

Doctoral Thesis

**Study on Conservation Management of
Sea Turtles by Using Genetic Information**

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Abstract

Sea turtles have long history since they appeared in the Cretaceous, in addition to the migratory life history containing some cryptic features. For conservation management of these endangered migratory species, it is important to understand the evolutionary and demographic underpinning of contemporary biodiversity, and to reveal the connectivity between populations and aggregations that relates to their migrations and behaviors. Genetic information is useful for uncovering these features in wide range of temporal and spatial scales. This study uncovered relationships between individuals and populations of sea turtles in the Yaeyama Islands of Japan and the Pacific, and made proposals for conservation.

Firstly, the patterns of mitochondrial DNA (mtDNA) variation in green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles nesting in the Yaeyama Islands, which represent the northern limit of their nesting in the western Pacific Ocean, indicated historical introgressions by individuals with divergent haplotypes from both Pacific colonies and Indian and Southeast Asian colonies.

Secondly, the population genetic structure of nesting green turtles in the Yaeyama Islands revealed the significant genetic differentiation, at least between southwestern Iriomote Island and Ishigaki Island based on mtDNA, indicating precise female natal philopatry compared to other Pacific and Indian regions. On the other hand, microsatellite analyses of four markers revealed no significant genetic differentiation, indicating the existence of male mediated gene flow.

Thirdly, the genetic compositions of consecutive Japanese feeding aggregations of green turtles along the Kuroshio Current were

examined by mixed-stock analyses of mtDNA control-region sequences. The results indicated that the southern feeding aggregation around Yaeyama was sourced from various Pacific rookeries in the Yaeyama, Ogasawara, Western Pacific, and Indian Oceans and Southeast Asia. Among northern feeding aggregations, the Ginoza aggregation was also sourced from the Western Pacific Ocean, but the Nomaie, Muroto, Kanto, and Sanriku aggregations were contributed mostly by the closer Ogasawara rookeries. The reduced contribution from tropical Pacific rookeries to northern feeding aggregations and the significant correlation between genetic differentiation and geographical distance matrices of feeding aggregations indicated that most hatchlings from these regions transported by the Kuroshio Current settle in upstream feeding grounds along the Japanese archipelago, implying that current flow influences the composition of feeding aggregations.

Lastly, historical patterns of population dynamics and differentiation in green and hawksbill turtles in the Pacific were estimated based on mtDNA sequence polymorphisms. Phylogenetic relationships of the haplotypes indicated that both turtles in the Pacific underwent very similar patterns and processes of population dynamics over the last several million years, with population subdivision during the early Pleistocene and population expansion after the last glacial maximum. These significant contemporary historical events were suggested to have been caused by climatic and sea-level fluctuations. On the other hand, regional differences in historical population dynamics compared to the Atlantic are suggested.

The above results indicate the following proposals for conservation management:

- 1) Nesting females of green turtles in the Yaeyama Islands have precise natal philopatry at ecological timescales although they had been formed by emigration from divergent tropical colonies at

historical timescales. Considering that natal philopatry is related to their response to changes in availability of nesting sites over time, Yaeyama nesting populations may have a high priority in conservation. Despite relatively high male mediated gene flow, this study indicated phylogeographic features and conservation priority of northwest Pacific green turtles.

2) Differences in the composition among the feeding aggregations of green turtles in the Japanese coastal areas indicate that both regional and multinational conservation strategies are needed. hazards that affect declining nesting populations in Ogasawara may also affect a wide range of Japanese feeding aggregations, especially northern feeding aggregations. This indicates the importance of regional management in Japan. On the other hand, the Yaeyama and Ginoza feeding aggregations of Japan are estimated to have migrated from remote Pacific rookeries. Therefore, any source of mortality in nesting rookeries in the tropical Pacific is likely to affect remote feeding aggregations in Japan. Conversely, effects on feeding aggregations in Japanese waters could affect nesting populations in other countries because of natal philopatry.

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Chapter 1

Background

1.1 Introduction

Recent progress of information and communication technology has resulted in a flood of various kinds of data that include both useful and useless information for us. In this information society, it is necessary to uncover knowledge from a vast quantity of data by using computational and informational approaches. Biological data are no exceptional and are flooding in at an unprecedented rate. For example, advancement of remote monitoring of physiological and behavioral variables, known as biotelemetry and/or bio-logging, has enabled us to understand the physiology, behavior, and energetic status of unrestrained organisms in their natural environment (Boyd et al. 2004, Cooke et al. 2004). In addition, innovations in molecular genetic techniques (e.g. the development of polymerase chain reaction (PCR) which amplifies specified stretches of DNA to usable concentrations and automated sequencing techniques) (Hedrick & Miller 1992, Sunnusk 2000) have provided a great amount of genetic information. Actually, new sequence data and genotypic data of various organisms have become apparent one after another as represented by human and other genome projects (Hedrick 2001). However, there is a considerable gap between the availability of genetic data and a scientific understanding of that information (Fogel & Corne 2003), for example genetic characterization of genes and conservation of genetic diversity. This gap can be filled through the interdisciplinary approach bringing together biology, computer science, statistics, and information theory to analyze biological data for interpretation and prediction (Fogel & Corne 2003).

This study explored the use of genetic information in biological conservation, providing the important example of effective utilization of vast amounts of biological information. Using the genetic markers with computer-based simulations and statistics, this study uncovered

relationships between individuals and populations of sea turtles, species of conservation interest, and made proposals for conservation. In the following sections, I overviewed the importance of biological conservation, genetic information, status of sea turtles, and then summarized the aims of this study.

1.2 Importance of Biological Conservation

Because of the increase in the human population and the expansion of human-related activity in recent decades, many species have become extinct or are on the verge of extinction (Hedrick & Miller 1992, Frankham et al. 2002). Increased human activity has directly and indirectly led to exploitation and dramatic habitat fragmentation and loss; hence, extinction rates have arisen alarmingly over the last few decades (Hedrick & Miller 1992, Frankham et al. 2002). Many species now require benign human intervention to improve their management and ensure their survival.

The reason for biological conservation is that humans derive many direct and indirect benefits from the living world. Thus, we have a stake in the conservation for the resources we use, for the ecosystem services it provides for us, for the pleasure we derive from living organisms and for ethical reasons (Frankham et al. 2002). Bioresources include all of our food, many pharmaceutical drugs, clothing fibres (wool and cotton), rubber and timber for housing and construction, etc (Frankham et al. 2002). Their value is many billions of dollars annually (Frankham et al. 2002). Further, the natural world contains many potentially useful novel resources. Ecosystem services are essential biological functions that are provided free of charge by living organisms and which benefit humankind (Frankham et al. 2002). They include oxygen production by plants, climate control by forests, nutrient cycling, natural pest control, pollination of crop plants, etc (Frankham et al. 2002). Moreover, humans derive pleasure from living organisms (aesthetics), as expressed in growing ornamental plants, keeping pets, visits to zoos and nature reserves, and ecotourism (Frankham et al. 2002). This translates into direct economic value.

When we deal in biological conservation, it is essential to preserve the three levels of biodiversity, genetic diversity, species diversity, and ecosystem diversity as recommended by the World Conservation Union (IUCN) (Frankham et al. 2002, Reed & Frankham 2002). Conservation of genetic diversity is recommended by the two reasons (Reed & Frankham 2002): (1) genetic diversity is required for populations to evolve in response to environmental changes and (2) heterozygosity levels are linked directly to reduced population fitness via inbreeding depression. Because genetic variation is a basis for evolution via natural and artificial selection (Frankham et al. 2002), populations with high genetic diversity will tend to survive through the environmental changes. For example, newly-emerged disease organisms require that species maintain the ability to adapt to the changes in the features of the disease organisms (Carius et al. 2001). It is also applied to changes due to human activities, for example global climate change which is forcing species to move or adapt (Hughes 2000).

The detrimental effects of inbreeding, or the mating between the close relatives, on the fitness of a species have been long known. Early in the development of Mendelian genetics, researchers realized that the increased homozygosity resulting from inbreeding caused a loss in fitness, a phenomenon termed inbreeding depression (Hedrick & Miller 1992, Freeland et al. 2011). Inbreeding leads to inbreeding depression in virtually all species studied thus far and reduces reproductive fitness (Reed & Frankham 2002, Freeland et al. 2011). Thus, the loss of genetic diversity or heterozygosity has a deleterious effect on population fitness (Reed & Frankham 2002).

1.3 Genetic Information

The success of wildlife management strategies is contingent on understanding the evolutionary and demographic underpinning of contemporary biodiversity (Moritz et al. 2002). In this context, one of the major uses of genetic techniques in conservation is to use variation to uncover relationship between individuals, groups, populations, races, or

species (Moritz 1994, Marshall et al. 1999, Frankham et al. 2002). Genetic information is involved directly in the genetic diversity (Section 1.2) and is a crucial factor in species conservation. Therefore, understanding the genetic diversity based on genetic information, such as the number of haplotypes or alleles, heterozygosity, and genetic diversity indices (Freeland et al. 2011), is the first step of biological conservation.

In addition to the fact that genetic information is necessary for understanding the genetic diversity of animals, it is also useful for providing new insights about ecology and evolution (Freeland et al. 2011). DNA sequence polymorphisms and patterns in the genetic diversity of natural populations represent their genealogy and the relative impacts of historical, geographic, and demographic events (Avice 2000, Nordborg & Innan, 2002), useful for estimating population dynamics in evolutionary time scales. Gene genealogies provide insight into the relative impact of historical dispersal events on the contemporary distribution of evolutionary lineages (i.e. phylogeography; Avice 2000) and occurrence of genetic bottleneck (populations severely reduced in size) that can increase demographic stochasticity, rate of inbreeding, loss of genetic variation, and fixation of deleterious alleles and, thereby, reduce adaptive potential and increase the probability of population extinction (Frankham et al. 2002, Luikart & Cornuet 1998).

In ecological time scales, quantifying population subdivision and gene flow can be used for estimating the behaviors of animals, including philopatry in migration. Gene flow is one of the most important determinants of genetic differentiation and essentially the movement of genes between populations (Freedman et al. 2011). When female animals nest at their natal areas, genetic differentiation among rookeries can be observed in mitochondrial DNA (mtDNA) that is maternally inherited (Encalada et al. 1996, Bowen & Karl 2007). By comparing the population genetic structure investigated by mtDNA markers and biparentally inherited nuclear DNA (nDNA) markers, we can assess the level of male-mediated gene flow and the breeding strategy of males (FitzSimmons

et al. 1997a, b).

Furthermore, genetic isolation among populations has also afforded an opportunity to link populations of migratory species in different places (Pella & Masuda 2001, Bolker et al. 2003). Therefore, genetic information is useful for revealing cryptic features of animals, enabling us to establish adequate conservation management strategies. For example, migratory species go beyond our range of vision, but linkage between locations can be estimated by genetic information.

1.4 Sea Turtles

1.4.1 Overview

Sea turtles (Fig. 1.1) are highly adapted to marine environment and distributed in tropical, temperate, and even sub-arctic waters worldwide (Pritchard 1997). They are basically creatures that spend their entire lives in marine or estuarine habitats and their only reptilian ties to terrestrial habitats are for nesting and restricted cases of basking (Musick & Limpus 1997). A typical life history pattern of sea turtles is shown in Fig. 1.2 and below based on Miller (1997) and Bolten (2003). All sea turtles move immediately to the sea after hatching, usually after dark, and swim actively offshore (Wyneken & Salmon 1992). Most then undertake a mostly passive, denatant migration drifting pelagically in oceanic gyre systems, but active swimming may play an important role in forming the settlement patterns of young juveniles (Okuyama et al. 2011, Scott et al. 2012, Manisfield & Putman 2013). Subsequently, after a period of years, these now larger and older juveniles actively recruit to demersal neritic developmental habitats in the tropical and temperate zones. When approaching maturity, pubescent turtles move into adult foraging habitats where are geographically distinct from juvenile developmental habitats in some populations. Upon maturity as the nesting season approaches adults make a contranatant migration toward the nesting beaches. Most mating occurs at poorly defined courtship areas that are close to the nesting beaches relative to the distant foraging areas. After mating the females move to their respective nesting beaches.

During the nesting season, females usually become resident in the interesting habitat in the vicinity of the nesting beach.

Sea turtles have a long and ancient history (Pritchard 1997). They arose in the early Mesozoic, and for the next 100 million years, during the rise and reign of the dinosaurs, they shared the oceans with a rich diversity of other air-breathing marine reptiles. By the end of the Cretaceous era, the sea-going ichthyosaurs and plesiosaurs were extinct, but sea turtles tenaciously survived and flourished up very recent times. But today, their numbers are dramatically reduced to the point that all seven remaining species of sea turtles are considered either threatened or endangered on a worldwide basis (Lutcavage et al. 1997). They have extreme vulnerability to mankind, from loss of nesting beach and foraging habitats to mortalities on the high seas through pelagic fishing, and their inclusion on most lists of threatened or endangered species is a reflection primarily of past overexploitation and current need for better management rather than to inherently poor adaptation to post-Pleistocene conditions (Pritchard 1997). The Convention on International Trade in Endangered Species of Flora and Fauna (CITES) lists all sea turtles on its Appendix I (i.e. prohibited from international trade from or to signatory countries). The IUCN has listed all sea turtle species (except flatback turtles, *Natator depressus*) as Vulnerable, Endangered, or Critically Endangered (Seminoff & Shanker 2008, IUCN 2013) (Table 1.1).

On the other hand, sea turtles have various economic and noneconomic values (Campbell 2003, Witherington & Frazer 2003). Firstly, they provide bioresources for us. For example, tortoiseshell, traditionally obtained from the hawksbill turtle (*Eretmochelys imbricata*), has ranked among the world's luxury goods since earliest recorded times (Campbell 2003). Japan has a long history of crafting hawksbill shell (*bekko*) into various decorative items, some of which have been found in ruins of a seventh-century city. *Bekko* is crafted using traditional techniques and tools thought to be the same as those used 300-1000 years ago. Secondly, sea turtles are potential resources for non-consumptive recreation tourism, providing esthetic merit

(Wilson & Tisdell 2001, Witherington & Frazer 2003). Sea turtles lend themselves well to being watched, and in this admiration, there is an economic enterprise. The enterprise known as ecotourism can include sea turtles when visitors huddle around a female turtle on a beach to closely watch her nesting behavior, when tourists watch groups of hatchling scrambling from nest to sea, or when boat or diving tours bring people within sight of turtles in the water. The effect of turtle-watching visitors on the local economy was calculated to be approximately 800,000 AUD (0.45 million USD) per year in Mon Repos, Queensland, Australia (Witherington & Frazer 2003). Thirdly, sea turtles are obligate terrestrial nesters that are ecologically important in both marine and terrestrial systems (Bjorndal & Jackson 2003). For example, sea turtles can transfer substantial quantities of nutrients and energy from nutrient-rich foraging grounds to nutrient-poor nesting beaches because they occur in oceanic and neritic habitats from the tropics and subarctic waters and venture onto terrestrial habitats to nest or bask in tropical and temperate latitudes (Bouchard & Bjorndal 2000).

1.4.2 Sea Turtles in Japan

Due to increasing concern about the conservation of sea turtles the demand of *bekko*, various projects have been carried out for conserving and enhancing the sea turtle population in Japan, including mitigation for their incidental captures with fisheries (Gilman et al. 2010), coastal management for conserving their nesting habitat (Watanabe 2010), and head-starting projects (Okuyama et al. 2010). In Japan, three species of sea turtles, loggerhead (*Caretta caretta*), green (*Chelonia mydas*), and hawksbill turtles, are mainly observed to nest. Loggerhead turtles nest on beaches widely in Japanese Archipelago and have been relatively well studied (Bowen et al. 1995, Sakamoto et al. 1997, Hatase et al. 2002a, 2002b, Kamezaki et al. 2003, Watanabe et al. 2011). On the other hand, there has been relatively less attention to green turtles and hawksbill turtles in Japan that have been reported to nest in restricted subtropical regions in Japan, mainly in Ogasawara and Ryukyus (green turtle; Hatase et al. 2006,

Hamabata et al. 2009) or Ryukyus (hawksbill turtle; Kamezaki 1989).

In the Yaeyama islands of Ryukyu Archipelago, consistent number of green turtles nest (estimated number of total nesting females in Ishigaki Island of Yaeyama Islands is 75 at most; Abe et al. 2003). Whereas a majority of nesting on the island is done by green turtles, hawksbills also nest on at a lower frequency. Between 1995 and 2003, 14 hawksbill nests were observed in comparison with 427 green turtle nests (Abe et al. 2003). Despite the low level of hawksbill nesting, the coastal areas around the Yaeyama Islands including Ishigaki Island are considered a major foraging ground for both green turtles (Hamabata et al. 2009) and hawksbill turtles (Okayama et al. 1999). Therefore, information on behaviors and populations of sea turtles in this northwestern Pacific region is important for prioritization of sea turtle conservation in the Pacific.

Table 1.1 Summary of current status of sea turtles on the IUCN Red List (modified from Seminoff & Shanker (2008)).

| Species | Red List status | Year |
|---|-----------------------|------|
| Leatherback (<i>Dermochelys coriacea</i>) | Critically Endangered | 2000 |
| Hawksbill (<i>Eretmochelys imbricata</i>) | Critically Endangered | 1996 |
| Kemp's ridley (<i>Lepidochelys kempii</i>) | Critically Endangered | 1996 |
| Olive ridley (<i>Lepidochelys olivacea</i>) | Vulnerable | 2007 |
| Loggerhead (<i>Caretta caretta</i>) | Endangered | 1996 |
| Green (<i>Chelonia mydas</i>) | Endangered | 2004 |
| Flatback (<i>Natator depressus</i>) | Data deficient | 1996 |



Fig. 1.1 A nesting green turtle.

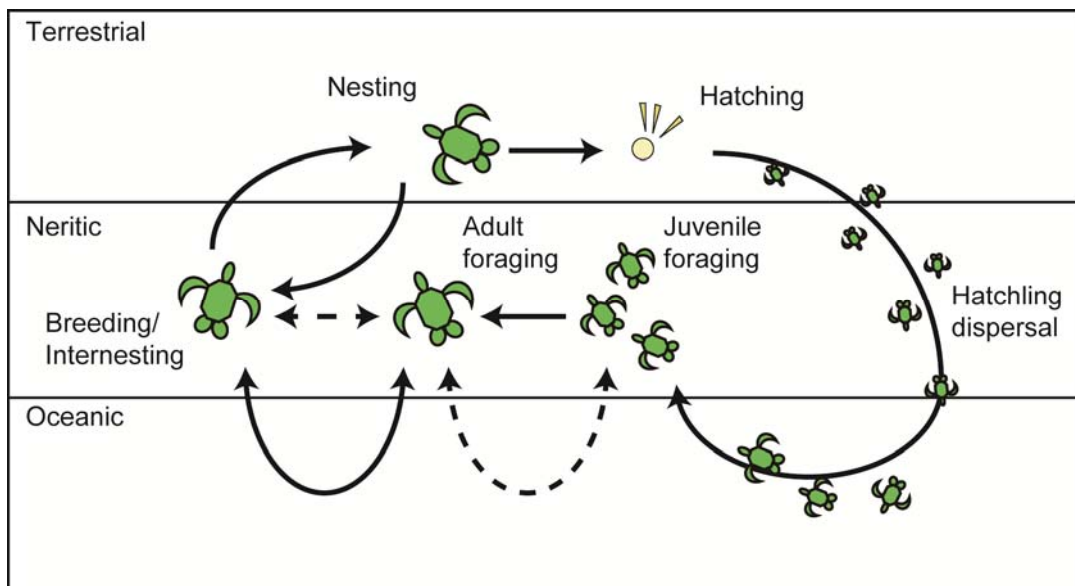


Fig. 1.2 A typical life history pattern of sea turtles illustrating the sequence of ecosystems inhabited (modified from Miller (1997) and Bolten (2003)). Some species or at least populations show different patterns: Leatherback (*Dermodochelys coriacea*) and at least some populations of olive ridley turtles (*Lepidochelys olivacea*) remain pelagic foragers throughout their lives, and flatback turtles (*Natator depressus*) remain neritic throughout their lives (Bolten 2003).

1.5 Objectives

Sea turtles have long history since they appeared in the Cretaceous, in addition to the migratory life history containing some cryptic features (Section 1.4). Therefore, for understanding the evolutionary and demographic underpinning of contemporary biodiversity, and revealing the connectivity between populations and aggregations relating to their migrations and behaviors, genetic information is considered to be useful. This study focused on the sea turtles observed in the Yaeyama Islands and in the Japanese coastal areas because information on behaviors and populations in this northwestern Pacific region is important for prioritization of sea turtle conservation in the Pacific. Green turtles were mainly analyzed in this study, but hawksbill turtles were also analyzed when possible, enhancing our understanding of sea turtles.

I set two axes, temporal scale and spatial scale, for revealing the evolutionary and ecological implications for conservation of sea turtles. Firstly, this study worked on the phylogenetic origin of nesting turtles in the Yaeyama Islands, which enables us to estimate the historical colonization from equatorial regions and whether historical bottleneck had occurred in this region (Chapter 2). Secondly, more recent dynamics and migrations of nesting populations in the Yaeyama Islands were inferred from the information on the genetic subdivision and population structure (Chapter 3). Next, I expanded the spatial scale from the nesting sites in the Yaeyama to broad Northwest Pacific. This involved the analysis of feeding aggregation, and estimated the origin and migration from natal beaches to the feeding grounds statistically by mixed-stock analysis based on Bayesian approaches (Chapter 4). Covering both nesting populations and feeding aggregations is important because surveys of multiple life stages are desirable to resolve conservation implications in migratory marine species (Bowen et al. 2005). Because the feeding aggregations were estimated to contain individuals originating multiple rookeries in the Pacific, further analysis of the feeding aggregations inferred the historical population dynamics of the Pacific sea turtles (Chapter 5).

The questions answered in this study can be summarized below:

1. How did sea turtles colonize nesting sites in the Yaeyama Islands? From the conservation perspective, did they experience bottleneck by founder effect? (Chapter 2)
2. What is the degree of genetic divergence between and among nesting populations, and how does this relate to natal philopatry of sea turtles? This question is important of setting the management units and conservation strategies of nesting sea turtles. (Chapter 3)
3. How are the genetic structure of feeding aggregations around Japanese coastal areas and the contribution of individual nesting beaches to offshore feeding aggregations? This invokes the conservation of estimated corridors of migration. (Chapter 4)
4. What patterns of historical population contraction have sea turtles in the Pacific experienced? The comparison with the Atlantic sea turtles (Reece et al. 2005) can provide insight into the features of genetic diversity of sea turtles in the Pacific. (Chapter 5)

Based on the results of Chapters 1-5, I discussed the conservation management of sea turtles in the Yayama and Pacific regions in Chapter 6. This thesis might be an important comprehensive study about conservation of migratory marine species.

Chapter 2

Phylogenetic Origin of Nesting Turtles in the Northwest Pacific

Summary

For conservation of endangered species, understanding genetic diversity is important. A factor influencing the genetic diversity is bottleneck (populations severely reduced in size) that can increase demographic stochasticity, rate of inbreeding, loss of genetic variation, and fixation of deleterious alleles and, thereby, reduce adaptive potential and increase the probability of population extinction. Bottlenecks can occur not only by the recent human activity, but in the beginning process of historical population colonization as a founder effect. Therefore, it is important to assess the relationships of biota in separate geographical regions and the relative impact of historical dispersal events on the contemporary distribution of evolutionary lineages (i.e. phylogeography) by the intraspecific gene genealogies. The Yaeyama nesting populations of green (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*), representing the northern limit of their nesting in the western Pacific Ocean, are thought to exhibit a genetic structure reflecting colonization from equatorial regions. In this chapter, the patterns of mitochondrial DNA (mtDNA) variation in nesting green (n = 67) and hawksbill turtles (n = 4) revealed haplotypes from distinct lineages in both species. Genetic diversity values were within the high end of the range found for other sea turtle populations. The results indicated historical introgressions by individuals with divergent haplotypes from both Pacific colonies and Indian and Southeast Asian colonies after glacial periods. In addition, populations of green and hawksbill turtles in the Pacific appear to have experienced very similar patterns and processes of distribution and subdivision over the last several million years.

The detail of this chapter was published in:

- [1] Nishizawa H, Abe O, Okuyama J, Kobayashi M, Arai N (2011)
Population genetic structure and implications for natal philopatry of nesting green turtles (*Chelonia mydas*) in the Yaeyama Islands, Japan. *Endangered Species Research* 14: 141-148
- [2] Nishizawa H, Okuyama J, Abe O, Kobayashi M, Arai N (2012)
Mitochondrial DNA variation in hawksbill turtles (*Eretmochelys imbricata*) nesting on Ishigaki Island, Japan. *Marine Turtle Newsletter* 132: 1-2

Chapter 3

Genetic Population Structure in the Nesting Populations: Philopatry in the Northwest Pacific

Summary

Population genetic structure inside the Yaeyama Islands will provide important information for their conservation. The reason is that genetic differentiation among the rookeries reflects migration of sea turtles in ecological timescales, implications for natal philopatry. Female turtles are known to nest at their natal areas at ecological timescales, resulting in genetic differences in mitochondrial DNA (mtDNA), but the geographical specificity of homing is uncertain. Resolution of natal homing is essential to understanding how green turtles respond to changes in the availability of nesting sites over time. In addition, to investigate the breeding strategy of males, nuclear DNA (nDNA) markers can be used to assess the level of male-mediated gene flow and population subdivision. In this chapter, the genetic differentiation among Yaeyama nesting rookeries was examined, providing insight into the degree of natal homing and male-mediated gene flow in this region. The population genetic structure of nesting green turtles in the Yaeyama Islands revealed the significant genetic differentiation, at least between southwestern Iriomote Island ($n = 26$) and Ishigaki Island ($n = 41$) based on mtDNA, indicating precise female natal philopatry compared to other Pacific and Indian regions. On the other hand, microsatellite analyses of four markers revealed no significant genetic differentiation, indicating the existence of male mediated gene flow.

The detail of this chapter was published in:

- [1] Nishizawa H, Abe O, Okuyama J, Kobayashi M, Arai N (2011)
Population genetic structure and implications for natal philopatry of
nesting green turtles (*Chelonia mydas*) in the Yaeyama Islands, Japan.
Endangered Species Research 14: 141-148

Chapter 4

Genetic Composition of Feeding Aggregations: Estimating the Migration in the Pacific Ocean

Summary

The movements of migratory marine vertebrates from their natal sites and the composition of feeding aggregations have historically been difficult to elucidate. Recently, however, examination of differences in mitochondrial DNA (mtDNA) haplotype frequencies caused by genetic isolation among nesting populations has afforded an opportunity to link feeding populations back to their rookery of origin and to estimate the contributions of genetically differentiated nesting populations to foraging assemblages using mixed-stock analysis. The results of MSA are expected to be useful for understanding the migrations of sea turtles. The coastal waters of the Japanese archipelago provide feeding grounds for the green turtle along the strong Kuroshio Current. Feeding habitats in Japan may be occupied by individuals from these Japanese rookeries, but also carried by the Kuroshio Current from multiple tropical rookeries in the Pacific. Therefore, the genetic compositions of consecutive Japanese feeding aggregations of green turtles along the Kuroshio Current were examined by mixed-stock analyses of mtDNA control-region sequences. The results indicated that the southern feeding aggregation around Yaeyama was sourced from various Pacific rookeries in the Yaeyama, Ogasawara, Western Pacific, and Indian Oceans and Southeast Asia. Among northern feeding aggregations, the Ginoza aggregation was also sourced from the Western Pacific Ocean, but the Nomaike, Muroto, Kanto, and Sanriku aggregations were contributed mostly by the closer Ogasawara rookeries. The reduced contribution from tropical Pacific rookeries to northern feeding aggregations and the significant correlation between genetic differentiation and geographical distance matrices of feeding aggregations indicated that most hatchlings

from these regions transported by the Kuroshio Current settle in upstream feeding grounds along the Japanese archipelago, implying that current flow influences the composition of feeding aggregations.

The detail of this chapter was partly published in:

- [1] Nishizawa H, Naito Y, Suganuma H, Abe O, Okuyama J, Hirate K, Tanaka S, Inoguchi E, Narushima K, Kobayashi K, Ishii H, Tanizaki S, Kobayashi M, Goto A, Arai N (2013) Composition of green turtle feeding aggregations along the Japanese Archipelago: implications for changes in composition with current flow. *Marine Biology* 160: 2671-2685

Chapter 5

Historical Perspectives on Population Genetics of Pacific Sea Turtles

Summary

DNA sequence polymorphisms and patterns in the genetic diversity of natural populations represent their genealogy and the relative impacts of historical, geographic, and demographic events. In particular, mitochondrial DNA (mtDNA) polymorphisms have been used extensively in the study of maternal lineages and population structures. The Yaeyama Islands, located in southwestern Japan, are an important feeding ground for both green and hawksbill turtles. Considering that both green and hawksbill turtles nest and hatch on beaches in tropical regions and that their hatchlings undertake mostly passive migrations, the foraging aggregation of green and hawksbill turtles around the Yaeyama Islands may also comprise individuals from widespread rookeries throughout the Pacific. Therefore, the mtDNA haplotypes observed in the turtle feeding aggregations in the Yaeyama Islands may represent those of widespread areas of the Pacific as a whole, and would thus enable us to estimate at least part of the genealogy and historical dynamics of hawksbill and green turtles in the Pacific. In this chapter, historical patterns in population dynamics and differentiation of Pacific hawksbill and green turtles from the Yaeyama feeding ground were estimated by neutrality test statistics, observed distributions of pairwise nucleotide differences, and comparative phylogenies. Phylogenetic relationships of the haplotypes indicated that both turtles in the Pacific underwent very similar patterns and processes of population dynamics over the last several million years, with population subdivision during the early Pleistocene and population expansion after the last glacial maximum. These significant contemporary historical events were suggested to have been caused by climatic and sea-level fluctuations. On the other hand, regional differences in historical population dynamics

compared to the Atlantic are suggested.

The detail of this chapter was published in:

- [1] Nishizawa H, Okuyama J, Kobayashi M, Abe O, Arai N (2010) Comparative phylogeny and historical perspectives on population genetics of the Pacific hawksbill (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*), inferred from feeding populations in the Yaeyama Islands, Japan. *Zoological Science* 27: 14-18

Chapter 6

General Discussion: Conservation Implications for Sea Turtles in the Pacific

6.1 Genetic Diversity and Conservation Priority

In the Chapter 5, the regional difference in historical population dynamics in the Pacific sea turtles compared with those in the Atlantic (Reece et al. 2005) would support the need for regionally varying conservation strategies (Seminoff & Shanker 2008). Chapter 2 and Chapter 3 revealed that the Yaeyama green turtle nesting populations exhibit relatively high genetic diversity, likely as a result of historical emigration from divergent tropical colonies, despite relatively small or obscure number of nesting turtles at least in Ishigaki Island (Abe et al. 2003). In Japan, green turtles as well as hawksbill turtles on nesting beaches have relatively high genetic diversity. Recently, regional management units have been suggested to provide a framework for assessing high diversity areas and evaluating conservation status of sea turtles (Wallace et al. 2010). Considering that green turtle nesting populations of Wan-an Island in Taiwan, another nesting rookery in Northwestern Pacific, also has high genetic diversity (Cheng et al. 2008) and significantly differentiated from those of Yaeyama islands, regional management unit of Northwestern Pacific green turtles (Wallace et al. 2010) are indicated to be important in terms of the conservation of genetic diversity. Moreover, the significant genetic differentiation among the Yaeyama Islands as revealed by mtDNA analysis may indicate precise female natal philopatry, as among Taiwanese rookeries. The findings indicate that nesting females of green turtles in the Yaeyama Islands have precise natal philopatry at ecological timescales although they had been formed by emigration from divergent tropical colonies at historical timescales. Considering that natal philopatry is related to their response to changes in availability of nesting sites over time (Dethmers et al. 2006), Yaeyama nesting populations may have a high priority in conservation.

Despite relatively high male-mediated gene flow, this study indicated some phylogeographic features and conservation priority of northwest Pacific green turtles.

6.2 Conservation Strategies based on the Migration

The management of green turtles based on linkages between their feeding aggregations and rookeries will be needed for their conservation (Bowen et al. 2007). In the Pacific Ocean, the Hawaiian feeding aggregation of green turtles was estimated to originate mostly from Hawaiian rookeries, indicating a distinct regional population for management (Dutton et al. 2008). On the other hand, the Colombian feeding aggregation in the eastern Pacific was estimated to be recruited from distant sites, indicating the importance of multinational conservation strategies (Amarocho et al. 2012). In Japanese feeding aggregations, both of these types of sourcing were observed (Chapter 4). The estimated compositions of Japanese feeding aggregations have conservation implications. The Ogasawara nesting rookery was estimated to contribute significantly to all Japanese feeding aggregations analysed in this study. Among them, northern feeding aggregations from Japan, Nomaie, Muroto, Kanto, and Sanriku were estimated to have contributions primarily from Ogasawara. Therefore, hazards that affect declining nesting populations in Ogasawara may also affect a wide range of Japanese feeding aggregations, especially northern feeding aggregations. This indicates the importance of regional management in Japan. On the other hand, the Yaeyama and Ginoza feeding aggregations of Japan are estimated to have migrated from remote Pacific rookeries. Therefore, any source of mortality in nesting rookeries in the tropical Pacific is likely to affect remote feeding aggregations in Japan. Conversely, effects on feeding aggregations in Japanese waters could affect nesting populations in other countries because of natal philopatry (Chapter 3). It emphasizes the necessity for multinational conservation strategies for green turtles in the western Pacific, as in other regions (Amarocho et al. 2012) and for other sea turtle species (Bowen et al. 2007).

Although only small amount of green turtles born in Hawaii or the Eastern Pacific rookeries may be transported to the Japanese coastal area, particularly the Sanriku coastal area (Chapter 4), and return to their natal beaches to breed, the effects on the Sanriku feeding aggregations could influence remote nesting populations in Hawaii or the Eastern Pacific.

6.3 Conclusions

By using genetic information, this study uncovered relationships between individuals and populations of sea turtles in the Yaeyama and the Pacific Ocean: phylogeography, historical population dynamics, natal philopatry, and migrations. In this way, genetic information is valuable for understanding the cryptic features in wide range of temporal and spatial scales. These kinds of information include conservation implications for endangered sea turtles contributing to the management of genetic diversity or local populations. Exploring the effective utilization of important data for biological conservation from genetic information by using computer-based simulations and statistical methods is necessary for resolving social issues concerning the conflicts between humans and other animals. This study offers an important case of conservation management of endangered species based on genetic information. Techniques of this study can be applicable to other endangered species and/or migratory species.

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