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Genetic Diversity and Population Structure in the Yaeyama flying fox

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Abstract

There are 122 mammal species in Japan, and 37 of them are bats. Especially, the species of genus *Pteropus* have big body size and the ability to fly hundreds of kilometers and have important roles in pollination and seed dispersal. The Ryukyu flying fox (*Pteropus dasymallus*) is one of the *Pteropus* species in Japan, distributed in the Ryukyu archipelago, Taiwan, and possibly the Philippines, and is divided into 5 subspecies. Although they are listed as VU (vulnerable) in IUCN Red List, few genetic analyses have been conducted for their conservation. The purpose of this study is to evaluate genetic diversity and investigate the genetic structure of Yaeyama flying fox, one of the subspecies of the Ryukyu flying fox. We conducted mtDNA haplotype analysis and microsatellite analysis with the 18 markers we developed.

mtDNA analysis was conducted with the samples collected in 8 islands (Miyako, Ishigaki, Kohama, Kuroshima, Hateruma, Taketomi, Iriomote, Yonaguni) We identified 39 haplotypes in 526bp of the control region of 142 samples. 14 haplotypes were shared between some islands, and haplotype network for the 8 islands did not show any clear genetic structure. However, haplotype diversity was higher in Ishigaki and Iriomote compared to other islands, and some haplotypes were only found in particular islands, so there might be some genetic structure which could not be revealed by mtDNA analysis.

Therefore, we also conducted microsatellite analysis with 155 samples collected in 6 islands (Miyako, Ishigaki, Kohama, Taketomi, Iriomote, Yonaguni). As a result of genetic diversity analysis, PCoA, STRUCTURE, and calculation of *Fst*, Yonaguni (the west end of the distribution area) population showed clear genetic differentiation from other populations, low genetic diversity, and a high inbreeding level. Ishigaki, Kohama, Taketomi, and Iriomote (the center of the distribution area) populations had gene flow between them and high genetic diversity. Miyako (the east end of the distribution area) population showed slight genetic differentiation and had the middle level of genetic diversity. Gene flow between Ishigaki and Miyako through islands between them might be preventing inbreeding of Miyako population.

We revealed genetic diversity, and genetic differentiation and gene flow between islands of Yaeyama flying fox for the first time. These results will be useful for setting

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of conservation units and conservation of populations in each island based on genetic structure.

1. Introduction

Bats are highly diverse groups of mammals. In Japan, 37 of 122 mammal species are bats. Especially, flying foxes, i.e. paleotropical fruit bats belonging to the *Pteropus* genus, have important roles in pollination and seed dispersal with their ability to fly hundreds of kilometers. Indeed, it is reported a *Pteropus* species flew 130km in 2 hours and they could fly between islands (Epstein et al., 2009). However, they are arguably the most endangered group of bats worldwide and are most threatened on islands (Vincenot, Florens and Kingston, 2017).

The Ryukyu flying fox (*Pteropus dasymallus*) is distributed across the Ryukyu archipelago in Japan, on two small islands of Taiwan, and possibly in the Philippines (Vincenot, Collazo and Russo, 2017). *P. dasymallus* mainly eats fruits, nectar, and sometimes leaves, and plays an important role in pollination and seed dispersal (Lee et al. 2009). It is listed as Vulnerable in the IUCN Red List (Vincenot 2017). There are 5 subspecies of *P. dasymallus*. One of the subspecies, P. d. yaeyamae, has thousands of individuals and is distributed in Yaeyama islands and Miyako islands.

Considering the largely insular distribution range of these bats, genetic information such as gene flow and genetic differentiation between islands is important for their conservation. However, few genetic studies have been conducted for this species.

In this study, we conducted mtDNA haplotype analysis of *P. d. yaeyamae* for evaluating genetic diversity and genetic differentiation between islands. Also, we developed species-specific microsatellite markers since there were no highly polymorphic markers for this species. With the developed markers, we conducted microsatellite analysis for revealing population structure and gene flow between islands.

2. Marterials and methods

2.1. mtDNA haplotype analysis

2.1.1. Samples

142 tissue and fecal samples were collected in 8 islands of Yaeyama and Miyako islands. The number of samples of each island is shown in Table 1.

2.1.2. DNA analysis

DNA extraction was conducted with QIAGEN DNeasy Blood and Tissue Kit (QIAGEN) for tissue samples and QIAamp DNA Stool Mini Kit (QIAGEN) for fecal samples. A part

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of control region of mtDNA was amplified through PCR. ppM01F (5'-accagaaaaggggarcaacc-3') and ppmtCR-RS2(5'-caagcatcccccaaaaatta-3') (Vincenot, unpublished) were used as primers and PCR System 9700 (GeneAmp) was used as a thermal cycler. The PCR conditions were: 95°C for 2 min; 40-45 cycles at 95°C for 30s, 50-55 °C for 30s, 74°C for 1 min; then a 10 min final extension at 74°C. PCR products were purified with High Pure PCR Product Purification Kit (Roche). After sequencing reaction, 526bp of control region was sequenced by ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

2.1.3. Data analysis

SNPs were detected through sequence alignment by MEGA7 (Kumar S, Stecher G and Tamura K, 2016). Haplotypes were identified based on the SNPs, and the haplotype network (Bandelt H, Forster P and Röhl A, 1999) was constructed by PopART (Leigh J and Bryant D, 2015). Also, *h* (haplotype diversity) and *hr* (haplotype richness) were calculated by Contrib (Petit R, Mousadik A and Pons O, 1998) (Kuroshima, Taketomi, Hateruma, and Kohama were not included since they had less than 5 samples).

2.2. Microsatellite marker development

A DNA sample was extracted using QIAGEN DNeasy Blood and Tissue Kit (QIAGEN) from the muscle tissue of a dead Ryukyu flying fox collected on Ishigaki-island in the Ryukyu archipelago, Japan. DNA shotgun sequencing was conducted on Miseq (Illumina). Ninety primer-pairs were designed using Krait (Du et al., 2018). Amplification and polymorphism were assessed with 24 DNA samples extracted from 1 blood, 1 ear, 7 wings, and 15 hair samples collected on Ishigaki-island, and 18 markers were selected for microsatellite analysis.

2.3. Microsatellite analysis

2.3.1. Samples

155 tissue and fecal samples collected in 6 islands of Yaeyama and Miyako islands were used for microsatellite analysis. The number of analyzed individuals of each island is shown in Table 2.

2.3.2. DNA analysis

DNA extraction was conducted with QIAGEN DNeasy Blood and Tissue Kit (QIAGEN) for tissue samples and QIAamp DNA Stool Mini Kit (QIAGEN) for fecal samples. 18 microsatellite loci were amplified through PCR with the developed markers. PCR System 9700 (GeneAmp) was used as a thermal cycler. Forward primers were synthesized with an M13 tag sequence (5'-GTTGTAAAACGACGGCCAGT-3') for fluorescent labeling.

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PCR was conducted in a final volume of 10 µl, containing 1 µl DNA, 5 µl Multiplex PCR Master Mix (QIAGEN), 0.2 µM of M13-tailed forward primer, reverse primer and a M13 fluorescent primer labeled with FAM, NED, or HEX, and 0.1 µg of T4 gene 32 Protein (Nippon Gene, Tokyo, JPN). The PCR conditions were: 94°C for 5 min; 45 cycles at 94°C for 30s, 60 °C for 45s, 72°C for 45s; then 8 cycles for M13 at 94 °C for 30s, 53 °C for 45s, 72°C for 45s, and a 10 min final extension at 72°C. Amplicon size was measured using the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) and Peak Scanner (Applied Biosystems).

2.3.3. Data analysis

Genetic diversity analysis and Principal Coordinate Analysis (PCoA) were conducted by GenAlEx (Peakall R and Smouse PE, 2006; Peakall R and Smouse PE, 2012). Also, we investigated genetic structure with STRUCUTE (Pritchard JK, Stephens M and Donnelly P, 2000). Finally, *Fst* was calculated by Genepop on the Web (Raymond M and Rousset F, 1995; Rousset F, 2008) to check genetic differentiation between islands. Taketomi was included in only PCoA because the number of analyzed individuals was not enough for the other analyses.

3. Results

3.1. mtDNA haplotype analysis

3.1.1 SNPs and haplotypes

45 SNPs were detected in 526bp of 142 samples. Based on the SNPs, 39 haplotypes (RFF1~RFF39) were defined. The SNPs of each haplotype are shown in Figure 1, and the number of samples of each haplotype in each island is shown in Table 3.

3.1.2. Genetic differentiation

In the haplotype network, haplotypes of each island did not form a cluster and no clear genetic structure was detected (Figure 2).

Haplotype distribution is shown in Figure 3. 14 out of 39 haplotypes were shared between multiple islands. While some haplotypes (RFF14, RFF36) were found in both Miyako (the east end of the distribution) and Yonaguni (the west end of distribution), others were only found in certain areas. RFF16 and RFF21 were only found in Iriomote and Yonaguni (the west area), while RFF7, RFF24, RFF30, RFF35, and RFF38 were only found in Miyako and Ishigaki (the east area).

3.1.3. Genetic diversity

The number of haplotypes, the number of unique haplotypes, haplotype diversity (*h*), and haplotype richness (*hr*) of 4 islands are shown in Table 4. *h* of Ishigaki and Iriomote

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were 0.96 and 1.00, while that of Miyako and Yonaguni were 0.68 and 0.61, respectively. Also, *hr* of Ishigaki and Iriomote were 7.51 and 9.00, while that of Miyako and Yonaguni were 3.66 and 2.74, respectively. Genetic diversity was higher in Ishigaki and Iriomote compared to Miyako and Yonaguni.

3.2. Microsatellite analysis

3.2.1. Genetic structure

PCoA shows genetic distance between individuals, and there were 3 clusters: Yonaguni cluster, center cluster (Ishigaki, Taketomi, Kohama, Iriomote and a part of Miyako), and Miyako cluster (Figure 4).

In STRUCTURE, LnP (D) was almost maximized at K=4 (Figure 5). The result of STRUCTURE at K=4 is shown in Figure 6. Ishigaki, Kohama, Iriomote and a part of Miyako populations had the same genetic composition, while the rest of Miyako and Yonaguni populations had each different genetic structure.

Fst between Yonaguni and the other islands were generally high (Table 5), and it shows Yonaguni population is genetically differentiated from the other islands' populations.

3.2.2. Genetic diversity

The number of analyzed individuals (*n*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and inbreeding coefficient (*F*) of each island are shown in Table 6. Ho of Ishigaki, Kohama, Iriomote, Miyako were 0.666, 0.650, 0.631, 0.512, respectively. On the other hand, *Ho* of Yonaguni was 0.388 and *F* was 0.355. Yonaguni population had low genetic diversity and showed high level of inbreeding compared to the other islands.

4. Discussion

4.1. Genetic differentiation

In mtDNA haplotype analysis, no clear genetic differentiation between islands was detected. However, in microsatellite analysis, populations of 6 islands were genetically divided into 3 groups: Miyako group (Miyako), the center group (Ishigaki, Taketomi, Kohama, and Iriomote), and Yonaguni group (Yonaguni). Populations in the center group had the same genetic composition, and Miyako group was partly differentiated from them. Yonaguni group was highly differentiated from the other 2 groups.

4.2. Genetic diversity

Genetic diversity was high in the center group and low in Yonaguni group. Miyako group had the middle level of genetic diversity. Also, inbreeding coefficient was high in

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Yonaguni group. This indicates that genetic diversity has been getting low due to inbreeding depression in Yonaguni group.

4.3. Gene flow between islands

The main habitats of the Yaeyama flying fox are Ishigaki island and Iriomote island. Populations in these 2 islands have high genetic diversity, and other populations of the center group might be keeping high genetic diversity because of gene flow with Ishigaki and Iriomote populations. Miyako group is geographically far from the center group, but there are some islands like Tarama island between the center group and Miyako group. There could be gene flow between the 2 groups through the islands between Ishigaki and Miyako, and that might be why Miyako group was partly differentiated from the center group and had the middle level of genetic diversity. On the other hand, there is no island between Yonaguni group and the center group. It could be said that Yonaguni group is isolated and genetically differentiated from the other populations, and that is causing low genetic diversity due to inbreeding depression.

4.4. Conservation implication

Considering the results of this genetic research, the Yaeyama flying fox could be divided into 3 conservation units: Miyako unit (populations in Miyako islands), the center unit (populations in the center of distribution including Ishigaki, Taketomi, Kohama, and Iriomote populations), and Yonaguni unit (Yonaguni population). The center unit has many individuals and high genetic diversity, and gene flow between the center unit and Miyako unit might be preventing Miyako unit from extinction. However, Yonaguni unit is isolated from other populations and could be highly endangered. Recovering of genetic diversity is important for population viability of Yonaguni unit.

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Base No.	8	42	61	69	86	100	109	122	126	143	145	146	163	164	165	173	175	176	177	179	180	181	182	184	211	213	217	221	225	247	254	280	296	297	302	336	337	374	378	386	402	422	423	429	489
Consensus	C	A	G	T	T	T	T	T	A	C	A	T	A	C	T	A	A	A	T	T	G	A	A	C	A	T	G	A	C	C	A	G	C	G	C	C	T	A	C	C	A	G	T	C	C
RFF1	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T
RFF2	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	T	•	•	•	•	•	•	•	•	T	•
RFF3	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•
RFF4	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF5	•	•	•	•	C	•	•	•	•	•	•	•	G	•	C	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	T
RFF6	•	•	•	•	•	C	•	•	•	•	•	•	•	T	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF7	•	•	•	•	•	C	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF8	•	•	•	•	•	•	C	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF9	•	•	•	•	•	•	•	C	•	•	•	C	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•
RFF10	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF11	•	•	•	•	•	•	•	•	•	T	•	•	•	•	C	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF12	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•
RFF13	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF14	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	T	•	•	•	•	•	•	•	•	T	•
RFF15	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•
RFF16	•	•	•	•	•	•	•	•	•	•	•	C	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	A	•	T	•
RFF17	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•
RFF18	•	•	•	•	•	•	•	•	•	•	•	•	•	T	C	•	G	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF19	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF20	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	T	•	A	C	T	•
RFF21	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	T	•	•	•	•	•	•	•	•	T	•
RFF22	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•
RFF23	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	G	T	•	•	•	•	T	•
RFF24	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	T	•	•	•	•	T	•
RFF25	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF26	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	T	•	A	•	T	•
RFF27	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF28	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	A	•	•	T	•	•	•	•	•	•	•	•	T	•
RFF29	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	T	•
RFF30	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T
RFF31	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF32	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	T
RFF33	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF34	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T
RFF35	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
RFF36	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	T	•	•	•	•	•	•	•	•	T	•
RFF37	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•
RFF38	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T
RFF39	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Figure 1 SNPs of each mtDNA haplotype

Base No shows positions in 526bp.

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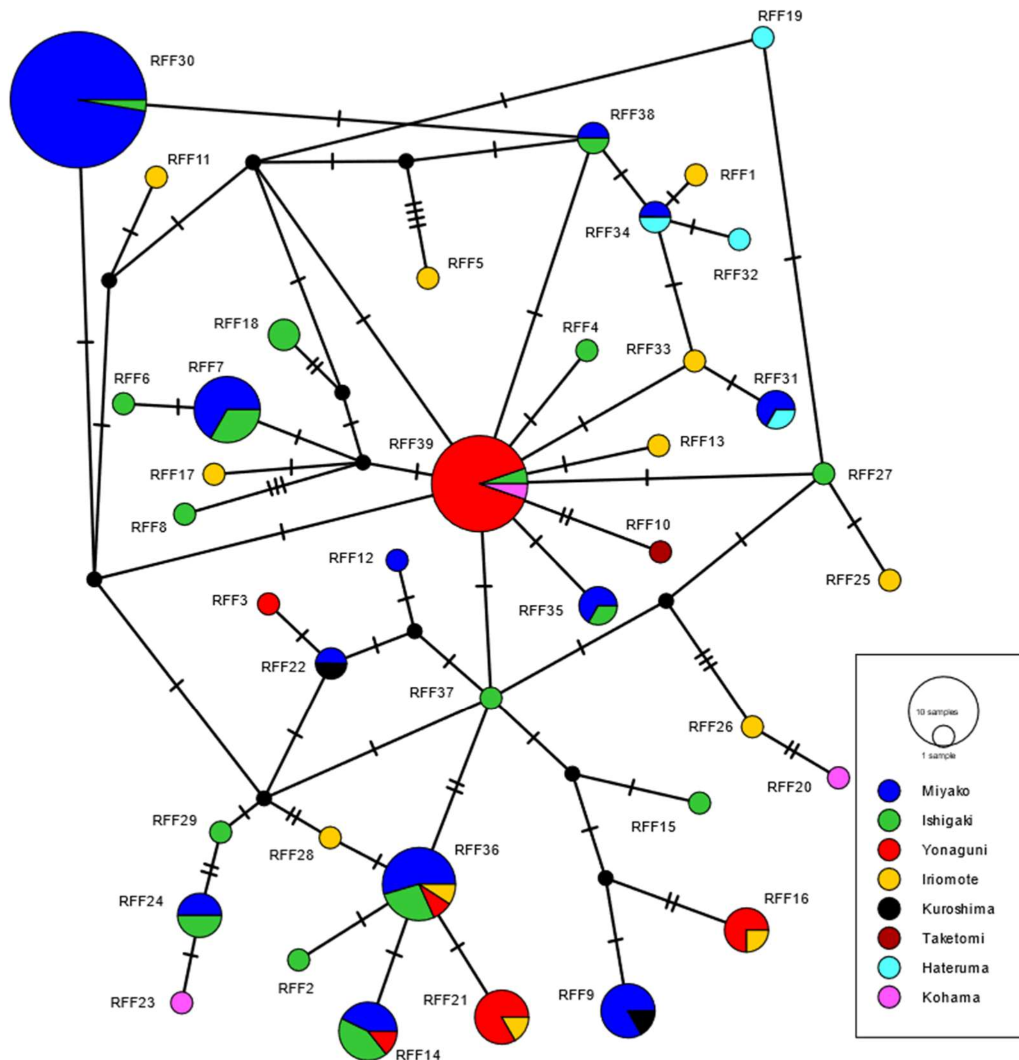

Figure 2 mtDNA haplotype network

Each haplotype is shown as a circle with ratio of the number of samples of each island. The sizes of circles represent the sample sizes.

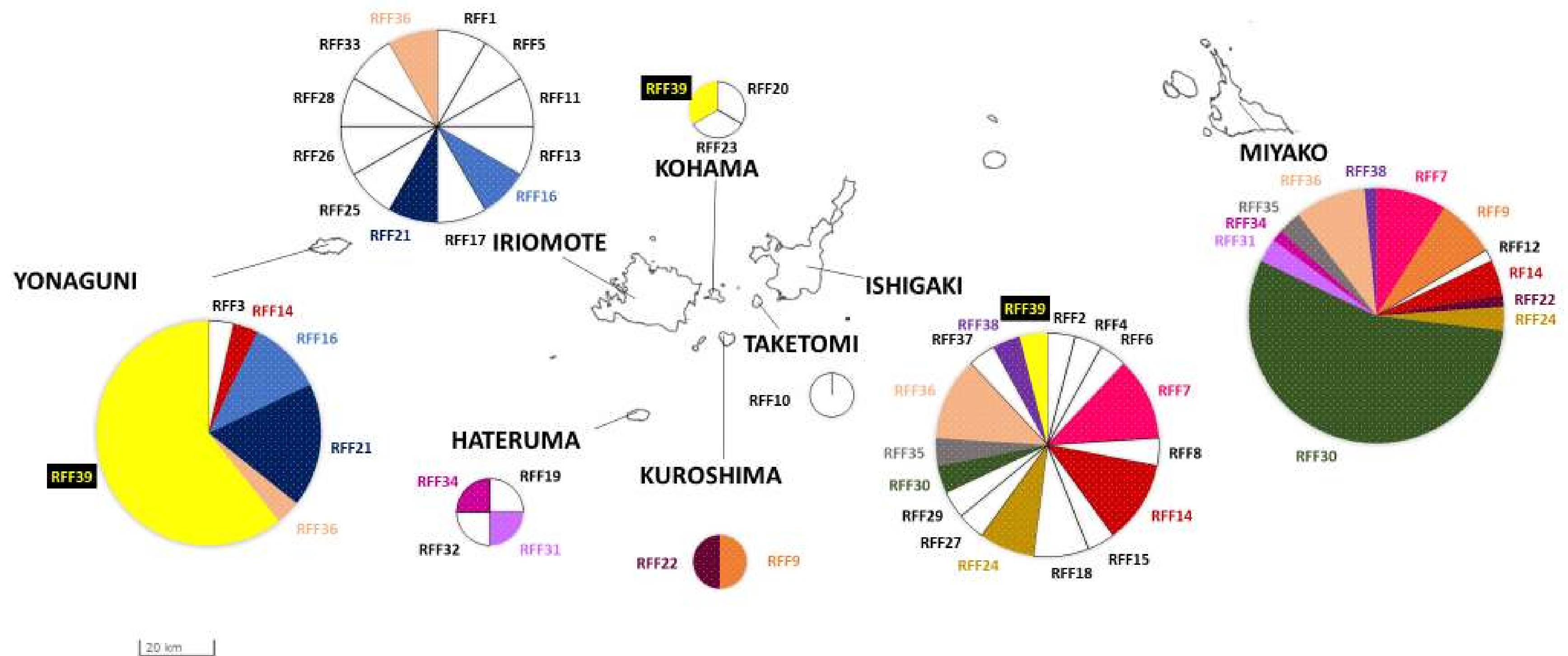

Figure 3 mtDNA haplotype distribution

Ratio of the number of samples of each haplotype is shown for each island on the map. The sizes of circles represent the sample sizes. Haplotypes shared between islands are shown with the same colors.

Source of the map: the Geospatial Information Authority of Japan

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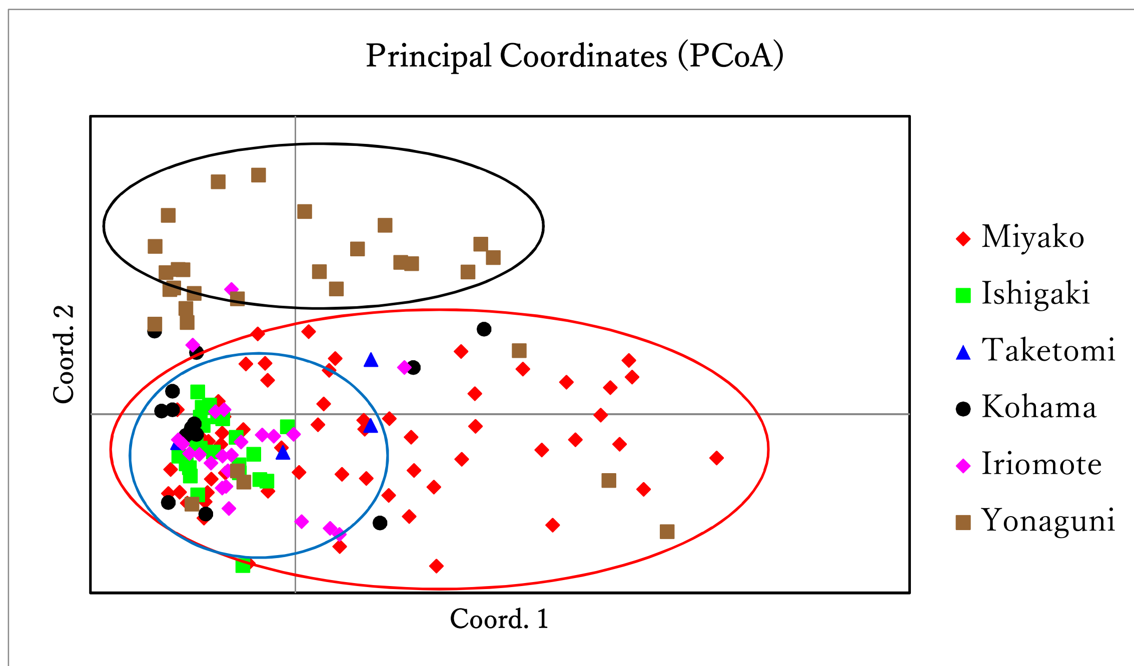

Figure 4 PCoA

Each dot represents an individual and populations are shown with colors. 3 clusters are shown with circles.

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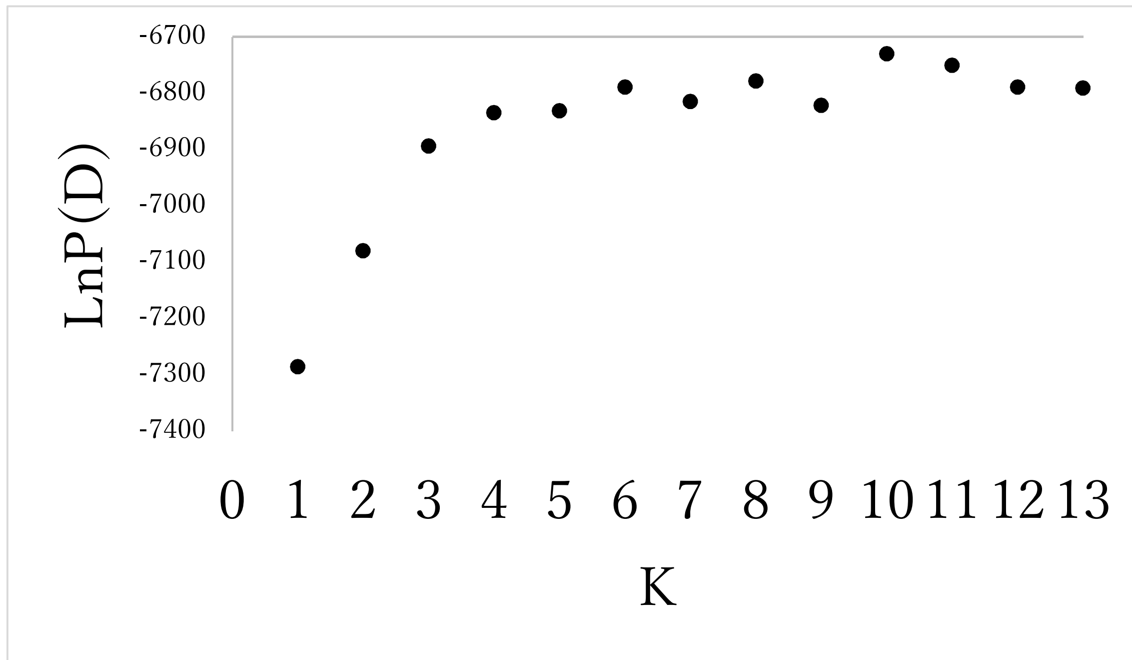

Figure 5 Selection of K for STRUCTURE

LnP(D) shows the accuracy of the analysis for each K. LnP(D) is almost maximized at K=4.

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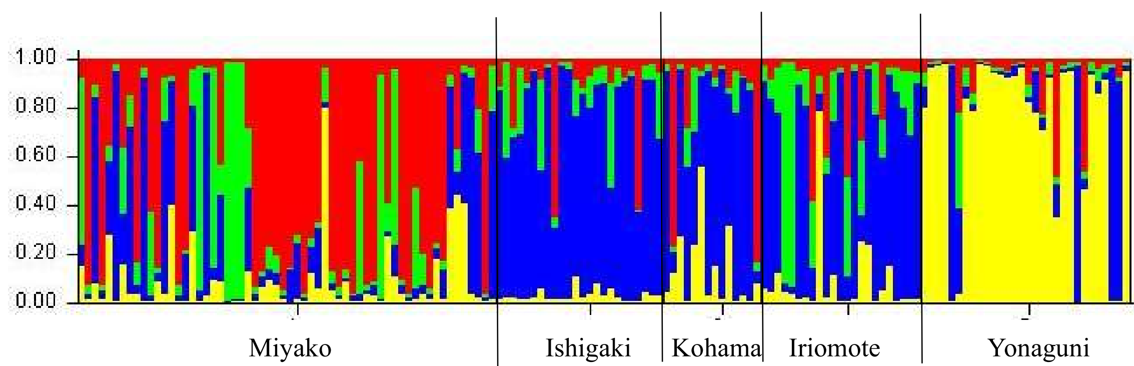

Figure 6 STRUCTURE

The result of STRUCTURE at K=4 is shown. K is the supposed number of ancestral genetic populations, and each ancestral population is shown with colors.

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Table 1 The number of samples of each population used for mtDNA analysis

Population	n
Miyako	67
Ishigaki	25
Taketomi	1
Kuroshima	2
Kohama	3
Iriomote	12
Hateruma	4
Yonaguni	28
Total	142

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Table 2 The number of analyzed individuals of each population for microsatellite analysis

Population	The number of individuals
Miyako	60
Ishigaki	24
Taketomi	4
Kohama	14
Iriomote	23
Yonaguni	30
Total	155

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Table 3 The number of samples of each mtDNA haplotype in each island

Haplotype	Miyako	Ishigaki	Taketomi	Kuroshima	*Kohama*	Iriomote	Hateruma	Yonaguni	Total
	n=67	*n*=25	*n*=1	*n*=2	*n*=3	*n*=12	*n*=4	*n*=28	*n*=142
RFF1	0	0	0	0	0	1	0	0	1
RFF2	0	1	0	0	0	0	0	0	1
RFF3	0	0	0	0	0	0	0	1	1
RFF4	0	1	0	0	0	0	0	0	1
RFF5	0	0	0	0	0	1	0	0	1
RFF6	0	1	0	0	0	0	0	0	1
RFF7	6	3	0	0	0	0	0	0	9
RFF8	0	1	0	0	0	0	0	0	1
RFF9	5	0	0	1	0	0	0	0	6
RFF10	0	0	1	0	0	0	0	0	1
RFF11	0	0	0	0	0	1	0	0	1
RFF12	1	0	0	0	0	0	0	0	1
RFF13	0	0	0	0	0	1	0	0	1
RFF14	3	3	0	0	0	0	0	1	7
RFF15	0	1	0	0	0	0	0	0	1
RFF16	0	0	0	0	0	1	0	3	4
RFF17	0	0	0	0	0	1	0	0	1
RFF18	0	2	0	0	0	0	0	0	2
RFF19	0	0	0	0	0	0	1	0	1
RFF20	0	0	0	0	1	0	0	0	1
RFF21	0	0	0	0	0	1	0	5	6
RFF22	1	0	0	1	0	0	0	0	2
RFF23	0	0	0	0	1	0	0	0	1
RFF24	2	2	0	0	0	0	0	0	4
RFF25	0	0	0	0	0	1	0	0	1
RFF26	0	0	0	0	0	1	0	0	1
RFF27	0	1	0	0	0	0	0	0	1
RFF28	0	0	0	0	0	1	0	0	1
RFF29	0	1	0	0	0	0	0	0	1
RFF30	37	1	0	0	0	0	0	0	38
RFF31	2	0	0	0	0	0	1	0	3
RFF32	0	0	0	0	0	0	1	0	1
RFF33	0	0	0	0	0	1	0	0	1
RFF34	1	0	0	0	0	0	1	0	2
RFF35	2	1	0	0	0	0	0	0	3
RFF36	6	3	0	0	0	1	0	1	11
RFF37	0	1	0	0	0	0	0	0	1
RFF38	1	1	0	0	0	0	0	0	2
RFF39	0	1	0	0	1	0	0	17	19

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Table 4 mtDNA haplotype diversity

h: haplotype diversity, *hr*: haplotype richness

Population	The number of haplotypes	The number of unique haplotypes	*h*	*hr*
Miyako	12	1	0.68	3.66
Ishigaki	17	9	0.96	7.51
Iriomote	12	9	1.00	9.00
Yonaguni	6	1	0.61	2.74
Average	11.75	5	0.81	5.73

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Table 5 *Fst* between populations

Population	Miyako	Ishigaki	Kohama	Iriomote
Ishigaki	0.0221			
Kohama	0.0577	0.0493		
Iriomote	0.0349	0.0296	0.0257	
Yonaguni	0.0665	0.0656	0.0740	0.0680

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Table 6 Genetic diversity of microsatellites

Ho: observed heterozygosity, *He*: expacted heterozygosity, *F*: inbreeding coefficient

Population	The number of analyzed individuals	*Ho*	*He*	*F*
Miyako	60	0.512	0.659	0.215
Ishigaki	24	0.666	0.688	0.042
Kohama	14	0.650	0.647	-0.020
Iriomote	23	0.631	0.710	0.114
Yonaguni	30	0.388	0.577	0.355