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Origin of Japanese White-eyes and Brown-eared Bulbuls on the Volcano Islands

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The Ogasawara Archipelago comprises two groups of oceanic islands: the Bonin Islands, formed in the Paleogene, and the Volcano Islands, formed in the Quaternary. These groups are located within a moderate distance (ca. 160–270 km) of one another; thus, most land bird species are not distinguished as different subspecies. Two land birds, however, show unusual distribution. The Japanese white-eyes *Zosterops japonicus* originally inhabited only the Volcano Islands, but has been introduced to the Bonin Islands. The brown-eared bulbuls *Hypsipetes amaurotis* are distributed as a different subspecies. We investigated their genetic differences and divergences in the Ogasawara Archipelago using mitochondria DNA. The Volcano population of white-eyes had four endemic haplotypes that were divergent from one another, except for the Bonin population, which shared three haplotypes with the Volcano, Izu, and Ryukyu Islands and did not have any endemic haplotype. This is the first genetic suggestion that the Bonin population is a hybrid of introduced populations. With respect to bulbuls, the Volcano and Bonin Islands each had a single endemic haplotype. The Volcano haplotype is closest to a haplotype shared with Izu, the Japanese mainland, Daito and Ryukyu, whereas the Bonin haplotype is closest to one endemic to the south Ryukyu Islands. This indicates that the sources of the two bulbul populations can be geologically and temporally distinguished. The populations of the two species in the Ogasawara Archipelago are irreplaceable, owing to their genetic differences and should be regarded as evolutionarily significant units. In order to prevent introgression between the two populations, we must restrict interisland transfers.

Key words: volcanic islands, Ogasawara Archipelago, COI, DNA barcoding, introduced species, introgression, reduced dispersibility

INTRODUCTION

The Ogasawara Archipelago comprising the Volcano and Bonin Islands are located ca. 1000–1250 km south of Tokyo on the Japanese mainland (Fig. 1). The oceanic islands have not previously been connected to any landmass. The Bonin Islands were created by submarine volcanic activity in the middle Paleogene (Umino, 1985; Umino and Nakano, 2007). The Volcano Islands are younger, formed by volcanic activity and uplift in the Quaternary (Nakano and Furukawa, 2009), and they remain volcanically active. The archipelago has a unique oceanic island ecosystem and biodiversity (Kawakami, 2010a) and was designated a United Nations Educational, Scientific, and Cultural Organization World Natural Heritage site in 2011. However, the islands have suffered detrimental effects to their ecosystem and biodiversity by invasive species and deforestation (Kawakami, 2010a), which has motivated the government, biologists and local inhabitants to implement conservation and restoration projects (Kawakami, 2010b). To establish effective conservation strategies for the Ogasawara Archipelago it is important to take into careful consideration the genetic differences and historical background of each species.

Nine land bird species currently breed in the Ogasawara Archipelago (Ornithological Society of Japan, 2012). Because the Volcano Islands are relatively close to the Bonin Islands (at their closest they are ca. 160 km apart), there are seven breeding land bird species that reside on both island groups (Ornithological Society of Japan, 2012). Various explorers and biologists have recorded the avifauna in the archipelago since the discovery of the islands in the 16th century. The distribution of land birds within the archipelago has transitioned over time (Higuchi, 1984). Several species, such as the Bonin grosbeak *Chaunoproctus ferreorostris* and Bonin wood pigeon *Columba versicolor* have entirely disappeared from the archipelago (Ornithological Society of Japan, 2012). The Bonin white-eye *Apalopteron familiare* was present throughout the Bonin Islands in the 19th century (Seebohm, 1890; Momiyama, 1930; Yamashina, 1930; Suzuki and Morioka, 2005), and is currently only

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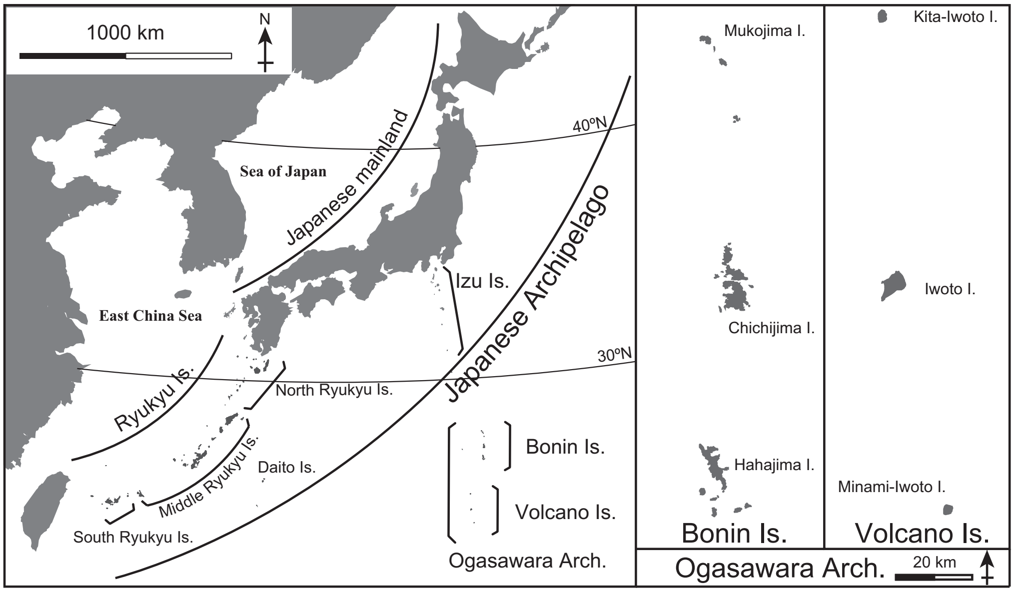

Fig. 1. Map showing the location of the Volcano and Bonin Islands.

found in the Hahajima island group (Ornithological Society of Japan, 2012). In contrast, the scaly thrush *Zoothera dauma* was not recorded in the archipelago until the mid-20th century except for one individual captured in the 1930s (Momiyama, 1930; Higuchi, 1984), but has now colonized the Bonin and Volcano Islands (Ornithological Society of Japan, 2012). The Japanese white-eye *Zosterops japonicus* had only been recorded in the Volcano Islands until the early 20th century (Seebohm, 1890; Seebohm, 1891; Uchida, 1911), but has subsequently been found in the Bonin Islands (Momiyama, 1930).

All land birds breeding in the Ogasawara Archipelago, except for Bonin white-eyes, are related to bird species on the Japanese Archipelago across the sea (Ornithological Society of Japan, 2012), but have few relationships with the birds of the Mariana Islands (Stinson, 1994). This suggests that land birds colonized the Ogasawara Archipelago from the older, larger landmass of the Japanese Archipelago. This theory would also apply within the Ogasawara Archipelago, in which land birds dispersed from the older (Bonin) to the younger islands (Volcano). The theory correlates with the results of a study on mitochondria DNA cytochrome c oxidase subunit I (COI) in Japanese bush-warblers *Cettia diphone*, in which the Volcano population was shown to be derived from the Bonin population base (Emura et al., 2013). In contrast, the Japanese white-eyes of Ogasawara exhibit an unexpected distribution in that the birds inhabit only the younger islands, the Volcano Islands, but not the older neighboring Bonin Islands (Seebohm, 1890, 1891). A similar distribution pattern was also recorded in two extinct populations that only inhabited the Volcano Islands: the white-browed crake *Porzana cinera* and the peregrine falcon *Falco peregrinus* (Seebohm, 1891).

The Japanese white-eye is widely distributed throughout the Japanese Archipelago and East Asia. The white-eye population on the Volcano Islands is classified as an endemic subspecies, *Z. j. alani* (Ornithological Society of Japan, 2012). Although the white-eye was not recorded in the Bonin Islands until the 1900s (Seebohm, 1890), it has become one of the most common bird species there. The Bonin population of white-eyes is classified as a subspecies, of *Z. j. stejnegeri* from the Izu Islands, or *Z. j. alani* from the Volcano Islands (Momiyama, 1930). Momiyama (1930) suggested that the Bonin population was a hybrid between *Z. j. stejnegeri* and *Z. j. alani*, due to its intermediate morphological traits in exposed culmen between the two subspecies. Based on oral reports, Momiyama (1930) surmised that the Bonin population was intentionally introduced from the Izu and Volcano population in the early 1900s due to their attractive song. To date, there is no genetic evidence to support Momiyama's theory, and the origin of the Bonin population of white-eyes remains unknown.

The brown-eared bulbul, which breeds mainly in the Japanese Archipelago, includes the native bulbul species that breed in the Volcano and Bonin Islands. The bulbul species in the Ogasawara Archipelago comprises two subspecies: *H. a. magnirostris* in the Volcano Islands and *H. a. squamiceps* in the Bonin Islands (Ornithological Society of Japan, 2012), which have morphological difference of bill size despite the islands being separated by a relatively short distance (Uchida, 1911). Of the extant bird species, the bulbul is the only species separated as a subspecies between the two island groups (Ornithological Society of Japan, 2012). The bulbuls inhabiting the Izu Islands are a subspecies, *H. a. amaurotis*, that is mainly distributed on the Japanese mainland and its offshore islands (Ornithological Society of Japan, 2012). A DNA barcoding study of Japanese birds revealed that in Japan the bulbul is divided into three groups with marked divergences in the COI gene sequence (Saitoh et al., 2015). The genetic difference within the Ryukyu Islands was shown to reach a maximum value of 3.57% using the Kimura 2 Parameter model (Saitoh et al., 2015). The COI sequence of the Bonin population is closest to that of populations of the Japanese mainland and south and north Ryukyu Islands.

The Japanese white-eye and brown-eared bulbul are widely distributed in the Japanese Archipelago. Both species have the advantage that large numbers of COI sequences were collected for them throughout the Japanese Archipelago in the DNA barcoding project (Saitoh et al., 2015). However, the barcoding library has lacked DNA samples from the populations of the Volcano Islands due to the difficulties posed by the islands being remote and uninhabited, and their use as a military base. Thus, the Volcano bird population represents a "missing piece of the puzzle" for understanding the phylogeography of Japanese birds. The objectives of the present study were to provide data on the white-eyes and bulbuls in the Ogasawara Archipelago based on COI sequences, which are important for guiding conservation projects in the islands. We also investigated genetic differences and genetic properties of the populations between the Japanese and Ogasawara Archipelagos.

MATERIALS AND METHODS

Study sites

The Ogasawara Archipelago was located on ca. 1,000 km east from the Ryukyu Islands and on ca. 600 km south from the Izu Islands (Fig. 1). The Bonin Islands have three island groups, the Mukojima island group, the Chichijima island group, and the Hahajima island group, which are ca. 40–50 km apart, respectively (Fig. 1). The Volcano Islands has three islands, Kita-Iwoto Island, Iwoto Island, and Minami-Iwoto Island, which are ca. 60–70 km apart (Fig. 1). Europeans and Polynesians first settled the Bonin Islands in the 1830s; subsequently migrants arrived from Japan in the 1860s. All residents were evacuated to the Japanese mainland in World War II in 1944. After the war, some of the former residents returned to the Bonin Islands, but Volcano residents have been prohibited from returning due to the use of Iwoto Island as a military base. Most previously cultivated areas on these islands have now become secondary growth forest (Shimizu, 2003). Although some islands are now abandoned, the ecosystems of the Ogasawara Archipelago have been highly affected by anthropogenic influences. Minami-Iwoto Island is the only remaining island in the archipelago that has never supported a human population; thus the Japanese government has classified the island as a strictly protected area.

Capturing and molecular experiments

Japanese white-eyes and brown-eared bulbuls were captured with mist nets on all three of the Volcano Islands from 2008 to 2010 (see Supplementary Table S1 online). Blood samples of white-eyes were obtained from four birds on Minami-Iwoto, eight on Iwoto, and seven on Kita-Iwoto. Blood samples of bulbuls were taken from one bird on Minami-Iwoto, one on Iwoto, and three on Kita-Iwoto and preserved in 95% ethanol. All captured birds were released after being banded to avoid re-capture of the same individuals. To investigate the genetic differences between the Volcano population and those of other habitats, we also analyzed blood and pectoral muscle samples collected from various islands in the Japanese Archipelago, Anejima (n = 3), Aogashima (n = 3), Hahajima (n = 1), Kohzushima (n = 2), Mikurajima (n = 3), Miyakejima (n = 3), Miijima (n = 3), Okinawajima (n = 1), Yonagunijima (n = 1) for white-eyes, and Aogashima (n = 2), Hachijojima (n = 2), Kohzushima (n = 2), Mikurajima (n = 2), Kuyakejima (n = 3), Niijima (n = 2), Yonagunijima (n = 2) for bulbuls, which were stored at –80°C at the National Museum of Nature and Science, Tokyo (NSMT; see Supplementary Table S1). DNA was extracted from the blood samples from Volcano Island and the frozen blood and tissue samples from the NSMT were obtained using a standard phenol-chloroform procedure.

A section of the cytochrome c oxidase I in the mtDNA (ca. 650 bp) was amplified using the primer set, L6697Bird (5′-TCAACYAACCACAAAGAYATCGGYAC-3′) and H7390Thrush (5′-ACGTGGGARATRATTCCAAATCCTG-3′) (Saitoh et al., 2015). The 25-µL polymerase chain reaction (PCR) reaction mix comprised 19.2 µL ultrapure water, 1.0 U Taq polymerase (Ex Taq, TaKaRa, Shiga, Japan), 2.5 µL PCR buffer, 0.3 µL of each primer (0.24 mM), 2.5 µL of each dNTP (2.5 mM), and 0.5 µL of DNA. The amplification protocol was as follows: 94°C for 3 min followed by five cycles at 94°C for 30 s, 48°C for 30 s, 72°C for 60 s, and subsequently 30 cycles at 94°C for 30 s, 51°C for 30 s, 72°C for 60 s, and a final extension at 72°C for 5 min. The PCR products were visualized on a 1.5%

Table 1. Haplotype frequency of the 533 bp cytochrome c oxidase I (COI) and genetic characteristics of Japanese white-eyes. Number of haplotypes (k), haplotype diversity (*h*) ± SD and nucleotide diversity (π) ± SD per population.

Haplotype	Volcano Is.			Bonin Is.	Izu Is.	Japanese mainland	Ryukyu Is.	Total
	Minami-Iwoto	Iwoto	Kita-Iwoto					
Zj01	4							
Zj02		1						
Zj03		3	7	2				
Zj04		4		1				
Zj05				3	3		24	
Zj06					2	7	9	
Zj07					5		1	
Zj08					4			
Zj09					1			
Zj10					1			
Zj11					1			
Zj12						1		
Zj13							3	
Zj14							11	
Zj15							9	
Zj16							7	
Zj17							3	
Zj18							1	
Zj19							1	
Zj20							4	
Zj21							1	
k	1	3	1	3	7	2	12	
h		0.6667 ± 0.0843		0.7333 ± 0.1552	0.8529 ± 0.0527	0.2500 ± 0.1802	0.8270 ± 0.0284	0.8886 ± 0.0157
π		0.0036 ± 0.0024		0.0024 ± 0.0019	0.0028 ± 0.0020	0.0005 ± 0.0007	0.0028 ± 0.0019	0.0035 ± 0.0022

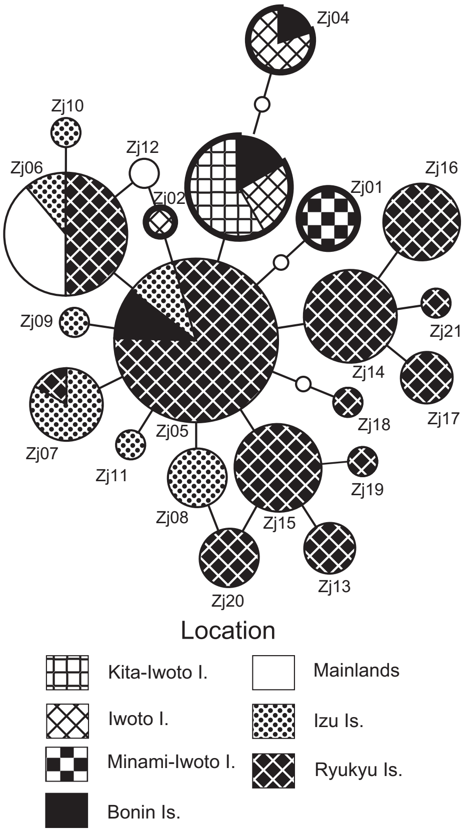

Fig. 2. Haplotype network of Japanese white-eyes *Zosterops japonicus*. Sample size is represented by the size of the circles. Small white dots on the branches represent inferred lost haplotypes. Volcano populations are circled with bold lines.

agarose gel stained with ethidium bromide, and purified by Exo-SAP-IT (Amersham Biosciences, Buckinghamshire, UK), and were sequenced using BigDye Terminator v1.1 (ABI, Paisley, UK) according to the standard protocol. Analysis was performed on an ABI 3130 Genetic Analyzer (ABI).

We added COI sequence data for white-eyes and bulbuls from previous studies (Hamao et al., 2013; Saitoh et al., 2015) to our data set to estimate the degree of genetic difference among Volcano and other habitat populations (Supplementary Table S1). The sequences of 121 white-eye and 88 bulbul individuals were aligned using the CLUSTALW module in MEGA 6.02 (Tamura et al., 2013). The numbers of available sequences of COI were 533 base pairs in white-eyes and 580 base pairs in bulbuls. To visualize the

Table 2. F_{ST}s for pariwise comparisons of haplotypes between Japanese white-eye populations and related significance.

	Volcano Is.	Bonin Is.	Izu Is.	Japanese Mainland	Ryukyu Is.
Volcano Is	–	NS	***	***	***
Bonin Is.	–0.026	–	***	***	***
Izu Is.	0.321	0.186	–	***	***
Mainland	0.524	0.645	0.402	–	***
Ryukyu Is.	0.346	0.187	0.136	0.376	–

NS: not significant, ***: $P < 0.001$

Table 3. F_{ST}s for parewise comparisons of haplotypes between Japanese white-eye populations in the Volcano Islands and related siginificance (upper symbols).

	Minami-Iwoto	Iwoto	Kita-Iwoto
Mimami-Iwoto	–	***	***
Iwoto	0.728	–	NS
Kita-Iwoto	1.000	0.317	–

NS: not significant, ***: $P < 0.001$

Table 4. Haplotype frequency of the 580 bp cytochrome c oxidase I (COI) and genetic characteristics of collected samples of brown-eared bulbuls. Number of haplotypes (k), haplotype diversity (h) ± SD and nucleotide diversity (π) ± SD per population.

Haplotype	Volcano Is.			Bonin Is.	Izu Is.	Japanese mainland	North and South Ryukyu Is.	Middle Ryukyu Is.	Daito Is.	Total
	Minami-Iwoto	Iwoto	Kita-Iwoto							
Ha01	1	1	3							
Ha02				4						
Ha03					2					
Ha04					1					
Ha05					16	6	1	1	1	
Ha06						2				
Ha07						1				
Ha08							2	6		
Ha09							9			
Ha10							8			
Ha11							4			
Ha12							2			
Ha13							1			
Ha14							1			
Ha15							1			
Ha16							1			
Ha17								8		
Ha18								1		
Ha19								1		
Ha20								1		
Ha21								1		
Ha22									1	
k	1	1	3	1	3	3	10	7	2	
h	0.0000 ± 0.0000			0.0000 ± 0.0000	0.2924 ± 0.1274	0.5556 ± 0.1653	0.8345 ± 0.0432	0.7684 ± 0.0689	1.0000 ± 0.5000	0.8838 ± 0.0226
π	0.0000 ± 0.0000			0.0000 ± 0.0000	0.0008 ± 0.0008	0.0011 ± 0.0010	0.0094 ± 0.0052	0.0086 ± 0.0049	0.0310 ± 0.0319	0.0142 ± 0.0074

geographic distribution pattern and estimate the degree of genetic divergence on Volcano, the range of white-eyes was divided into five regions representing the Volcano Islands, the Bonin Islands, the Izu Islands, the Japanese mainland, and the Ryukyu Islands. The range of bulbuls was divided into seven regions based on the findings of high genetic divergence within the Ryukyu Islands (Saitoh et al., 2015): the Volcano Islands, the Bonin Islands, the Izu Islands, the Japanese mainland, the south and north Ryukyu Islands (excluding islands between Miyako Island and Amami-Oshima), the middle Ryukyu Islands, and the Daito Islands (Fig. 1). Each haplotype network was estimated with TCS 1.2.1 software using the statistical parsimony method (Clement et al., 2000). The haplotype diversity (*h*) and nucleotide diversity (π) in each region and pairwise F_{ST} values between regions were calculated with ARLEQUN 3.5 software (Excoffier and Lischer, 2010). The pairwise F_{ST} values among the white-eye populations of the three Volcano Islands were also calculated.

RESULTS

Japanese white-eye

A total of 533 base pairs were obtained from COI sequence sections from 121 sampled white-eye individuals and were treated as a homologous region for genetic analysis. We found no genetic polymorphism with insertion or deletion mutations. A total of 21 haplotypes were recovered from white-eyes in the Japanese Archipelago and the Ogasawara Archipelago (Table 1 and Fig. 2). There was a maximum number of five substitutions between haplotypes, which occurred between haplotypes from Iwoto Island and the Bonin Islands and from the Ryukyu Islands. A haplotype (Zj05) from the Bonin, Izu, and Ryukyu Islands was located in the center in the haplotype network (Fig. 2).

All white-eye individuals from Minami-Iwoto Island had only one endemic haplotype, Zj01 (Table 1 and Fig. 2). In the populations in the Volcano Islands, the maximum number of substitutions was five, which was found between haplotype Zj01 from Minami-Iwoto Island and Zj04 from Iwoto Island (Fig. 2). All F_{ST} values between the Volcano population and each of the other populations, with the exception of the Bonin population, were significantly greater than zero (Table 2). The F_{ST} values between the Minami-Iwoto population and the other island populations were also significantly greater than zero (Table 3). The Iwoto population had three haplotypes, two of which were shared with the Bonin population (Table 1 and Fig. 2). Seven haplotypes were found on Izu and twelve on Ryukyu, one of which was shared with the Bonin population (Table 1 and Fig. 2). There was no endemic haplotype in the Bonin population.

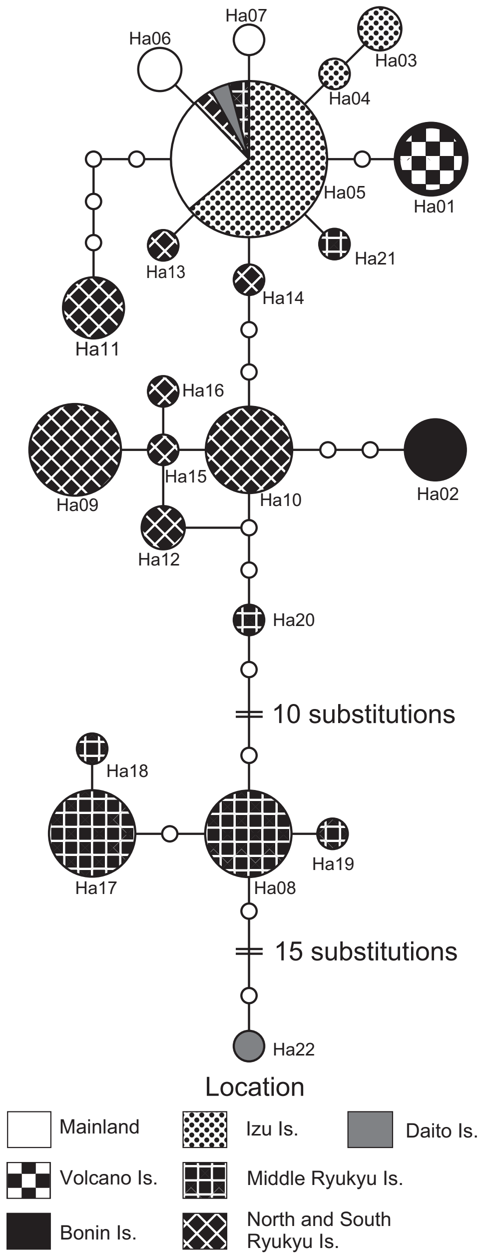

Fig. 3. Haplotype network of brown-eared bulbuls *Hypsipetes amaurotis*. Sample size is represented by the size of circles. Small white dots on branches represent inferred lost haplotypes. Volcano populations are circled with bold lines.

Table 5. F_{ST}s for pairwise comparisons of haplotypes between brown-eared bulbul populations and related significance (upper symbols).

	Volcano Is.	Bonin Is.	Izu Is.	Mainland	North and South Ryukyu Is.	Middle Ryukyu Is.	Daitoh Is.
Volcano Is.	–	**	***	***	***	***	*
Ogasawara Is.	1	–	**	***	**	***	*
Izu Is.	0.835	0.924	–	NS	***	***	NS
Japanese mainland	0.831	0.918	0.096	–	***	***	NS
North and South Ryukyu Is.	0.459	0.334	0.417	0.352	–	***	NS
Middle Ryukyu Is.	0.754	0.725	0.794	0.74	0.619	–	*
Daitoh	0.593	0.564	0.75	0.6	0.37	0.54	–

NS: not significant, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$

Brown-eared bulbul

We obtained 580 base pairs in the sections of the COI region from 88 sampled bulbuls. No insertion or deletion mutations were found. A total of 22 haplotypes were found in the COI sequences of bulbuls in the Japanese Archipelago and the Ogasawara Archipelago (Table 4 and Fig. 3). Each of the populations of bulbuls on Volcano and on Bonin had a single endemic haplotype (Table 3 and Fig. 3), which was not shared with any other populations. The haplotype Ha01 from Volcano was closest to haplotype Ha05 from Izu, the Japanese mainland, Daito, and Ryukyu, by two substitutions (Fig. 3). The haplotype Ha02 from Bonin was closest to haplotype Ha10, by three substitutions, which was endemic to the south Ryukyu Islands (Fig. 3 and Supplementary Table S1). There were nine substitutions between the Volcano and Bonin populations via three extant haplotypes: Ha05, Ha14, and Ha10. The F_{ST} values between the Volcano population and the other populations were greater than zero (Table 5).

DISCUSSION

The haplotype network of the Japanese white-eyes revealed a structure of the white-eyes genetically radiating from ancestral haplotypes lying in the center of the network (Zj05 from the Izu Islands, the Japanese mainland, and the Ryukyu Islands). Each population of white-eyes was genetically distinguishable from other populations based on pairwise F_{ST} values, except for the relationship between the Volcano and Bonin populations, due to the intentionally introduced population of the latter (Table 2). The results showed that gene flow was restricted between the white-eye populations. The Volcano populations can be distinguished from other populations based on the morphological traits in bill size and plumage color (Uchida, 1911), and the genetic features observed in the present study. Thus, the Volcano populations should be treated as a distinct population, of evolutionarily significant units (ESUs), following the definition of ESU by Crandall et al. (2000). Furthermore, the Volcano population was genetically divergent from Zj05 by one to three substitution mutations and has three lineages within the Volcano Islands (Fig. 2). Although the Volcano population had a moderate value of haplotype diversity (h) compared with other populations, it had the highest value of nucleotide diversity (π) (Table 2), suggesting that the population living in the most remote habitat of the Volcano Islands had obtained a set of specific genetic properties involved in different lineages. The pairwise F_{ST} values among the three Volcano Islands showed that gene flow from the Minami-Iwoto population was highly restricted to the other populations (Table 2). Hence, it is important to prevent interisland transfer from the Bonin to the Volcano populations to avoid introgression.

Momiyama (1930) suggested that the Bonin population of the white-eye was a hybridized population of two subspecies from the Izu and Volcano Islands. Although our genetic analysis results are consistent with Momiyama's theory, it is possible that the Ryukyu populations could also be involved in the origin of the Bonin population (Table 1 and Fig. 2). The number of species on an island depends on the size and age of the island (Whittaker et al., 2008). Japanese white-eyes and Bonin white-eyes coexist sympatrically on relatively small islands (Hahajima and several offshore islands), which may be sufficiently small in size to bring on the extinction of one related species through competition. The Japanese white-eye introduced to Hawaii was treated as an invasive introduced species that had negative effects on the survival and growth of juveniles of native bird species by competition for foods (Freed and Cann, 2009). No obvious interspecific competition between the two bird populations has been confirmed to date (Kawakami and Higuchi, 2003). The details of quantitative estimates of the origin and data on the introduction of the Bonin population, and the degree of hybridization using nuclear DNA and morphological analysis should be considered in the development of strategies to prevent introgression.

In the brown-eared bulbuls, multiple haplotypes that had vanished in the past were observed in the haplotype network (Fig. 3), indicating that the bulbul population underwent a bottleneck and/or founder effect in the past and subsequently increased in number. We also reconfirmed the complex genetic structure with marked genetic divergences in the bulbul population (Fig. 3) described by Saitoh et al. (2015). The phylogenetic pattern of the bulbuls was divided into three groups based on genetic distance (> 2% K2P): (i) the mainland group, including the Ogasawara Archipelago, Izu Islands, Japanese mainland, and the south and north Ryukyu Islands, (ii) the middle Ryukyu Island group, and (iii) and the Daito Island group (Saitoh et al., 2015). The genetic relationships between the Volcano population and the other populations were previously unknown; however, the present study reveals that a haplotype of the Volcano population was related to the haplotypes belonging to the mainland group. Each of the Volcano and Bonin populations was morphologically separated into different subspecies based on bill size (Uchida, 1911), as genetically divergent from the other populations (Table 5). Our results show that each of the Volcano and Bonin populations can be genetically distinguished and is irreplaceable; as such, these populations should be treated as ESUs for conservation purposes.

Comparatively large genetic differences, by nine substitutions, were found between the Volcano and Bonin Island bulbul populations (Fig. 3), despite the fact that the islands are relatively close in distance (160–270 km). Both haplotypes from Volcano and Bonin belonged to haplotypes of the Japanese mainland group, but the Volcano population did not directly diverge form the Bonin population, which is in agreement with a similar pattern observed in the Japanese bush-warbler (Emura et al., 2013). The haplotype of the Volcano bulbul population was relatively close to a haplotype of Japanese mainland and Izu individuals, whereas the haplotype of the Bonin population was relatively close to haplotypes of the north and south Ryukyu individuals (Fig. 3). These results suggested that each of the Volcano and Bonin Island populations was derived from at least two ancient populations distinguished by different lineage and source location. Furthermore, the north and south Ryukyu haplotypes related to those of the Bonin population would have had a sequence of more ancestral characteristics than those of the Izu Islands and Japanese mainland related to the Volcano population (Fig. 3). We propose the following scenario for the origin of Ogasawara bulbuls: a single bulbul population first colonized the older islands, namely the

Bonin Islands, and a second population may have subsequently independently colonized the younger islands, the Volcano Islands. Reduction of dispersibility is one of the characteristics of island form (Whittaker and Fernández-Palacios, 2007). The two bulbul populations can fly, but their gene flow has been restricted owing to loss of communal flight ability between islands. Loss of communal flight ability could also explain the restriction of gene flow among Japanese white-eye populations in the Volcano Islands. These differences in genetic structure between the two species, despite their similar distribution pattern, emphasize the phylogeographical peculiarity of the bulbul populations in the Ogasawara Archipelago.

Previous studies revealed genetic differences in five of eight land birds in the Ogasawara Archipelago (Kawakami et al., 2008; Emura et al., 2013; Ando et al., 2014). The magnitude of gene flow among the islands varies by species. Bonin white-eyes that have limited distribution in the Hahajima Islands and its two nearby islands were suggested to rarely migrate between islands based on the different frequency of occurrence of haplotypes among control regions on each island (Kawakami et al., 2008). The Volcano Island population of the Japanese bush-warbler did not share any COI haplotypes with its Bonin population, suggesting limited gene flow from the Volcano to the Bonin populations (Emura et al., 2013). In the Japanese wood pigeon *Columba janthina*, because the genetic difference was low between its Volcano and Bonin populations based on mtDNA control regions and nuclear DNA, both populations should be regarded as a single ESU (Ando et al., 2014). The genetic characteristics of the Ogasawara bird species are consistent with the hypothesis that diversification within an archipelagos results from the appropriate correspondence of geography and dispersal ability (Losos and Ricklefs, 2009).

Marked genetic divergences between the Ogasawara region and the Japanese mainland have been found in two species, the Japanese wood pigeon based on mtDNA control region sequences (Seki et al., 2007; Ando et al., 2014), and the Oriental greenfinch *Chloris sinica* based on COI sequences (Saitoh et al., 2015). The populations of both species can therefore be treated as separate species. The scaly thrush, which has recently colonized the Ogasawara Archipelago, had the same COI haplotype as the population of the Japanese mainland (Saitoh et al., 2015). Japanese bush-warblers (Emura et al., 2013), Japanese white-eyes and brown-eared bulbuls (this study) of Ogasawara had moderate genetic differences. The three bird species with their moderate genetic differences are assigned to endemic subspecies of the Volcano and/or Bonin Islands, which appears to be appropriate taxonomically.

Two key results were provided from our genetic analyses; one is the first genetic suggestion that Japanese white-eyes of the Bonin Islands are a hybrid of the Izu, Volcano, and perhaps Ryukyu populations, and the other is the genetic evidence that brown-eared bulbuls of the Volcano and Bonin Islands are derived from different populations that colonized the islands independently. The native populations of the two Ogasawara birds had endemic DNA sequences that were not exchangeable among populations. It is thus important to establish a management plan for conservation of biodiversity and ecosystems in the region that includes preventive measures for interisland introgression of the populations. Regulations based on contingency and determinism (Losos and Ricklefs, 2009) would protect the complex distribution pattern and the various degrees of genetic differences of the birds colonized in the Ogasawara Archipelago.

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