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Evolutionary ecology of sea turtles

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Chapter 1

Introduction

Jurjan P. van der Zee

The genetic diversity and structure of natural populations

Ecological and evolutionary processes fundamentally shape the genetic diversity and structure of natural populations (Hewitt, 2000). When populations become isolated, gene flow between these populations ceases and leads to genetic differentiation at neutral loci through random genetic drift and mutation (Wright, 1951). By contrast, gene flow counteracts genetic differentiation. The level of genetic diversity in populations is also correlated with population size (Watterson, 1975). Demographic processes affecting the size and structure of populations, such as range expansions, contractions and changes in population size, affect the spatial distribution-, and level of genetic diversity (Taberlet et al., 1998; Hewitt, 2000). By investigating the genetic structure and diversity of natural populations, fundamental knowledge of the underlying *extrinsic* and *intrinsic* processes can be gained that are of critical importance to understanding species' ecology and evolution (Lister, 2004). In addition, knowledge of the spatial distribution of genetic diversity can contribute to developing effective conservation and management strategies, such as the delineation of evolutionary significant units and management units (Moritz, 1994; Palsbøll, Bérubé & Allendorf, 2007; Wallace et al., 2010).

Extrinsic processes, such as the formation of biogeographic barriers or environmental fluctuations driven by climate change, can drive population structuring, contractions and expansions (Hewitt, 2000; Lister, 2004). The closure of the Panama Isthmus approximately 3.5 million years ago (Coates et al., 1992) restricted tropical marine connectivity between the Atlantic and Pacific Ocean and resulted in strong genetic divergence between Atlantic and Pacific lineages in many marine species (Bowen et al., 2001; Lessios, Kessing & Pearse, 2001). The cold Benguela Current near southern Africa has long been hypothesized to represent a biogeographic barrier to gene flow for tropical marine species since it formed around 2.5 million years ago (Dwyer et al., 1995), though various studies have reported gene flow around the southern point of Africa (Duncan et al., 2006; Bourjea et al., 2007). Many terrestrial species at higher latitudes became isolated in glacial refugia during the last glaciation, and subsequently expanded when global climate warmed and recolonized areas previously covered by continental ice sheets (Taberlet et al., 1998). In the marine environment, past sea level changes impacted connectivity among different regions (Ludt & Rocha, 2015). For example, lowered sea levels resulted in the exposure of the Sunda and Sahel shelves in the Indo-Malayan Archipelago and

restricted gene flow between the West Pacific-, and Indian oceans, such as in the reef fish *Cephalopholis argus* (Gaither et al., 2011). Sea level regression also affected the availability of marine habitat (Ludt & Rocha, 2015) and signatures of population bottlenecks have been observed in species associated with habitats lost during glaciations (Fauvelot, Bernardi & Planes, 2003).

Population structure can also develop due to *intrinsic processes*, such as ecological specialization or migratory behaviour. For example, dietary specialization has driven genetic differentiation among sympatric killer whales, *Orcinus orca* (Hoelzel et al., 2007; Foote et al., 2010). Philopatry towards breeding or feeding grounds can also lead to population structuring. Maternally directed site fidelity towards feeding grounds plays a role in maintaining genetic differentiation in mitochondrial DNA (mtDNA) among different feeding grounds in the humpback whale, *Megaptera novaeangliae* (Palsbøll et al., 1995). Many reef fishes also exhibit natal homing, resulting in restricted connectivity among geographically proximate regions despite a high potential for dispersal during larval stages (Thorrold et al., 2001). Natal homing has also been observed in bluefin tuna, *Thunnus thynnus* (Rooker et al., 2008) and sea turtles (Meylan, Bowen & Avise, 1990). Genetically differentiated populations may temporarily overlap in certain species, which is observed in salmon (Waples et al., 1990) and sea turtles (Bowen et al., 1996; Lahanas et al., 1998).

The genetic structure and diversity of natural populations can be investigated with molecular markers to unveil the underlying intrinsic and extrinsic processes that shaped the evolution of contemporary natural populations. The advent of Sanger's chain termination method during the late 1970's can be considered the cradle of modern DNA sequencing techniques (Sanger, Nicklen & Coulson, 1977). The development of automated fluorescence detection (Smith et al., 1986) and polymerase chain reaction (PCR) techniques (Mullis & Faloona, 1987; Saiki et al., 1988) further revolutionized DNA sequencing, ultimately culminating in the sequencing of the first human reference genome in the early 2000's (International Human Genome Sequencing Consortium, 2001). Rapid developments in DNA sequencing technologies continued and resulted in dramatic increases in sequencing throughput at ever-decreasing costs; a 100-fold decrease in sequencing costs was reported only a few years after the Human Genome Project (Wheeler et al., 2008). Furthermore, the development of restriction-site associated DNA (RAD) sequencing techniques (Miller et al., 2007; Baird et al., 2008; Peterson et al., 2012) has enabled generating thousands

Box 1.1. Sea turtle dispersal and migration

Sea turtles are characterized by complex life histories involving transitions among different developmental habitats and long-distance reproductive migrations among nesting, breeding and feeding grounds (Carr, Carr & Meylan, 1978; Carr, 1987; Reich, Bjorndal & Bolten, 2007). The early life history of most sea turtles consists of an oceanic stage where post-hatchlings recruit to pelagic oceanic developmental habitats and a neritic stage after juveniles recruit to feeding grounds located in coastal waters (Carr, Hirth & Ogren, 1966; Carr, 1987; Bolten, 2003; Reich, Bjorndal & Bolten, 2007). Relatively few data exists on the behaviour and ecology of sea turtle hatchlings during the oceanic stage (Carr, 1980; Reich, Bjorndal & Bolten, 2007; Putman & Naro-Maciel, 2013), but green turtle hatchlings have been observed in floating *Sargassum* communities where they opportunistically feed on *Sargassum*, molluscs and crustaceans (Carr & Meylan, 1980). Less is known about the distribution of hawksbill turtle hatchlings, though they are assumed to follow the oceanic-neritic life history pattern observed in loggerhead and green turtles (Bolten, 2003). Exceptions to the oceanic-neritic life history pattern are the flatback turtle, where an oceanic stage seems to be absent (Walker & Parmenter, 1990), the leatherback turtle, which retains a predominantly oceanic distribution (Bolten, 2003), and olive ridley sea turtles in the eastern Pacific, which seem to occupy oceanic feeding habitats during juvenile life stages (Polovina et al., 2004). Dispersal during the oceanic stage is mediated by a combination of passive drifting in ocean currents (Carr & Meylan, 1980; Luschi, Hays & Papi, 2003; Monzón-Argüello et al., 2010a; Witherington, Hirama & Hardy, 2012; Putman & Naro-Maciel, 2013) and active swimming (Putman et al., 2012, 2014; Putman & Naro-Maciel, 2013; Putman & Mansfield, 2015). Juvenile dispersal is challenging to assess because of the difficulties in monitoring the postnatal movements of hatchlings and juveniles (Carr & Meylan, 1980; Reich, Bjorndal & Bolten, 2007; Mansfield et al., 2014).

of single nucleotide polymorphism (SNP) markers at relatively low cost and holds great promise for studying the population structure and phylogeography of non-model species.

What are sea turtles?

Sea turtles are migratory (Box 1.1) and endangered (Box 1.2) marine reptiles that first appeared during the early Cretaceous (Zangerl, 1980; Hirayama, 1994). There are seven extant sea turtle species: the green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), the olive (*Lepidochelys olivacea*) and Kemp's ridley (*Lepidochelys kempi*), the loggerhead (*Caretta caretta*), the flatback (*Natator depressus*) and the leatherback

turtle (*Dermochelys coriacea*). The *Cheloniidae* family encompasses six out of seven sea turtle species characterized by hardened carapaces, while the remaining species, the leatherback turtle, is the only extant species in the *Dermochelyidae* family. A recent phylogenetic analysis using mitochondrial genomes suggested *Chelonidae* and *Dermochelyidae* diverged approximately 110 to 100 million years ago (Duchêne et al., 2012). Within the *Chelonidae*, two subfamilies exist, *Carettini* and *Chelonini* (Dutton et al., 1996), that diverged approximately 55 million years ago (Duchêne et al., 2012). The *Carettini* subfamily encompasses the hawksbill, loggerhead and olive and Kemp's ridley, while the *Chelonini* contains the green and flatback turtle. The phylogenetic position of the flatback turtle was initially poorly resolved (Bowen, Nelson & Avise, 1993; Dutton et al., 1996), but analysis of whole mitochondrial genomes suggested flatback turtles are a sister species to the green turtle (Duchêne et al., 2012).

Sea turtles occupy a wide range of ecological niches (Hendrickson, 1980). Green turtles are herbivores that graze seagrass or algae in tropical marine ecosystems

Box 1.2. Population decline in sea turtles

Sea turtles have been intensely hunted by humans for meat, eggs and carapace, resulting in dramatic declines in abundance (Jackson, 1997; Bjorndal & Jackson, 2003; McClenachan, Jackson & Newman, 2006). Caribbean green and hawksbill turtles are estimated to have declined by 95% and 99% respectively since the arrival of Columbus (Jackson, 1997; Meylan & Donnelly, 1999; McClenachan, Jackson & Newman, 2006), and are currently classified as 'endangered' and 'critically endangered' respectively (Meylan & Donnelly, 1999; Seminoff, 2004; Mortimer & Donnelly, 2008). The decline in green and hawksbill turtles raises concerns for the functioning and health of seagrass and coral reef ecosystems (Jackson et al., 2001; Worm et al., 2006; Waycott et al., 2009; Fourqurean et al., 2010). The remaining sea turtle species are listed as 'vulnerable' (olive ridley-, loggerhead-, and leatherback turtle) or 'critically endangered' (Kemp's ridley turtle) by the International Union for the Conservation of Nature (Abreu-Grobois & Plotkin, 2008; Wallace, Tiwari & Girondot, 2013; Casale & Tucker, 2017; Wibbels & Bevan, 2019). Sea turtles continue to be threatened despite widespread legislation against exploitation by, for example, climate change (Fish et al., 2005; Saba et al., 2007; Hawkes et al., 2007; Hays et al., 2010; Jensen et al., 2018), poaching (Koch et al., 2006) and entanglement in fishing gear (Lewison, Freeman & Crowder, 2004). However, signs of population recovery have recently been reported as well, highlighting the merit of conservation practices (Balazs & Chaloupka, 2004; Troëng & Rankin, 2005; Dutton et al., 2005; Chaloupka et al., 2008; Mazaris et al., 2017).

(Bjorndal, 1980) where they fulfil keystone functions by facilitating the transport of nutrients (Bouchard & Bjorndal, 2000) and affecting the growth, nutritional content and structure of seagrass beds (Bjorndal, 1980; Moran & Bjorndal, 2005, 2006). Hawksbill turtles are predominantly spongivorous in the Caribbean (Meylan, 1988; León & Bjorndal, 2002) but are also known to feed on algae (Bell, 2013), which suggests that hawksbill turtles are omnivorous (Bjorndal, 1980; Hendrickson, 1980). Hawksbill turtles are closely associated with coral reef ecosystems (León & Bjorndal, 2002). The flatback turtle seems to be carnivorous, possibly feeding on sea cucumbers (Hendrickson, 1980). The loggerhead turtle is a carnivore that feeds on benthic invertebrates, primarily molluscs (Hendrickson, 1980; Plotkin, Wicksten & Amos, 1993), while leatherback turtles feed almost exclusively on jellyfish in oceanic waters (Hendrickson, 1980). Olive ridley-, and Kemp's ridley turtles are carnivores that feed on benthic invertebrates, similar to the loggerhead turtle, but primarily predate on crustaceans (Hendrickson, 1980; Colman et al., 2014). However, more opportunistic feeding was later suggested for the Kemp's ridley turtle (Witzell & Schmid, 2005).

Natal homing – geographic structure in mitochondrial DNA

Mitochondrial DNA has played an important role in sea turtle research during the last three decades, proving invaluable as a test for natal homing (Bowen, Meylan & Avise, 1989; Meylan, Bowen & Avise, 1990) and playing a major role in defining population boundaries (Wallace et al., 2010). Given that mitochondrial DNA is maternally inherited, limited gene flow in mitochondrial DNA is expected among rookeries if adult females return to natal nesting sites. Thus, mitochondrial DNA diversity is expected to be geographically structured under natal homing, while this is not expected under the social facilitation hypothesis (Bowen, Meylan & Avise, 1989; Meylan, Bowen & Avise, 1990). Under social facilitation, inexperienced females randomly encounter and follow experienced females to nesting sites, and then imprint upon these localities after a successful nesting experience (Hendrickson, 1958; Owens, Grassman & Hendrickson, 1982). Restriction fragment length polymorphism analysis demonstrated nearly fixed differences among green turtle rookeries in the Caribbean, providing strong support for natal homing in adult females (Meylan, Bowen & Avise, 1990). Natal homing was later also demonstrated in adult males (FitzSimmons et al., 1997; Vélez-Zuazo et al., 2008; Naro-Maciel et al., 2012) as was suggested by earlier tagging experiments (Limpus, 1993), and juveniles (Bowen et al.,

2004; Naro-Maciel et al., 2017).

Later studies repeatedly demonstrated the widespread geographic structure in mitochondrial DNA diversity in other regions and species, underlining the prevalence of natal homing in sea turtles (Bowen et al., 1993, 1997; Allard et al., 1994; Norman, Moritz & Limpus, 1994; Bass et al., 1996; Encalada et al., 1996, 1998; Dutton et al., 1999; Hatase et al., 2002; Shanker et al., 2004; Bjorndal, Bolten & Troëng, 2005; Formia et al., 2006, 2007; Dethmers et al., 2006; Carreras et al., 2007, 2013; Bourjea et al., 2007; Garofalo et al., 2009; Shamblin et al., 2011, 2014, 2015; Leroux et al., 2012; Naro-Maciel et al., 2012, 2014b; Gaos et al., 2016; Vargas et al., 2016; Jensen et al., 2019). Genetic studies have also demonstrated there is typically limited genetic differentiation between rookeries separated by less than 500 kilometres (Dethmers et al., 2006; Vargas et al., 2016; Jensen et al., 2019). Limited genetic differentiation among geographically proximate rookeries has been attributed to 'imperfect natal homing', i.e. where females also occasionally nest elsewhere and which represents a possible adaptive mechanism that facilitates the colonization of novel nesting habitats (Carr, Carr & Meylan, 1978).

The natal origins of sea turtles observed at feeding grounds

Mitochondrial DNA has also proven useful for tracking the natal origins of sea turtles (Awise & Bowen, 1994). In the simplest form, mitochondrial DNA haplotypes at feeding grounds can be compared with those observed at rookeries to test for statistical differences in genetic composition (Bowen et al., 1995, 1996). More sophisticatedly, maximum likelihood or Bayesian mixed stock analysis can be applied to estimate the proportional contribution from an arbitrary number of rookeries that represents the best fit to the mitochondrial DNA haplotype composition observed at a feeding ground (Pella & Masuda, 2001; Bolker et al., 2003, 2007; Okuyama & Bolker, 2005).

Analysis of juvenile dispersal using mitochondrial DNA has revealed extensive interconnectivity among rookeries and feeding grounds (Awise & Bowen, 1994; Bowen et al., 1995, 1996, 2007; Lahanas et al., 1998; Bass, Lagueux & Bowen, 1998; Bass & Witzell, 2000; Bass, Epperly & Braun-McNeill, 2004, 2006; Luke et al., 2004; Naro-Maciel et al., 2007, 2012, 2014a; Bjorndal & Bolten, 2008; Monzón-Argüello et al., 2009, 2010a,b, 2012; Blumenthal et al., 2009; Prosdocimi et al., 2012, 2014, 2015; Proietti et al., 2012; Anderson, Shaver & Karel, 2013; Clusa et al., 2013; LaCasella et al.,

2013; Joseph et al., 2014, 2016; Jordão et al., 2015; Read et al., 2015; Yang, Wang & Chen, 2015; Jensen et al., 2016; Shamblin et al., 2017, 2018; Patrício et al., 2017a,b; Tolve et al., 2018; Dutton et al., 2019; Labastida-Estrada et al., 2019; Hancock et al., 2019). Mitochondrial DNA analysis also highlighted the mixing of sea turtles originating from different rookeries at feeding grounds (Fig. 1.1), underlining the need to assess which nesting populations utilize a feeding ground (Bowen et al., 2005). Analysis of mitochondrial DNA at feeding grounds also yielded insights in the factors influencing juvenile dispersal, such as the number of adult females annually nesting at rookeries (Lahanas et al., 1998; Bass, Lagueux & Bowen, 1998), geographic distance among rookeries and feeding grounds (Bass & Witzell, 2000; Bowen et al., 2007), ocean currents (Luke et al., 2004; Bass, Epperly & Braun-McNeill, 2006; Bowen et al., 2007; Naro-Maciel et al., 2012) and natal homing behaviour (Bowen et al., 2004; Bass, Epperly & Braun-McNeill, 2006; Naro-Maciel et al., 2007, 2017; Monzón-Argüello et al.,

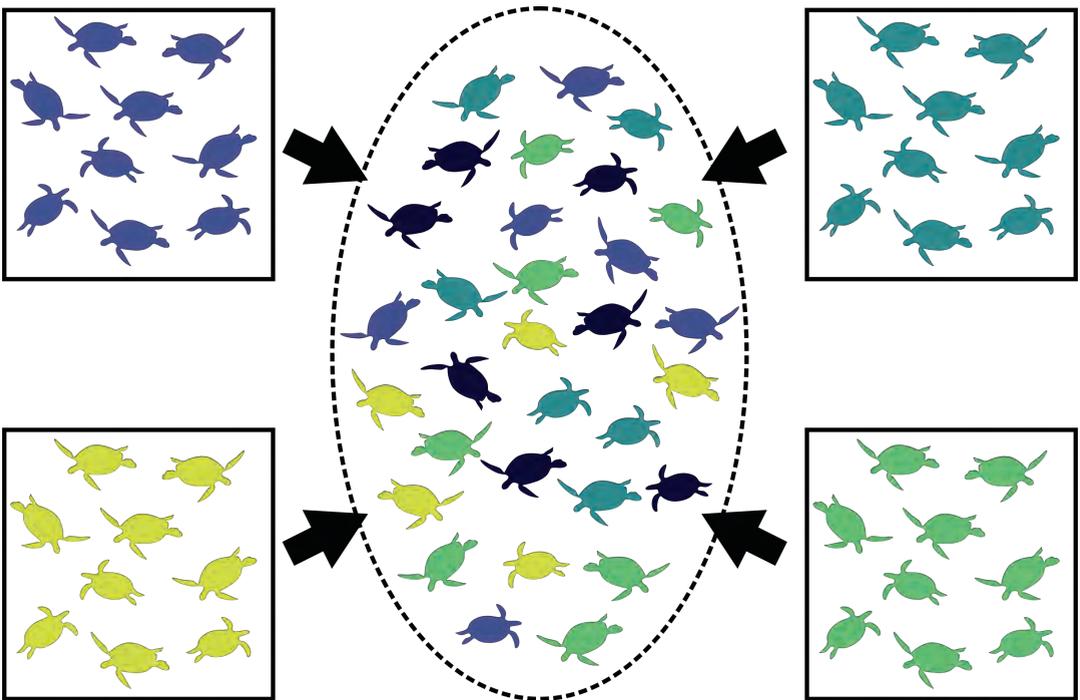


Figure 1.1. An illustration of the mixing of individuals originating from different sea turtle rookeries at a shared feeding ground. The different colours indicate genetic differences among rookeries observed using mitochondrial DNA markers.

2010a). Furthermore, recruitment of juveniles to feeding grounds can fluctuate through time due to environmental stochasticity (Bjørndal & Bolten, 2008) and long-term demographic changes at rookeries (Jensen et al., 2016).

Male-mediated gene flow – reduced geographic structure in nuclear DNA

In contrast with mitochondrial DNA, studies using nuclear markers have typically reported limited population structure in sea turtles (Karl, Bowen & Avise, 1992; FitzSimmons et al., 1997; Roberts, Schwartz & Karl, 2004). Early studies revealed intra-oceanic gene flow estimated from mitochondrial DNA (Bowen et al., 1992) was lower compared to estimates from restriction fragment length polymorphisms in anonymous single-copy nuclear DNA (Karl, Bowen & Avise, 1992) and microsatellites (Roberts, Schwartz & Karl, 2004) in Atlantic green turtles. Reduced geographic structure in nuclear DNA, relative to mitochondrial DNA, was also reported in other regions and species, such as Australian green turtles (FitzSimmons et al., 1997), Mediterranean loggerhead turtles (Carreras et al., 2007; Garofalo et al., 2013), Atlantic leatherback turtles (Dutton et al., 2013) and Caribbean hawksbill turtles (Levasseur et al., 2019).

Reduced population structure in nuclear diversity has been attributed to male-mediated gene flow (Karl, Bowen & Avise, 1992; FitzSimmons et al., 1997; Roberts, Schwartz & Karl, 2004). Male-mediated gene flow may be caused by mating that occurs prior or during reproductive migrations in areas where different breeding populations overlap, such as migration corridors or on feeding grounds (Karl, Bowen & Avise, 1992; FitzSimmons et al., 1997; Roberts, Schwartz & Karl, 2004), and given that female sea turtles can store sperm for prolonged periods of time (Gist & Jones, 1989). The occurrence of mating during reproductive migrations is supported by opportunistic mating behaviour of male sea turtles, which is underlined by widespread multiple paternity (FitzSimmons, 1998; Moore & Ball, 2002; Hoekert et al., 2002; Ireland et al., 2003; Lee & Hays, 2004; Zbinden et al., 2007; Stewart & Dutton, 2011; Duran et al., 2015; Howe et al., 2018) and the relatively common hybridization among all six *Cheloniidae* species (Karl, Bowen & Avise, 1995; Seminoff et al., 2003; Lara-Ruiz et al., 2006; Reis, Soares & Lôbo-Hajdu, 2010; Vilaça et al., 2012; Kelez, Vélez-Zuazo & Pacheco, 2016).

The Isthmus of Panama - a biogeographic barrier between the Atlantic and Pacific Oceans

The formation of the Isthmus of Panama approximately 3.5 million years ago (Coates et al., 1992) played a major role in the evolutionary history of sea turtles. Divergence between mitochondrial lineages in Kemp's and olive ridley turtles suggested a separation around 2.5 to 3.5 million years ago (Bowen et al., 1997), consistent with the formation of the Panama Isthmus. However, molecular phylogenies based upon multiple markers (Naro-Maciel et al., 2008) and whole mitogenomes (Duchêne et al., 2012) suggested a more ancient divergence approximately 4.8 to 5.2 million years ago between Kemp's and olive ridley turtles. In the green turtle, divergence times between Atlantic and Indo-Pacific mitochondrial lineages range from 1.5 to 3.0 million years ago (Bowen et al., 1992), 1.8 to 3.7 million years ago (Encalada et al., 1996), 7.0 million years ago (Naro-Maciel et al., 2008), 3.1 million years ago (Duchêne et al., 2012). Hawksbill turtles in the Atlantic and Indo-Pacific divergence time estimates range from approximately 3.6 million years (Naro-Maciel et al., 2008), 4.3 million years (Vargas et al., 2016) to 5.6 million years ago (Duchêne et al., 2012). Similarly, two mitochondrial lineages were detected in loggerhead turtles in the Atlantic and Indo-Pacific ocean basins (Bowen et al., 1994; Encalada et al., 1998). Divergence between the two loggerhead turtle mitochondrial lineages has been dated to 3.5 million years ago based upon multiple mitochondrial and nuclear markers (Naro-Maciel et al., 2008) and 4.1 million years ago based upon whole mitogenomes (Duchêne et al., 2012).

The above-mentioned divergence times appear consistent with the timing of the formation of the Panama Isthmus, with the exception of the estimate by Naro-Maciel et al. (2008) for green turtles. Possible explanations include a relative small sample size compared to the studies of Bowen et al. (1992) and Encalada et al. (1996), and the relative small proportion of the mitochondrial genome that was screened compared to the study of Duchêne et al. (2012) that involved whole mitogenomes. However, the timing of the closure of the Isthmus of Panama is still contentious. While many studies suggest the tropical marine connection between the Atlantic and Pacific was severed around 3.5 million years ago (Coates et al., 1992; Haug & Tiedemann, 1998), recent work suggested the closure of the Panama Isthmus started as early as 20 to 23 million years ago (Farris et al., 2011) and was accompanied by several distinct migration pulses (Bacona et al., 2015). Nonetheless, divergence between

Atlantic and Indo-Pacific lineages in extant sea turtle species highlights an important role for the Panama Isthmus as a biogeographic barrier to gene flow.

The Benguela Current - a permeable barrier between the Atlantic and Indian Oceans

The waters around the southern tip of Africa represent the primary avenue for tropical marine connectivity between the Atlantic and Indo-Pacific ocean basins after the Panama Isthmus formed (Teske et al., 2011). However, the cold Benguela Current, which developed around 2.5 million years ago (Dwyer et al., 1995), is believed to represent a biogeographic barrier to tropical marine connectivity (Bowen et al., 2016). Occasionally, gyres of tropical water from the warm Agulhas Current are transported to the Atlantic Ocean (Penven et al., 2001; Hutchings et al., 2009), which has been hypothesized (the 'Agulhas leakage' hypothesis) to facilitate westward dispersal, i.e., from the Indo-Pacific Ocean to the Atlantic Ocean, in tropical marine species (Bowen et al., 2016). Phylogeographic evidence seems to support the Agulhas leakage hypothesis, with genetic signatures of westward colonization of the Atlantic Ocean by Indian Ocean lineages in certain marine species (Buonaccorsi, McDowell & Graves, 2001; Rocha et al., 2005). However, the opposite pattern, i.e. eastward colonization of the Indian Ocean by Atlantic lineages, has also been reported (Duncan et al., 2006; Gaither et al., 2015).

Mitochondrial DNA analysis suggested Indian Ocean olive ridley turtles colonized the Atlantic Ocean during the last 300,000 years (Bowen et al., 1997; Duchêne et al., 2012). By contrast, Southwest Indian Ocean green turtles show genetic signatures of a recent eastward colonization of the Indian Ocean, though the timing of this event remained unclear (Bourjea et al., 2007). An Atlantic mitochondrial lineage, i.e., mitochondrial haplotype CM-A08, was detected in green turtle rookeries located in the southern Mozambique Channel (Bourjea et al., 2007). In addition, a break in mitochondrial DNA diversity was observed across the Mozambique Channel, which was attributed to isolation driven by complex local oceanographic conditions (Bourjea et al., 2007). These findings supported a scenario where the Southwest Indian Ocean was colonized by Atlantic green turtles in the recent past, and genetic structure was maintained by a combination of natal homing and isolation due to the complex oceanography of the Mozambique Channel.

Although there is genetic evidence for recent colonization in both directions by green and olive ridley turtles, the cold Benguela Current seems to represent a biogeographic barrier to tropical sea turtles. Whether Agulhas leakage facilitates dispersal is unclear; given that both westward and eastward past colonization has been reported (Bowen et al., 1997; Bourjea et al., 2007). Restricted tropical marine connectivity due to the Benguela Current is further underlined by the phylogeography of the tropical hawksbill turtle, and more temperate loggerhead and leatherback turtle (Bowen et al., 1994; Encalada et al., 1998; Dutton et al., 1999; Leroux et al., 2012; Vargas et al., 2016). Mitochondrial DNA analysis of Atlantic (Leroux et al., 2012) and Indo-Pacific (Vargas et al., 2016) hawksbill turtles revealed several distinct mitochondrial clades that were geographically partitioned according to ocean basin and no signatures of recent gene flow around southern Africa. Two distinct mitochondrial lineages co-occur within loggerhead turtle rookeries located in the western Atlantic, which has been attributed to recent inter-oceanic colonization facilitated by a more temperate distribution compared to tropical sea turtle species (Bowen et al., 1994; Encalada et al., 1998; Shamblin et al., 2014). The leatherback shows a relative shallow structuring in global mitochondrial DNA diversity (Dutton et al., 1999), which is probably a consequence of its highly migratory potential and extensive distribution up to cold-temperate waters at high latitudes (Pritchard, 1976).

Pleistocene climate change - isolation in glacial refugia and post-glacial population expansion

During the last glacial maximum, sea levels were around 120 meters lower than current levels and temperate climate zones were compressed towards the equator (Hewitt, 2000; Bintanja, Wal & Oerlemans, 2005; Clark et al., 2009). A cooler global climate and lowered sea levels likely severely impacted the availability of sea turtle nesting and feeding habitat (Pike, 2013; Ludt & Rocha, 2015). For example, mitochondrial DNA analysis provided strong evidence that the Ascension Island green turtle was established after sea levels were restored to present-day levels during the Holocene (Bowen, Meylan & Avise, 1989). Sea turtles likely experienced dramatic alterations in habitat availability, range contractions and isolation in glacial refugia during glacial periods, followed by subsequently population expansion and colonization of previously inaccessible habitats during interglacial periods.

Two mitochondrial DNA lineages are found in Atlantic green turtles, corresponding to A) the eastern Caribbean, southern Atlantic and eastern Atlantic regions B) and the western Caribbean and Mediterranean, supporting the past existence of two glacial refugia (Encalada et al., 1996). A later study corroborated the presence of two glacial refugia in Atlantic green turtles, but also reported discordance between mitochondrial and nuclear markers in the eastern Caribbean (Naro-Maciel et al., 2014b). Mitochondrial DNA diversity in the eastern Caribbean is associated with the southern Atlantic, while nuclear variation groups the eastern Caribbean with the northern Atlantic, highlighting this region as an area of secondary contact between the two Atlantic refugial populations (Naro-Maciel et al., 2014b). In the Indo-Pacific, rookeries in the Southwest Pacific are highly genetically distinct, which was likely the result of the exposure of the Torres Strait during periods with lowered sea levels (Dethmers et al., 2006; Jensen et al., 2019).

Atlantic hawksbill turtles show a more complex phylogeographic history (Bass et al., 1996; Leroux et al., 2012). The most recent common ancestor of three mitochondrial lineages in Atlantic hawksbill was dated to approximately 2.0 million years ago, suggesting the onset of the glacial cycles during the Pleistocene played an important role in shaping the phylogeography of hawksbills in the Atlantic Ocean. Signatures of demographic expansion were also detected in two clades (Leroux et al., 2012). However, the lack of a clear geographic pattern in the distribution of mitochondrial lineages confounds our understanding of Atlantic hawksbill turtle phylogeography (Bass et al., 1996; Leroux et al., 2012). The presence of three mitochondrial clades suggested isolation in glacial refugia, but the geographic distribution of these clades provides no insights in where these putative glacial refugia were located. Instead, the phylogeography of Atlantic hawksbill turtles suggests a complex pattern of isolation, possible extirpation of rookeries and recolonization by individuals from distant rookeries driven by repeated glaciation events (Leroux et al., 2012). Similar observations were made in the Indo-Pacific, where hawksbill turtle lineages also show a highly complex phylogeographic pattern (Vargas et al., 2016).

Harnessing the power of genomic tools

Advances in DNA sequencing now enable the cost-effective genome-wide characterization of potentially ten-thousands of single nucleotide polymorphisms

(SNPs), greatly enhancing the number of markers potentially available for studying population structure and phylogeography (Morin et al., 2004). Genomic resources represent a relatively untapped resource for sea turtles (Komoroske et al., 2017), though the ability to generate large numbers of SNPs at relatively low costs have great potential for enhancing our understanding of sea turtle population structure and phylogeography. Mitochondrial DNA has been very useful for studying sea turtle population structure and phylogeography, but there are two major limitations associated with the singular use of mitochondrial markers. First, the genealogy estimated from mitochondrial DNA reflects the evolutionary history of mitochondria, representing the maternal line in animal species, and doesn't necessarily fully capture population history (Birky, Fuerst & Maruyama, 1989). Second, reliable estimates of demographic parameters that are essential for understanding the phylogeography of species require the use of many unlinked markers (Edwards & Beerli, 2000). However, the mitochondrial genome inherits as a single non-recombining unit (Birky, Fuerst & Maruyama, 1989), thus essentially representing a single marker. Mitochondrial DNA should therefore be combined with nuclear markers (Edwards & Beerli, 2000; Rubinoff & Holland, 2005) and in recent years this has increasingly been done in animals (Toews & Brelsford, 2012).

Outline of the thesis

The present thesis investigated questions regarding the intrinsic and extrinsic processes that shaped the genetic diversity and structure of sea turtles, focusing on the green turtle, *Chelonia mydas*, and hawksbill turtle, *Eretmochelys imbricata*, in the Atlantic and Southwest Indian Ocean. The thesis is divided into an ecological section (Chapters 2 and 3) and an evolutionary section (Chapters 4 and 5). The ecological section is concerned with the contemporary intrinsic and extrinsic processes affecting present dispersal and recruitment dynamics. The evolutionary section revolves around the influence of past climate and environmental change on the evolution of sea turtles. The following questions were addressed:

- How do changes in population dynamics affect juvenile dispersal and recruitment in sea turtles? (Chapter 2)
- How do ocean currents influence juvenile sea turtle dispersal? (Chapter 3)

- What is the significance of genetic monitoring for studying marine dispersal and recruitment in? (Chapters 2 and 3)
- How did climate change associated with the glaciations of the Pleistocene influence the evolution of sea turtles? (Chapters 4 and 5)
- How did past sea level fluctuations affect the evolution of sea turtles? (Chapter 5)

In Chapter 2, “Population recovery changes population composition at a major southern Caribbean juvenile developmental habitat for the green turtle, *Chelonia mydas*”, we investigated how changes in population dynamics at rookeries influenced juvenile recruitment to feeding grounds using mitochondrial DNA control region sequences. Changes in the genetic composition of a juvenile green turtle population were monitored between 2006 and 2016 at a major feeding ground located in the southern Caribbean. Temporal changes in rookery contributions were estimated using Bayesian mixed stock analysis and correlated with regional and rookery-specific population recovery trends. Population recovery trends were estimated from published nesting trends and population growth data from the Caribbean.

In Chapter 3, “Southward currents restrict northward juvenile dispersal from southern green turtle (*Chelonia mydas*) rookeries in the Southwest Indian Ocean”, the impact of ocean currents on juvenile dispersal was investigated in green turtles in the Southwest Indian Ocean using mitochondrial DNA control region sequences. Bayesian mixed stock analysis was conducted to estimate the contribution of Southwest Indian Ocean green turtle rookeries to a feeding ground located in the Barren Isles, off the western coast of Madagascar in the southern Mozambique Channel.

In Chapter 4, “Pre-glacial origins, glacial isolation and post-glacial admixture characterize the phylogeographic history of Atlantic and Southwest Indian Ocean green turtles, *Chelonia mydas*”, the influence of past climate and environmental change on the evolutionary history of green turtles was investigated using next-generation sequencing data. Double-digested restriction site associated DNA sequencing was employed to characterize a large number of genome-wide SNPs in green turtles from three distinct geographic regions: the Caribbean, Southeast Atlantic and Southwest Indian Ocean. Model-based clustering was used to investigate population structure and admixture patterns, and coalescent model selection was applied to evaluate the fit of different evolutionary models and estimate demographic parameters for the best-

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supported model.

In Chapter 5, “The rise and fall of Pleistocene sea turtles was driven by sea level fluctuations affecting feeding habitat area”, the impact of past sea level fluctuations on coastal tropical marine species was investigated using next-generation sequencing data. A modified double-digested restriction site associated DNA sequencing approach was used to account for potential biases caused by PCR enrichment. Maximum likelihood estimates of the folded site frequency spectrum were obtained from genome-wide SNP data and used to estimate past changes in genetic diversity in Caribbean hawksbill turtles. Past changes in genetic diversity were correlated with fluctuations in shallow marine habitat area during the last 125 thousand years.

In chapter 6, I provide a synthesis of all chapters as well as a final concluding remark.

