Short Note (短 報)

Genetic Profile of Deko-chan, an Unringed Short-tailed Albatross in Torishima, Izu Islands, and the Implications for the Species' Population StructureMasaki Eda^{*, 1)}, Hiroko Koike^{**, 2)}, Fumio Sato^{***} and Hiroyoshi Higuchi^{*}

Abstract. The Short-tailed Albatross *Diomedea albatrus* is a vulnerable seabird that breeds on Torishima, one of the Izu Islands, and on Minami- and Kita-kojima islands in the Senkaku Islands. An unringed bird has been observed at the Hatsunezaki colony on Torishima every year since 1996, even though since 1979 almost all birds hatched on Torishima have been ringed on both legs. This unringed individual was named Deko-chan as, until the 2005/06 breeding season, it made a nest every year near a decoy that was set to attract the birds to Hatsunezaki. Given that losing the rings from both legs while still having sub-adult plumage is unlikely, the natal site of the bird was suspected to be the Senkaku Islands. The identification of Deko-chan's natal site is important to assess whether birds from the Senkaku Islands are coming to Torishima and whether birds from the two islands are pairing. Our previous studies using control region 2 (CR2) of the mitochondrial DNA sequence suggested that the species includes two distinct populations (clades 1 and 2), with birds breeding on Torishima mostly consisting of descendants of clade 1 individuals, and those sampled in the Senkaku Islands comprised of descendants of clade 2 individuals. In this study, we analysed CR2 obtained from down collected from the nest of Deko-chan. The results reveal that Deko-chan has a CR2 sequence that differs from any reported to date, but indicates that it belongs to clade 2, supporting the suggestion that the natal site of the bird is the Senkaku Islands, not Torishima. Although the bird had paired with a ringed bird and reared two young by the 2009/10 breeding season, further studies using bisexually transmitted molecular markers are required to confirm whether the two lineages are able to mix.

Key words: Control region, Genetic profile, Mitochondrial DNA, Short-tailed Albatross *Diomedea albatrus*.

キーワード: 制御領域, 遺伝のプロファイル, ミトコンドリア DNA, アホウドリ *Diomedea albatrus*.

The Short-tailed Albatross *Diomedea albatrus* is a vulnerable seabird that breeds on remote islands in the northwestern Pacific during winter and spends the summer in the

Received 5 January 2011, Revised 10 March 2011, 2nd Revision 29 March 2011, Accepted 30 March 2011.

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North Pacific Ocean (Tickell 2000, Hasegawa 2003, BirdLife International 2008, U.S. Fish and Wildlife Service 2008). It is estimated that in the late nineteenth century, there were several million birds in more than 13 breeding colonies. Since then, however, their numbers have declined drastically due to fowling (Tickell 2000, Hasegawa 2003, BirdLife International 2008, U.S. Fish and Wildlife Service 2008). In the mid-twentieth century, only about 50 individuals and a single breeding site were left on Torishima, one of the Izu Islands. Currently, however, as a result of conservation efforts, the population on Torishima is increasing. These efforts have included managing the conditions of the breeding sites on Cape Tsubamezaki by transplanting native plants, and establishing a new breeding colony at Cape Hatsunezaki by using decoys and recorded playback of breeding-colony sounds (Hasegawa 2003). Recently, another breeding site has been recognised, roughly 1,700 km from Torishima. It is located on Minami- and Kita-kojima islands in the Senkaku Islands. In the 2007/08 nesting season, the total albatross population was estimated at approximately 2,400, and the numbers of breeding pairs on Torishima and the Senkaku Islands were estimated to be 375 and 75, respectively (U.S. Fish and Wildlife Service 2008).

Fig. 1. Distribution of breeding sites of the Short-tailed Albatross and of the sampled archaeological site (Hamanaka 2 site).

On Torishima, an unringed bird has been observed at the Hatsunozaki colony every year since 1996, even though almost all birds hatched on Torishima since 1979 have been ringed on both legs (Sato 1999, Yamashina Institute for Ornithology 2006). Until the 2005/06 breeding season, the bird made a nest near decoy No. 22 every year and showed mating displays towards the decoy. It was named Deko-chan, after this behaviour (Sato 1999). Given that Deko-chan's plumage was sub-adult when first observed, it is unlikely that it hatched on Torishima before 1979 or that it had lost both rings. Thus, the natal site of the bird was suspected to be the Senkaku Islands rather than Torishima (Sato 1999).

Previously, we analysed CR2 obtained from modern and archaeological samples of Short-tailed Albatrosses and revealed that the species includes two distinct populations (clades 1 and 2); birds sampled in the Senkaku Islands consist of descendants of clade 2 birds; and birds breeding on Torishima are mostly descendants of clade 1 birds, although approximately 7% of the birds (3 of 41 studied birds) were clade 2 descendants (Eda *et al.* 2010, Kuro-o *et al.* 2010, Eda *et al.* in press). Because clade 2 birds are rare on Torishima and no clade 1 bird has been observed in the Senkaku Islands, determination of the CR2 haplotype of Deko-chan would be useful to confirm the natal site of the bird. Clarification of Deko-chan's natal site is important to assess whether birds from Senkaku Islands are coming to Torishima and whether birds from the two islands are pairing. Herein, we also address the implications of our results and future perspectives with regard to genetic studies of the Short-tailed Albatross.

A down feather was collected from the nest of the unringed Short-tailed Albatross in the Hatsunozaki colony, Torishima, in November 2001. We are certain that the feather was from Deko-chan because no other birds were observed near the nest during 17 days of continuous observations prior to collecting the sample. The whole feather was sliced into small fragments with clean scissors and homogenised in liquid nitrogen. The sample was then placed in a solution containing 450 μ l of lysis buffer, 25 μ l of 10% SDS, and 25 μ l of 20 mg/ml Proteinase K for the protein digestion, and incubated for 4 h at 55°C on a rotator. Nucleic acids were extracted using an IsoQuick Nucleic Acid Extraction Kit (ORCA Research, Inc.). A blank extraction containing all buffers but lacking any sample was carried out to detect any contamination within the buffers and solutions.

We set a target region in domain I of CR2 (139 bp) of mtDNA and conducted semi-nested PCR using the primer pairs Lcon2.dio (5'-TAGGAAAAACATTTAATGCATGTACYACG-3') and Hcon3.dio (5'-TACCATTTCARRGTATGYTGCTCRAGC-3') for the first PCR and Lcon3.dio (5'-ATTTAATGCATGTACTACGCACATASAC-3') and Hcon3.dio for the second PCR to increase the specificity of the fragment (Eda *et al.* 2010, Eda *et al.* unpubsied data). The PCR amplifications were carried out in 25- μ l reaction volumes consisting of 1 μ l of the DNA extract, 0.2 μ M of each nucleotide, 0.1 μ M of each primer, 0.2 mg/ml of bovine serum albumin and 1.25 units of *Ex Taq* (Takara) in a 1 $\times$ concentration of the supplied reaction buffer. PCR cycling was conducted as follows: step 1, 94°C for 1 min; step 2, 94°C for 30 s, 50°C for 45 s, 72°C for 45 s, and a final extension at 72°C for 1 min. The number of cycles was set at 40 for the first PCR and at 30 for the second PCR. An amplification blank (negative control) was performed for the PCR reaction. Following purification of the template using a PCR

Product Pre-sequencing Kit (USB), the PCR product was sequenced in both directions using a CEQ 2000 DNA Analysis System (Beckman Coulter) with the same primers as used for the second PCR.

The sequence obtained was aligned with sequences from *D. albatrus* individuals sampled on Torishima, the Senkaku Islands, or an archaeological site (Hamanaka 2 site, Rebun Island, northern Hokkaido, c. 1,000 y.b.p.) (DDBJ/EMBL/GenBank Accession No. AB276044—AB276063 and AB551841—AB551868) using Clustal-X in MEGA 4.0 (Tamura *et al.* 2007). A statistical parsimony network was generated using TCS version 1.2.1 (Clement *et al.* 2000) with sequences of modern and archaeological Short-tailed Albatrosses. Because the nesting algorithm terminated with the non-parsimonious union of two clades in the TCS, the least genetic distance between the two clades was calculated using MEGA. The newly observed sequence was deposited into the DDBJ/EMBL/GenBank databases with Accession No. AB604649.

A CR2 sequence from the down of Deko-chan was obtained successfully without any positive PCR products in the negative controls. The sequence obtained was different from the previously observed sequences, as it included at least one variable site from the archaeological samples and at least four variable sites from the modern samples (Table 1). The statistical parsimony network clearly showed that the sequence belonged to clade 2 (Fig. 2). The results are in agreement with the unringed status of the bird and suggest strongly that the bird was hatched on the Senkaku Islands, not on Torishima.

After the 2006/07 breeding season, the project to establish a new colony at Cape Hatsunezaki ended and the decoys were removed. In the next breeding season, Deko-chan paired with a ringed (*i.e.*, demonstrably hatched on Torishima) adult albatross. The pair reared chicks in the 2007/08 and 2009/10 breeding seasons, although they failed to fledge a chick in the 2008/09 breeding season (Sato unpublished data). The breeding success of the pair (two of three) seems to be comparable to that of all pairs in the Torishima population (50% to 73% for the last decade (U.S. Fish and Wildlife Service 2008)), with no symptoms of any genetic disorder. Although this observation is sufficient to suggest the pairing of birds from Torishima and the Senkaku Islands, it is insufficient to confirm the breeding success of Deko-chan or a pair from different islands. This is because the chicks might result from extra-pair mating, which is not uncommon in albatrosses (approximately 25% and 11% in the Waved Albatross *D. irrorata* and the Wandering Albatross *D. exulans*, respectively (Huyvaert *et al.* 2000, Jouventin *et al.* 2007)). To resolve this point, paternity tests for the chicks using bisexually transmitted molecular markers, such as microsatellite DNA, are required.

In our study, we suggested that birds in Torishima and the Senkaku Islands are descendants from different populations, between which the genetic distance is 11.46% (Eda *et al.* in press). This distance is larger than that between a sister-species pair of the Black-browed Albatross *D. melanophris* and Campbell Albatross *D. impavida* (7.16%), between which pre-mating selection was suggested (Moore *et al.* 1997, Burg & Croxall 2001). This may indicate that divergence between the two populations of the Short-tailed albatross is large enough to achieve pre-mating isolation. Actually, pre-mating isolation is implied by differences observed in courtship displays between Deko-chan and some ringed

Table 1. Observed haplotype, variable nucleotide positions, and haplotype frequency at each sampling site. Haplotype names in Figure 2 and accession numbers in DDBJ/EMBL/GenBank are also shown. Nucleotide positions are numbered starting from the first nucleotide of the control region fragment studied in this study. Tr: Torishima, Sn: Senkaku, HM: Hamanaka 2 site (archaeological site).

[illegible]

Fig. 2. The statistical parsimony network of the haplotypes from living (Deko-chan, Torishima and Senkaku Islands) and archaeological (Hamanaka 2 site) Short-tailed Albatrosses. The size of each circle is proportional to the number of albatrosses sharing that haplotype. Numbers in the network show haplotype names corresponding to Table 1.

birds (Sato unpublished data, H. Hasegawa personal communication) and by a difference in the breeding season (approximately two weeks earlier on the Senkaku Islands; H. Hasegawa, personal communication). It is important to note that even if the paternity test proves Deko-chan's paternity, it is still insufficient to prove mixing between individuals from the two populations. This is because some ringed birds may be the descendants of birds that immigrated from Senkaku and bred on Torishima. DNA analysis of the chicks and mate of Deko-chan is required to evaluate whether these two populations are mixing.

Acknowledgements

Special thanks to Hiroshi Hasegawa, who provided unpublished data for Short-tailed Albatrosses on Torishima and in the Senkaku Islands. Masaki Kuro-o provided valuable comments on the DNA analysis. Ryo Maeyama, Yuki Watanabe, Hayao Murakami and Yuko Masuda helped with field research. We also thank Isao Nishiumi and two anonymous reviewers for their helpful comments on the manuscript.

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鳥島で観察される未標識アホウドリ・デコちゃんの遺伝的プロファイルと そのアホウドリの集団構造における意味合い

アホウドリ *Diomedea albatrus* は伊豆諸島の鳥島と尖閣諸島の南小島や北小島で繁殖する危

急種の海鳥である。1979 年以降、鳥島で生まれたほとんどの個体が両脚に標識をされているにもかかわらず、1996 年以降、鳥島の初寝崎において未標識の 1 個体が観察されている。2005 年度の繁殖期まで、アホウドリ誘致用に設置された特定のデコイのそばに毎年巣をつくったこの鳥は、デコイにちなんで「デコちゃん」と呼ばれている。若鳥のうちに標識が両脚から外れることは考えにくいので、この鳥は尖閣諸島で生まれた個体であると考えられてきた。近年の私たちのミトコンドリア DNA の制御領域 2 を用いた研究によって、アホウドリには 2 つの系統的に離れた集団（クレード 1 とクレード 2）が含まれていたこと、尖閣諸島で採集された資料はクレード 2 の個体からなること、鳥島で生まれた個体の多くがクレード 1 に属することが示唆されている。この鳥の巣で羽毛を採取して解析した結果、この鳥の制御領域 2 の配列は、これまでに知られていた配列とは異なるものの、クレード 2 に属することが明らかになった。このことは、デコちゃんの出生地が鳥島ではなく、尖閣諸島であることを支持するものである。デコちゃんは鳥島で生まれた個体とつがいを形成し、2009 年度の繁殖期までに 2 羽の雛を巣立たせている。しかし、2 つの系統が交配しているかどうかを判断するためには、両性遺伝する遺伝子マーカーによる研究が必要である。

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