mating in fish. J Fish Biol 61: 739-750.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York, NY, USA.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution 29: 1-10.

Norman JA, Moritz C, Limpus CJ (1994) Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. Molecular Ecology 3: 363-373.

Olsson M, Madsen T (2001) Promiscuity in sand lizards (Lacerta agilis) and adder snakes (Vipera berus): causes and consequences. Journal of Heredity 92: 190-197.

Owens D, Ruiz G (1980) New methods for obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica 36: 17-20.

Owens DW, Grassman MA, Hendrickson JR (1982) The imprinting hypothesis and sea turtle reproduction. Herpetologica 38: 124-135.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4:347-354.

Paetkau D, Slade R, Burden M, Estoup A (2004) Direct, real-time estimation of migration rate using assignment methods: a simulation-based exploration of accuracy and power. Molecular Ecology 13: 55-65.

Pardini AT, Jones CS, Noble LR, Kreiser B, Malcolm H, Bruce BD, Stevens JD, Cliff G, Scholl MC, Francis M, Duffy CAJ, Martin AP (2001) Sex-biased dispersal of great white sharks. Nature 412: 139-140.

Pearce AF (2001) Contrasting population structure of the loggerhead turtle (Caretta caretta) using mitochondrial and nuclear DNA markers. MS Thesis. University of Florida.

Peare T, Parker PG (1996) Local genetic structure within two rookeries of Chelonia mydas (the green turtle). Heredity 77: 619-628.

Pearse DE, Avise JC (2001) Turtle mating systems: behavior, sperm storage, and genetic paternity. Journal of Heredity 92:206–211.

Pearse DE, Crandall KA (2004) Beyond FST: analysis of population genetic data for conservation. Conservation Genetics 5: 585-602.

Pearse DE, Janzen FJ, Avice JC (2002) Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (Chrysemys picta) in nature. Behav Ecol Sociobiol 51:164-171.

Pella J, Masuda M (2001) Bayesian methods for analysis of stock mixtures from genetic characters. Fishery Bulletin 99: 151-167.

Perrin N, Mazalov V (2000) Local competition, inbreeding, and the evolution of sex-biased dispersal. The American Naturalist 144: 116-127.

Piry S, Alapetite A, Cornuet J-M et al. (2004) Geneclass2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95: 536-539.

Plotkin P (2003) Adult migrations and habitat use. In: The Biology of Sea Turtles (Eds. Lutz P, Musick J, Wyneken J). CRC Press, Boca Raton, Florida, pp. 225-241.

Prosser MR, Weatherhead PJ, Gibbs HL, Brown GP (2002) Genetic analysis of the mating system and opportunity for sexual selection in northern water snakes (Nerodia sipedon) Behavioral Ecology 13: 800-807.

Pusey A (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. Trends in Ecology and Evolution 2: 295-299.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43: 258-275.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences USA 94: 9197-9221.

Raup DM (1975) Taxonomic diversity estimation using rarefaction. Paleobiology 1: 333-342.

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49: 1280-1283.

Reece JS, Ehrhart LM, Parkinson CL (2006) Mixed stock analysis of juvenile loggerheads (Caretta caretta) in Indian River Lagoon, Florida: implications for conservation planning. Conservation Genetics 7: 345-352.

Reis EC, Soares LS, Vargas SM, Santos FR, Young RJ, Bjorndal KA, Bolten AB, Lôbo-Hajdu G (2010) Genetic composition, population structure and phylogeography of the loggerhead sea turtle: colonization hypothesis for the Brazilian rookeries. Conservation Genetics 11: 1467-1477.

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225.

Richards C, Leberg PL (1996) Temporal changes in allele frequencies and a population's history of severe bottlenecks. Conservation Biology 10: 832-839.

Rowe L (1992) Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Animal Behaviour 44: 189-202.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487–91.

Schroeder BA, Foley AM, Bagley DA (2003) Nesting patterns, reproductive migrations, and adult foraging areas of loggerhead turtles. In: Loggerhead Sea Turtles (eds. Bolten AB, Witherington BE). Smithsonian Books, Washington. Pp. 114-124.

Schroth W, Streit B, Schierwater B (1996) Evolutionary handicap for turtles. Nature 384: 521-522.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters 9:615-629.

Shamblin BM, Faircloth BC, Dodd M, Wood-Jones A, Castleberry SB, Carroll JP, Nairn JC (2007) Tetranucleotide microsatellites from he loggerhead turtle (Caretta caretta). Molecular Ecology Notes 7: 784-787.

Slatkin M (1991) Inbreeding coefficients and coalescence times. Genetics Research 58: 167-175.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462.

Soucy S, Travis J (2003) Multiple paternity and population genetic structure in natural populations of the poeciliid fish, Heterandria formosa. Journal of Evolutionary Biology 16: 1328-1336.

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. Genetics 105: 437-460.

Tajima F (1993) Measurement of DNA polymorphism. In Mechanisms of Molecular Evolution. Introduction to Molecular Paleopopulation Biology (Eds. Takahata N, Clark AG). Tokyo, Sunderland, MA: Japan Scientific Societies Press, Sinauer Associates, Inc. P. 37-59.

Tamura K, Nei M (1993) Estimation of the number of substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512-526.

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of pheonotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619-633.

Theissinger K, FitzSimmon NN, Limpus CJ, Parmenter CJ, Phillot AD (2009) Mating system, multiple paternity and effective population size in the endemic flatback turtle (Natator depressus) in Australia. Conservation Genetics 10: 329-346.

Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge, Massachusetts.

Tipper JC (1979) Rarefaction and rarefiction - the use and abuse of a method in paleontology. Paleobiology 5: 423-434.

Toonen RJ, Hughes S (2001) Increased throughput for fragment analysis on an ABI 377 automated sequencer using a 100-lane RapidLoad membrane comb and STRand software. BioTechniques 31: 1320-1324.

Tregenza T, Wedell N (2000) Polyandrous females avoid costs of inbreeding. Nature 415: 71-73.

Trivers RL (1972) Parental investment and sexual selection. In Sexual Selection and the Descent of Man, 1871-1971 (ed. Campbell B). Chicago: Aldine-Atherton. Pp. 136-179.

Tucker AD, McCallum HI, Limpus CJ, McDonald KR (1998) Sex-biased dispersal in a long-lived polygynous reptile (Crocodylus johnstoni). Behavioral Ecology and Sociobiology 44: 85-90.

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

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538.

Wagner HW, Sefc KM (1999) IDENTITY 1.0. Centre for Applied Genetics. University of Agricultural Sciences, Vienna.

Wagner RH (1992) Behavioural and breeding-habitat related aspects of sperm competition in razorbills. Behaviour 123:1-25.

Wang J (2002) Application of the one-migrant-per-generation rule to conservation management. Conservation Biology 18: 332-343.

Watkins MW (2002). MacBonferroni (Computer software). State College, PA: Ed & Psych Associates.

Watson PJ, Arnqvist G, Stallmann RR (1998) Sexual conflict and the energetic costs of mating and mate choice in water striders. The American Naturalist 151:46-58.

Weir BS (1996) Genetic Data Analysis II: Methods for Discrete Population Genetic Data. Sinauer Associates, Inc., Sunderland, MA, USA.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Whitehead H, Dillon M, Dufault S, Weilgart L, Wright J (1998) Non-geographically based population structure of South Pacific sperm whales: dialects, fluke markings, and genetics. Journal of Animal Ecology 67: 253-262.

Witherington B, Kubilis P, Brost B, Meylan A (2009) Decreasing annual nest counts in a globally important loggerhead sea turtle population. Ecological Applications 19: 30-54.

Wright S (1931) Evolution in Mendelian populations. Genetics 16: 97-159.

Wright S (1943) Isolation by distance. Genetics 28: 114-138.

Wright S (1946) Isolation by distance under diverse systems of mating. Genetics 31: 39-59.

Wright S (1951) The genetical structure of populations. Annals of Eugenics 15: 323-354.

Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395-420.

Wright S (1978) Evolution and the Genetics of Population, Variability Within and Among Natural Populations. The University of Chicago Press, Chicago.

Yasui Y (2001) Female multiple mating as a genetic bet-hedging strategy when mate choice criteria are unreliable. Ecological Research 16:605-616.

Yntema CL, Mrosovsky N (1980) Sexual differentiation in hatchling loggerheads incubated at different controlled temperatures. Herpetologica 36:33-36.

Zaykin DV, Zhivotovsky LA, Westfall PH, Weir BS (2002) Truncated product method for combining p-values. Genetic Epidemiology 22: 170-185.

Zbinden J, Largiader C, Leippert F, Margaritoulis D, Arlettaz R (2007) High frequency of multiple paternity in the largest rookery of Mediterranean loggerhead sea turtles. Molecular Ecology 16: 3703-3711.

Zegura S, Karafet TM, Zhivotovsky LA, Hammer MF (2004) High-resolution SNPs and microsatellite haplotypes point to a single, recent entry of native American Y chromosomes into the Americas. Molecular Biology and Evolution 21: 164-175.

Zeh JA, Zeh DW (1996) The evolution of polyandry I: intragenomic conflict and genetic imcompatibility. Proceedings of the Royal Society of London Series B 263: 1711-1717.