Genetic lineage of the Amami islanders inferred from classical genetic markers

Yuri Nishikawa and Takafumi Ishida

Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan

Correspondence: Yuri Nishikawa, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan.

E-mail address: yuri.nishikawa@gmail.com

Abstract

The genetic structure of the people of mainland Japan and Okinawa has been gradually unveiled in recent years. However, previous anthropological studies dealing with people in the Amami islands, located between mainland Japan and Okinawa, were less informative because of the lack of genetic data. In this study, we collected DNAs from 104 subjects in two of the Amami islands, Amami-Oshima island and Kikai island. We analyzed the D-loop region of mtDNA, four Y-STRs, and four autosomal nonsynonymous SNPs to clarify the Amami islanders' genetic structure compared with peoples in Okinawa, mainland Japan, and other regions of East Asia. We found that the Amami islanders showed a genetically intermediate position between mainland Japan and Okinawa in mtDNA and Y-STR. However, the frequencies of several autosomal SNPs in the Amami islanders indicated a significant difference from mainland Japanese, which may be because of the gene flow from Okinawa but not natural selection. Moreover, extremely high or low frequencies of several alleles implied a founder effect in Kikai islanders. Note that there is room for the interpretation of the results because of the small sample size and number of alleles in the present study. Geographically broad and detailed samplings and genome-wide analyses are awaited.

Keywords: Japanese, Amami islanders, Classical genetic markers

1. Introduction

The genetic structure of the people of Japan has been gradually unveiled in recent years. The dual structure model (Hanihara, 1991) is a well-known hypothesis that predicts that the modern Japanese population was formed by the admixture of two populations, Jomon and Yayoi; Jomon peoples dwelled in the Japanese Archipelago since about 18,000 years ago, and Yayoi peoples immigrated from the Asian continent 2,000–3,000 years ago (Habu, 2004; Nakagome et al., 2015). This model is also supported largely by genomic/genetic studies (Jinam et al., 2012; Nakagome et al., 2015). According to this model, there are two populations assumed to be strongly contributed from Jomon peoples. One of these populations is the Ainus, indigenous in Hokkaido, the most northern part of Japan. The other population comprises people living in the Ryukyu Archipelago, southern end of Japan (Fig. 1).

This model has been supported by previous genetic studies among the Ryukyuans, Ainus, mainland Japanese, and other East Asian populations. Genetic similarity between the Ryukyuans and Ainus has been observed (Hammer et al., 2006; Koganebuchi et al. 2012), whereas the Ryukyuans are believed to be differentiated from mainland Japanese by the analyses of the Y chromosome (Hammer & Horai, 1995; Hammer et al., 2006), mtDNA (Horai et al., 1996; Tanaka et al., 2004), autosomal markers (Omoto & Saitou, 1997), and genome-wide SNP data (Yamaguchi-Kabata et al., 2008). Additionally, modern Ryukyuans are believed to have no direct genetic relations with the Taiwan aborigines (Matsukusa et al., 2010) or human skeletons in the late Pleistocene (Sato et al., 2014; Mizuno et al., 2021) that are older than Jomon peoples found in several sites in the Ryukyu Archipelago (Kobayashi et al., 1974; Nakagawa et al.,

2010). Furthermore, rich genetic variations among the residents on the islands of the Ryukyu Archipelago have been observed (Sato et al., 2014; Matsunami et al., 2021).

However, in those studies mentioned above, much attention was not paid to the Amami islands located in the northern Ryukyu Archipelago (Fig. 1). There have been historical differences between the Amami islands and the rest of the Ryukyu Archipelago (Okinawa prefecture). Some researchers claim that the Amami islands played important roles in the trade among East Asia, including mainland Japan and China, around the 7th–11th century, which distinguished the Amami islands from Okinawa (Takanashi, 2011; Yoshinari, 2011). In Kikai island, one of the Amami islands, there is the Gusuku Site group which is supposed to be the local agency of the government of mainland Japan in the 9th–13th century (Yoshinari, 2011; Takamiya et al., 2019), and this corresponds to the hypothesis that there was population migration into the Ryukyu Archipelago from the north via the Amami islands around the 11th–12th century (Takamiya, 2013). In the 15th century, the Amami islands were conquered by the Ryukyu Kingdom, which was an independent country centered on main-island Okinawa (Yoshinari & Fuku, 2007), but since 1609, the Amami islands were directly ruled by the Satsuma-Han, one of the feudal domains in mainland Japan; this has brought about administrative and cultural differences between the Amami islands and Okinawa (Tsuha, 2012).

Despite these non-negligible historical and cultural features, few anthropological studies, including genetic studies about the Amami islanders, have been conducted. Dodo et al. (1998) analyzed 22 cranial nonmetric traits of early modern Amami and Okinawa islanders and found that they shared morphological similarities. Nishiyama et al. (2012) indicated a low but significant

level of genetic difference between the Amami islanders and mainland Japanese using SNP data, but their research was limited to only two islands, Tokuno and Okinoerabu islands (Fig. 1), in the southern part of the Amami islands, and they concluded that studies that cover further north areas should be indispensable.

We then newly focused on two of the Amami islands, Amami-Oshima and Kikai islands, in this research. Amami-Oshima island (Fig. 1) is the largest island and has the largest population in the Amami islands (Kagoshima Prefecture, 2018a). Kikai island (Fig. 1) is a relatively small island with a population of about 7,000 (Kagoshima Prefecture, 2018b), located on the east of Amami-Oshima island, and assumed to have played a very important role in the relationship between the Ryukyu Archipelago and mainland Japan around the 11th–12th century (Takanashi, 2009; Yoshinari, 2011; Takamiya, 2013; Takamiya et al., 2019). On the basis of the previous studies and historical backgrounds described above, we hypothesized that people in these two islands might genetically be located in the intermediate position between Okinawa and mainland Japan. This study aims to clarify the genetic structure of the Amami islanders compared with peoples in Okinawa, mainland Japan, and other regions in East Asia.

105 2. Materials and methods

106

107 2.1. Subjects

108 A total of 104 Amami islanders (Fig. 1), 78 in Amami-Oshima island (49
109 males and 29 females) and 26 in Kikai island (15 males and 11 females), who were
110 not genetically related, were the subjects of this study. This research was
111 approved by the Research Ethics Committee of the University of Tokyo, and all
112 subjects provided informed consent. We collected nail or oral mucosa samples
113 from the subjects. Their origins were confirmed by an interview that at least one
114 of the grandparents of each subject was born in the Amami islands.

115

116 2.2. DNA extraction

117 We extracted DNA from nail samples using the ISOHAIR (Nippon Gene)
118 and oral mucosa samples using the High Pure PCR Template Preparation Kit
119 (Roche).

120

121 2.3. Genotyping

122 We amplified the D-loop region (16024–16569 and 1–41 of the rCRS;
123 Anderson et al., 1981; Andrews et al., 1999) of mtDNA by nested PCR to avoid
124 nuclear insert contaminations. The first PCR for 9 kb of mtDNA including the
125 D-loop region was conducted (primers: GENBANK_MT_26F &
126 GENBANK_MT_5R) with an initial denaturation at 94°C for 2 min, followed by
127 25 cycles of denaturation at 98°C for 10 s, annealing at 72°C for 30 s, extension at
128 68°C for 10 min. The second PCR for the D-loop region was conducted (primers:
129 L15996F & H408R) with an initial denaturation at 94°C for 2 min, followed by 25
130 cycles of denaturation at 98°C for 10 s, annealing at 56°C for 30 s, extension at

131 68°C for 1 min. After confirmation by 2% agarose gel electrophoresis, PCR
132 products were purified using the PCR Clean-Up Mini Kit (FAVORGEN) and
133 sequenced by outsourcing those to Fasmac (Kanagawa, Japan) or Eurofins
134 Genomics (Tokyo, Japan). The primers used for PCR amplification and sequencing
135 are listed in Supp. Table 1.

136 Four Y-STRs (DYS393, DYS19, DYS391, and DYS438) of which genetic
137 diversities differed between Honshu (Fig. 1) and Okinawa (Uchihi et al., 2003)
138 were selected. PCRs for these loci were conducted with an initial denaturation at
139 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s, annealing
140 at 53°C–59°C for 30 s, extension at 72°C for 1 min, followed by a final extension of
141 5 min at 72°C. After confirmation by 2% agarose gel electrophoresis, PCR
142 products were purified using the PCR Clean-Up Mini Kit (FAVORGEN) and
143 sequenced by outsourcing those to Fasmac (Kanagawa, Japan) or Eurofins
144 Genomics (Tokyo, Japan). The primers and annealing temperatures for each PCR
145 amplification are listed in Supp. Table 2.

146 We genotyped four nonsynonymous autosomal SNPs (rs3827760,
147 rs17822931, rs2070235, and rs14103), of which genotype frequencies differed
148 between the Hondo cluster, which includes most of the individuals in mainland
149 Japan and the Ryukyu cluster, which includes most of the individuals in Okinawa
150 (Yamaguchi-Kabata et al., 2008) by PCR-RFLP. PCRs were conducted with an
151 initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at
152 95°C for 15 s, annealing at 54°C–65°C for 30 s, extension at 72°C for 1 min,
153 followed by a final extension of 5 min at 72°C. After confirmation by 2% agarose
154 gel electrophoresis, PCR products were purified using the PCR Clean-Up Mini Kit
155 (FAVORGEN) and digested with an appropriate restriction enzyme at 37°C for 1 h.
156 Thereafter, we electrophoresed these digested products on 4% agarose gel for

genotype determination. The primers, annealing temperatures, and restriction enzymes for each locus are listed in Supp. Table 3.

2.4. Data analyses

The mtDNA sequences were aligned using MEGA X (Kumar et al., 2018) together with sequences of other Asian populations downloaded from the NCBI (National Center for Biotechnology Information) database. Net genetic distances (d_A) between populations were calculated from these sequences under the Tamura-Nei model (Tamura & Nei, 1993) with gamma distribution. Phylogenetic networks between populations based on d_A distances were constructed in the Neighbor-Net method (Bryant & Moulton, 2004) by using SplitsTree4 (Huson & Bryant, 2006). Pairwise F_{ST} between regions and nucleotide diversities within each population were calculated by using Arlequin ver 3.5.2.2 (Excoffier & Lischer, 2010), and Tajima's D (Tajima, 1989) within each population were calculated using DnaSP 5.10 (Librado & Rozas, 2009). The probabilities of Tajima's D were computed through 5,000 coalescence simulations.

We aligned the Y-STR sequences using MEGA X and counted the number of repeats. Pairwise R_{ST} between regions was calculated by using Arlequin ver 3.5.2.2 as well as gene diversities.

Fisher's exact test was used to compare the allele frequencies of the SNPs between populations.

3. Results

3.1. mtDNA

D-loop sequences (587 bp) were determined for a total of 102 subjects whose maternal grandmothers had been born in the Amami islands. Fig. 2 and Supp. Fig. 1 show the phylogenetic networks for 78 Amami-Oshima islanders, 24 Kikai islanders, and other Asian populations. Amami-Oshima and Kikai islands were close to each other in both networks, and the Amami islanders did not form a clear cluster with the residents of Honshu, Kyushu, the Okinawa islands, or any other populations. Table 1 shows the nucleotide diversities and Tajima's D in each population. Nucleotide diversities in the Amami islands were almost the same as those in other regions in Japan. Tajima's D values were significantly negative in the Amami islands (−1.613, P = 0.024) and Amami-Oshima island (−1.539, P = 0.038). Tajima's D value in Kikai island was negative but not significant (−1.494, P = 0.050). Table 2 shows the pairwise F_{ST} values between regions. F_{ST} value between Amami-Oshima and Kikai islands was not significant. F_{ST} values between Amami-Oshima island and Honshu, between Amami-Oshima island and Okinawa, between Amami-Oshima and Ishigaki islands, and between Kikai and Ishigaki islands were significant. Additionally, F_{ST} values between Korea and four regions in Japan (Amami-Oshima island, Kikai island, Kyushu, and Honshu) were not significant.

3.2. Y-STR

Y-STR sequences were determined for 58 male subjects whose paternal grandfathers had been born in the Amami islands. Twenty-five different haplotypes were observed in the Amami islands (Supp. Table 4). Three of them

205 were observed in Kikai island but not in Amami-Oshima island. The paternal
206 grandfather of one subject who had one of the three haplotypes (Ht11) was born in
207 Amami-Oshima island. This subject was counted as Amami-Oshima samples in
208 the Y-STR analyses. Comparing with other regions in Japan (Hashiyada et al.,
209 2008), six haplotypes observed in Amami-Oshima island were not in Okinawa,
210 and one haplotype was found only in Amami-Oshima island and Okinawa but not
211 in any other regions. The allele frequency distributions of four loci were similar
212 among regions (Supp. Table 5). Gene diversities of the four loci are shown in Supp.
213 Table 6. In Amami-Oshima island, gene diversities of any loci did not significantly
214 differ with Honshu or Okinawa. In Kikai island, gene diversities of the loci except
215 DYS438 did not differ with other regions, and that of DYS438 was significantly
216 higher than any other regions. Table 3 shows the pairwise R_{ST} values between
217 regions. R_{ST} value between Amami-Oshima and Kikai islands was significant. R_{ST}
218 values between Amami-Oshima island and Okinawa and between Kikai island
219 and Okinawa were not significant.
220
221 3.3. Autosomal SNPs
222 Genotypes of 91 subjects whose all four grandparents were born in the
223 Amami islands were determined, and allele frequencies were calculated.
224 The frequency of the T allele in rs3827760 (*EDAR*) in Amami-Oshima
225 island (0.385) was significantly higher than in the Hondo cluster (0.222) ($P <$
226 0.001) and not significantly different from the Ryukyu cluster (0.398) ($P = 0.426$).
227 The frequency of the T allele in Kikai island (0.647) was significantly higher than
228 in Amami-Oshima island, the Ryukyu cluster, and the Hondo cluster ($P = 0.005$, P
229 $= 0.004$, and $P < 0.001$, respectively) (Table 4).
230 The frequency of the G allele in rs17822931 (*ABCC11*) in Amami-Oshima

island (0.311) was significantly higher than in the Hondo cluster (0.121) ($P < 0.001$) but not significantly different from that in the Ryukyu cluster (0.258) ($P = 0.123$). The frequency of the G allele in Kikai island (0.118) was significantly lower than in the Ryukyu cluster ($P = 0.044$) and Amami-Oshima island ($P = 0.015$) but not significantly different from that in the Hondo cluster ($P = 0.612$) (Table 4).

The frequency of the G allele in rs2070235 (*MYBL2*) in Amami-Oshima island (0.324) was significantly higher than in the Hondo cluster (0.185) ($P < 0.001$) but not significantly different from that in the Ryukyu cluster (0.291) ($P = 0.228$). The frequency of the G allele in Kikai island was not significantly different from those in Amami-Oshima island, the Ryukyu cluster, and the Hondo cluster ($P = 0.324$, $P = 0.456$, and $P = 0.167$, respectively) (Table 4).

The frequency of the A allele in rs14103 (*C1orf212*) in Amami-Oshima island (0.791) was significantly lower than in the Hondo cluster (0.855) ($P = 0.022$) and not significantly different from that in the Ryukyu cluster (0.753) ($P = 0.187$), whereas that in Kikai island was not different from those in Amami-Oshima island, the Ryukyu cluster, and the Hondo cluster ($P = 0.287$, $P = 0.126$, and $P = 0.562$, respectively) (Table 4).

4. Discussion

Amami islanders showed a trend of Tajima's *D* values of mtDNA to be negative. This trend is commonly observed in the populations of East Asia, including main-island Okinawa, Miyako island, Ishigaki island, and Honshu (Oota et al., 2002; Matsukusa et al., 2010). Purifying selection or recent population expansion would generally be a reason for the negative Tajima's *D* values (Tajima, 1989), but the former is unsuitable in this case because D-loop is a non-coding region. Therefore, this result suggests the past population expansion and/or gene flow from the surrounding regions, *i.e.,* the Amani islanders were genetically not isolated for long.

Pairwise F_{ST} values and phylogenetic networks represent close genetic relations in the female lineages between Amami-Oshima and Kikai islands. The female lineages in Amami-Oshima island differed from Honshu, main-island Okinawa, and Ishigaki island, and those in Kikai island differed from Ishigaki island. In the phylogenetic network, the female lineages in Amami-Oshima and Kikai islands were apart from those in main-island Okinawa and Ishigaki island. Conversely, the female lineages in Amami-Oshima and Kikai islands did not differ from those in Miyako island, and those three were located close to each other in the phylogenetic network; however, no historical records that link geographically separated people in the Amami islands and Miyako island have been recognized. Furthermore, female lineages of Korea were not different from those of Amami-Oshima island, Kikai island, Kyushu, and Honshu, whereas female lineages of Ainu, Taiwan Han, and Taiwan aborigines were different from any other populations; this indirectly suggests that female lineages in the Amami islanders have been genetically affected by mainland Japanese strongly compared

276 with the genetic effect of mainland Japanese on female lineages of Okinawa.

277 Gene diversities and R_{ST} values of the four Y-STRs indicated that the male
278 lineages in Amami-Oshima island were located between those in Honshu and
279 Okinawa, but those in Kikai island showed some genetic deviation from these
280 three populations, including Amami-Oshima islanders; this is assumed to be due
281 to the founder effect that refers to the phenomenon that gene frequencies of a
282 small population migrated to a new location sometimes differ from those of the
283 original population because of chance bias (Mayr, 1954). In Kikai islanders, the
284 small population size (Kagoshima Prefecture, 2018b) and the degree of contacts
285 with mainland Japanese different from Amami-Oshima islanders (Takanashi,
286 2009; Yoshinari, 2011; Takamiya, 2013; Takamiya et al., 2019) may have caused
287 that.

288 Comparing the maternal (mtDNA) and paternal (Y-STR) genes (Table 5),
289 we found that the mean of pairwise R_{ST} values of Y-STR had a trend that the
290 larger the value is, the smaller population size is (0.034 in entire Japan, 0.055 in
291 the Ryukyu Archipelago and Kyushu, 0.073 in the Ryukyu Archipelago, and 0.113
292 in the Amami islands). Conversely, the mean F_{ST} values of mtDNA did not change
293 so much (0.020 in entire Japan, 0.021 in the Ryukyu Archipelago and Kyushu,
294 0.019 in the Ryukyu Archipelago, and − 0.007 in the Amami islands). These values
295 suggest that males are genetically more divergent than females because of
296 patrilocality; in fact, female migration was suggested frequently in the Ryukyu
297 Archipelago (Matsukusa et al., 2010), and we confirmed this in the Amami
298 islands.

299 Four autosomal SNPs whose allele frequencies differed between Okinawa
300 and the rest of Japan (Yamaguchi-Kabata et al., 2008) were selected to identify
301 the Amami islanders' genealogic position and search for the traits of selective

302 pressure if it ever exerted in these populations. The allele frequencies of the four
303 SNPs in Amami-Oshima islanders were similar to the Ryukyu cluster and
304 significantly different from the Hondo cluster, which may be because of gene flow
305 from Okinawa. Conversely, in Kikai islanders, the T allele frequency of rs3827760
306 (*EDAR*) was significantly higher than those in the other three populations, and
307 the G allele frequency of rs17822931 (*ABCC11*) was significantly lower than those
308 in the Ryukyu cluster and Amami-Oshima islanders but not lower than that in
309 the Hondo cluster. These can be explained by the founder effect, as observed in
310 Y-STR: DYS438. The presence of selective pressure is hard to recognize in these
311 loci for the following reasons.

312 The C allele of rs3827760 on the EDAR gene is associated with thicker
313 hair (Fujimoto et al., 2008a, b), straighter hair (Tan et al., 2013), multiple dental
314 traits (Kimura et al., 2009; Park et al., 2012; Kamberov et al., 2013), and high
315 density of sweat gland (Kamberov et al., 2013). The frequencies of the C allele are
316 very high in East Asians and Native Americans, whereas that allele is almost
317 absent in Europeans and Africans, and this implies strong positive selection in
318 Asia (Sabeti et al., 2007; Bryk et al., 2008; Xue et al., 2009; Grossman et al., 2010).
319 Adaptation to high humidity is assumed to be selective pressure (Kamberov et al.,
320 2013; Tan et al., 2013), but frequencies of the C allele in the Ryukyu cluster,
321 Amami-Oshima island, and Kikai island were significantly lower than that in the
322 Hondo cluster, despite hot and humid climate in the Ryukyu Archipelago (Table 4).
323 This suggests that selective pressure on the C allele has not been so strong in the
324 Ryukyu Archipelago, and low frequencies of the C allele in the Amami islands
325 may be due to gene flow from Okinawa.

326 Secondly, G/G and G/A genotypes of rs17822931 located on the ABCC11
327 gene are associated with wet earwax (Yoshiura et al., 2006) and axillary

328 osmidrosis (Nakano et al., 2009), which are assumed to be due to their enhanced
329 function of the apocrine gland. The frequencies of the A allele are very high in
330 East Asia. They decline when the latitude is getting lower in Asian, African, and
331 European populations, and this implies strong positive selection and can be
332 explained by adaptation to cold climate, such as less sweating (Yoshiura et al.,
333 2006; Ohashi et al., 2011). Therefore, the frequencies of the A allele are
334 significantly lower in the Ryukyu cluster and Amami-Oshima island than in the
335 Hondo cluster (Table 4) because selective pressure should not have existed under
336 hotter circumstances in the Ryukyu Archipelago.

337 In conclusion, Amami-Oshima and Kikai island populations showed a
338 genetically intermediate position between mainland Japan and Okinawa in
339 mtDNA and Y-STR. However, the frequencies of several autosomal SNPs in the
340 Amami islanders indicated a significant difference from mainland Japanese. The
341 *EDAR* and *ABCC11* of mainland Japanese are assumed to have been under
342 natural selection; however, those of the Amami islanders may have been affected
343 by the gene flow from Okinawa but not natural selection. Furthermore, the
344 extremely high or low frequencies of several alleles implied a founder effect in
345 Kikai island. It should be noted that we could not say with strong evidence
346 whether mtDNA and Y-STRs of the Amami islanders were close to Okinawa or
347 mainland Japan and whether autosomal SNPs of the Amami islanders have been
348 affected by gene flow from Okinawa because of the small sample size (especially in
349 Kikai island) and the number of alleles in the present study. Geographically broad
350 and detailed samplings and genome-wide analyses are awaited.

Declarations of interest: none

Acknowledgments

We would like to thank all anonymous subjects in Naze, Amami-shi, Kagoshima, in Kasari-cho, Amami-shi, Kagoshima, in Yamato-son, Oshima-gun, Kagoshima, and in Kikai-cho, Oshima-gun, Kagoshima for participating in this study. We are grateful to Dr. M. Suzuki (Ministry of the Environment, Japan), Prof. H. Takamiya (Kagoshima University), Ms. R. Koike, and anonymous cooperators for their help in sampling. We thank Enago (www.enago.jp) for the English language review.

References

Anderson, S., Bankier, A.T., Barrell, B.G., de Bruijn, M.H.L., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R., Young, I.G., 1981. Sequence and organization of the human mitochondrial genome. Nature 290, 457–465. https://doi.org/10.1038/290457a0

Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M., Howell, N., 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat. Genet. 23, 147–147. https://doi.org/10.1038/13779

Ayub, Q., 2000. Identification and characterisation of novel human Y-chromosomal microsatellites from sequence database information. Nucleic Acids Res. 28, 8e – 8. https://doi.org/10.1093/nar/28.2.e8

Bryant, D., Moulton, V., 2004. Neighbor-Net: An Agglomerative Method for the Construction of Phylogenetic Networks. Mol. Biol. Evol. 21, 255–265. https://doi.org/10.1093/molbev/msh018

Bryk, J., Hardouin, E., Pugach, I., Hughes, D., Strotmann, R., Stoneking, M., Myles, S., 2008. Positive Selection in East Asians for an EDAR Allele that Enhances NF-κB Activation. PLoS One 3, e2209. https://doi.org/10.1371/journal.pone.0002209

Butler, J.M., Schoske, R., Vallone, P.M., Kline, M.C., Redd, A.J., Hammer, M.F., 2002. A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. Forensic Sci. Int. 129, 10–24. https://doi.org/10.1016/S0379-0738(02)00195-0

de Knijff, P., Kayser, M., Caglià, A., Corach, D., Fretwell, N., Gehrig, C., Graziosi, G., Heidorn, F., Herrmann, S., Herzog, B., Hidding, M., Honda, K., Jobling,

388 M., Krawczak, M., Leim, K., Meuser, S., Meyer, E., Oesterreich, W., Pandya,
389 A., Parson, W., Penacino, G., Perez-Lezaun, A., Piccinini, A., Prinz, M.,
390 Schmitt, C., Schneider, P.M., Szibor, R., Teifel-Greding, J., Weichhold, G.,
391 Roewer, L., 1997. Chromosome Y microsatellites: population genetic and
392 evolutionary aspects. Int. J. Legal Med. 110, 134–140.
393 https://doi.org/10.1007/s004140050052
394 Dodo, Y., Doi, N., Kondo, O., 1998. Ainu and Ryukyuan cranial nonmetric
395 variation: Evidence which disputes the Ainu-Ryukyu common origin theory.
396 Anthropol. Sci. 106, 99–120. https://doi.org/10.1537/ase.106.99
397 Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of
398 programs to perform population genetics analyses under Linux and Windows.
399 Mol. Ecol. Resour. 10, 564–567.
400 https://doi.org/10.1111/j.1755-0998.2010.02847.x
401 Fujimoto, A., Kimura, R., Ohashi, J., Omi, K., Yuliwulandari, R., Batubara, L.,
402 Mustofa, M.S., Samakkarn, U., Settheetham-Ishida, W., Ishida, T., Morishita,
403 Y., Furusawa, T., Nakazawa, M., Ohtsuka, R., Tokunaga, K., 2008a. A scan for
404 genetic determinants of human hair morphology: EDAR is associated with
405 Asian hair thickness. Hum. Mol. Genet. 17, 835–843.
406 https://doi.org/10.1093/hmg/ddm355
407 Fujimoto, A., Ohashi, J., Nishida, N., Miyagawa, T., Morishita, Y., Tsunoda, T.,
408 Kimura, R., Tokunaga, K., 2008b. A replication study confirmed the EDAR
409 gene to be a major contributor to population differentiation regarding head
410 hair thickness in Asia. Hum. Genet. 124, 179–185.
411 https://doi.org/10.1007/s00439-008-0537-1
412 Grossman, S.R., Shylakhter, I., Karlsson, E.K., Byrne, E.H., Morales, S., Frieden,
413 G., Hostetter, E., Angelino, E., Garber, M., Zuk, O., Lander, E.S., Schaffner,

414 S.F., Sabeti, P.C., 2010. A Composite of Multiple Signals Distinguishes Causal
415 Variants in Regions of Positive Selection. Science (80-.). 327, 883 LP – 886.
416 https://doi.org/10.1126/science.1183863
417 Habu, J., 2004. Ancient Jomon of Japan. Cambridge University Press, Cambridge.
418 Hammer, M.F., Horai, S., 1995. Y chromosomal DNA variation and the peopling of
419 Japan. Am. J. Hum. Genet. 56, 951–962.
420 Hammer, M.F., Karafet, T.M., Park, H., Omoto, K., Harihara, S., Stoneking, M.,
421 Horai, S., 2006. Dual origins of the Japanese: Common ground for
422 hunter-gatherer and farmer Y chromosomes. J. Hum. Genet. 51, 47–58.
423 https://doi.org/10.1007/s10038-005-0322-0
424 Hanihara, K., 1991. Dual Structure Model for the Population History of the
425 Japanese. Japan Rev. 2, 1–33.
426 Hashiyada, M., Umetsu, K., Yuasa, I., Tamura, A., Matsusue, A., Suzuki, K.,
427 Kashimura, S., Funayama, M., 2008. Population genetics of 17
428 Y-chromosomal STR loci in Japanese. Forensic Sci. Int. Genet. 2, 69–70.
429 https://doi.org/10.1016/j.fsigen.2008.01.001
430 Hayashida, M., Ota, T., Iwao-Koizumi, K., Murata, S., 2010. Simple SNP
431 genotyping of hair thickness-determining gene EDAR and human
432 earwax-type gene ABCC11 for education of genetic tests. Bull. Mukogawa
433 Women's Univ. Nat. Sci. 58, 43–47. https://doi.org/10.14993/00000508
434 Horai, S., Murayama, K., Hayasaka, K., Matsubayashi, S., Hattori, Y., Fucharoen,
435 G., Harihara, S., Park, K.S., Omoto, K., Pan, I.H., 1996. mtDNA
436 polymorphism in East Asian populations, with special reference to the
437 peopling of Japan. Am. J. Hum. Genet. 59, 579–590.
438 Huson, D.H., Bryant, D., 2006. Application of Phylogenetic Networks in
439 Evolutionary Studies. Mol. Biol. Evol. 23, 254–267.

https://doi.org/10.1093/molbev/msj030

Jinam, T., Nishida, N., Hirai, M., Kawamura, S., Oota, H., Umetsu, K., Kimura, R., Ohashi, J., Tajima, A., Yamamoto, T., Tanabe, H., Mano, S., Suto, Y., Kaname, T., Naritomi, K., Yanagi, K., Niikawa, N., Omoto, K., Tokunaga, K., Saitou, N., 2012. The history of human populations in the Japanese Archipelago inferred from genome-wide SNP data with a special reference to the Ainu and the Ryukyuan populations. J. Hum. Genet. 57, 787–795. https://doi.org/10.1038/jhg.2012.114

Kagoshima Prefecture. (2018a) Amami-Oshima Kakeroma shotō no gaiyō. http://www.pref.kagoshima.jp/ac07/pr/shima/gaiyo/amami/amami_top.html (accessed 19 April 2021). (in Japanese)

Kagoshima Prefecture. (2018b) Kikaijima no gaiyō. http://www.pref.kagoshima.jp/ac07/pr/shima/gaiyo/kikai/kikai_top.html (accessed 19 April 2021). (in Japanese)

Kamberov, Y.G., Wang, S., Tan, J., Gerbault, P., Wark, A., Tan, L., Yang, Y., Li, S., Tang, K., Chen, H., Powell, A., Itan, Y., Fuller, D., Lohmueller, J., Mao, J., Schachar, A., Paymer, M., Hostetter, E., Byrne, E., Burnett, M., McMahon, A.P., Thomas, M.G., Lieberman, D.E., Jin, L., Tabin, C.J., Morgan, B.A., Sabeti, P.C., 2013. Modeling Recent Human Evolution in Mice by Expression of a Selected EDAR Variant. Cell 152, 691–702. https://doi.org/10.1016/j.cell.2013.01.016

Kayser, M., Caglià, A., Corach, D., Fretwell, N., Gehrig, C., Graziosi, G., Heidorn, F., Herrmann, S., Herzog, B., Hidding, M., Honda, K., Jobling, M., Krawczak, M., Leim, K., Meuser, S., Meyer, E., Oesterreich, W., Pandya, A., Parson, W., Penacino, G., Perez-Lezaun, A., Piccinini, A., Prinz, M., Schmitt, C., Schneider, P.M., Szibor, R., Teifel-Greding, J., Weichhold, G., De Knijff, P.,

466 Roewer, L., 1997. Evaluation of Y-chromosomal STRs: A multicenter study.
467 Int. J. Legal Med. 110, 125–133. https://doi.org/10.1007/s004140050051
468 Kimura, R., Yamaguchi, T., Takeda, M., Kondo, O., Toma, T., Haneji, K., Hanihara,
469 T., Matsukusa, H., Kawamura, S., Maki, K., Osawa, M., Ishida, H., Oota, H.,
470 2009. A Common Variation in EDAR Is a Genetic Determinant of
471 Shovel-Shaped Incisors. Am. J. Hum. Genet. 85, 528–535.
472 https://doi.org/10.1016/j.ajhg.2009.09.006
473 Kobayashi, H., Hirose, T., Sugino, M., Watanabe, N., 1974. University of Tokyo
474 Radiocarbon Measurements V. Radiocarbon 16, 381–387.
475 https://doi.org/10.1017/S0033822200059671
476 Koganebuchi, K., Katsumura, T., Nakagome, S., Ishida, H., Kawamura, S., Oota,
477 H., Ohashi, J., Yamamoto, T., Nishida, N., Tajima, A., Tanabe, H., Umetsu, K.,
478 Saitou, N., Tokunaga, K., Suto, Y., Hirai, M., Kimura, R., Mano, S.,
479 Kawamura, S., Oota, H., Omoto, K., 2012. Autosomal and Y-chromosomal
480 STR markers reveal a close relationship between Hokkaido Ainu and Ryukyu
481 islanders. Anthropol. Sci. 120, 199–208. https://doi.org/10.1537/ase.120322
482 Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular
483 evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35,
484 1547–1549. https://doi.org/10.1093/molbev/msy096
485 Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of
486 DNA polymorphism data. Bioinformatics 25, 1451–1452.
487 https://doi.org/10.1093/bioinformatics/btp187
488 Matsukusa, H., Oota, H., Haneji, K., Toma, T., Kawamura, S., Ishida, H., 2010. A
489 genetic analysis of the sakishima islanders reveals no relationship with
490 taiwan aborigines but shared ancestry with ainu and main-island Japanese.
491 Am. J. Phys. Anthropol. 142, 211–223. https://doi.org/10.1002/ajpa.21212

492 Matsunami, M., Koganebuchi, K., Imamura, M., Ishida, H., Kimura, R., Maeda, S.,
493 2021. Fine-scale genetic structure and demographic history in the Miyako
494 Islands of the Ryukyu Archipelago. Mol. Biol. Evol.
495 https://doi.org/10.1093/molbev/msab005
496 Mayr, E., 1954. Change of environment and evolution, in: Huxley, J., Hardy, A.C.,
497 Ford, E.B. (Eds.), Evolution as a Process. Allen and Unwin, London, pp.
498 157-180.
499 Mizuno, F., Gojobori, J., Kumagai, M., Baba, H., Taniguchi, Y., 2021. Population
500 dynamics in the Japanese Archipelago since the Pleistocene revealed by the
501 complete mitochondrial genome sequences. Sci. Rep. 1–11.
502 https://doi.org/10.1038/s41598-021-91357-2
503 Nakagawa, R., Doi, N., Nishioka, Y., Nunami, S., Yamauchi, H., Fujita, M.,
504 Yamazaki, S., Yamamoto, M., Katagiri, C., Mukai, H., Matsuzaki, H.,
505 Gakuhari, T., Takigami, M., Yoneda, M., 2010. Pleistocene human remains
506 from Shiraho-Saonetabaru Cave on Ishigaki Island, Okinawa, Japan, and
507 their radiocarbon dating. Anthropol. Sci. 118, 173–183.
508 https://doi.org/10.1537/ase.091214
509 Nakagome, S., Sato, T., Ishida, H., Hanihara, T., Yamaguchi, T., Kimura, R., Mano,
510 S., Oota, H., 2015. Model-Based Verification of Hypotheses on the Origin of
511 Modern Japanese Revisited by Bayesian Inference Based on Genome-Wide
512 SNP Data. Mol. Biol. Evol. 32, 1533–1543.
513 https://doi.org/10.1093/molbev/msv045
514 Nakano, M., Miwa, N., Hirano, A., Yoshiura, K.I., Niikawa, N., 2009. A strong
515 association of axillary osmidrosis with the wet earwax type determined by
516 genotyping of the ABCC11 gene. BMC Genet. 10, 1–5.
517 https://doi.org/10.1186/1471-2156-10-42

518 Nishiyama, T., Kishino, H., Suzuki, S., Ando, R., Niimura, H., Uemura, H., Horita,
519 M., Ohnaka, K., Kuriyama, N., Mikami, H., Takashima, N., Mastuo, K.,
520 Guang, Y., Wakai, K., Hamajima, N., Tanaka, H., 2012. Detailed Analysis of
521 Japanese Population Substructure with a Focus on the Southwest Islands of
522 Japan. PLoS One 7, e35000. https://doi.org/10.1371/journal.pone.0035000
523 Ohashi, J., Naka, I., Tsuchiya, N., 2011. The impact of natural selection on an
524 ABCC11 SNP determining earwax type. Mol. Biol. Evol. 28, 849–857.
525 https://doi.org/10.1093/molbev/msq264
526 Omoto, K., Saitou, N., 1997. Genetic origins of the Japanese: A partial support for
527 the dual structure hypothesis. Am. J. Phys. Anthropol. 102, 437–446.
528 https://doi.org/10.1002/(SICI)1096-8644(199704)102:4<437::AID-AJPA1>3.0.
529 CO;2-P
530 Oota, H., Kitano, T., Jin, F., Yuasa, I., Wang, L., Ueda, S., Saitou, N., Stoneking,
531 M., 2002. Extreme mtDNA homogeneity in continental Asian populations. Am.
532 J. Phys. Anthropol. 118, 146–153. https://doi.org/10.1002/ajpa.10056
533 Park, J.-H., Yamaguchi, T., Watanabe, C., Kawaguchi, A., Haneji, K., Takeda, M.,
534 Kim, Y.-I., Tomoyasu, Y., Watanabe, M., Oota, H., Hanihara, T., Ishida, H.,
535 Maki, K., Park, S.-B., Kimura, R., 2012. Effects of an Asian-specific
536 nonsynonymous EDAR variant on multiple dental traits. J. Hum. Genet. 57,
537 508–514. https://doi.org/10.1038/jhg.2012.60
538 Roewer, L., Epplen, J.T., 1992. Rapid and sensitive typing of forensic stains by
539 PCR amplification of polymorphic simple repeat sequences in case work.
540 Forensic Sci. Int. 53, 163–171.
541 https://doi.org/https://doi.org/10.1016/0379-0738(92)90193-Z
542 Sabeti, P.C., Varilly, P., Fry, B., Lohmueller, J., Hostetter, E., Cotsapas, C., Xie, X.,
543 Byrne, E.H., McCarroll, S.A., Gaudet, R., Schaffner, S.F., Lander, E.S., 2007.

Genome-wide detection and characterization of positive selection in human populations. Nature 449, 913–918. https://doi.org/10.1038/nature06250

Sato, T., Nakagome, S., Watanabe, C., Yamaguchi, K., Kawaguchi, A., Koganebuchi, K., Haneji, K., Yamaguchi, T., Hanihara, T., Yamamoto, K., Ishida, H., Mano, S., Kimura, R., Oota, H., 2014. Genome-wide SNP analysis reveals population structure and demographic history of the ryukyu islanders in the southern part of the japanese archipelago. Mol. Biol. Evol. 31, 2929–2940. https://doi.org/10.1093/molbev/msu230

Stanford Genome Technology Center, 2002. List of primers used for resequencing the mitochondrial genome. http://insertion.stanford.edu/primers.html (accessed 19 April 2021).

Tajima, A., Sun, C.-S., Pan, I.-H., Ishida, T., Saitou, N., Horai, S., 2003. Mitochondrial DNA polymorphisms in nine aboriginal groups of Taiwan: implications for the population history of aboriginal Taiwanese. Hum. Genet. 113, 24–33. https://doi.org/10.1007/s00439-003-0945-1

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.

Takamiya, H., 2013. Amami Okinawa shoto eno hito no idō, in: Intoh, H. (Eds.), Jinrui no idōshi. Rinsen Shoten, Kyoto, pp. 182–197. (in Japanese)

Takamiya, H., Katagiri, C., Yamasaki, S., Fujita, M., 2019. Human Colonization of the Central Ryukyus (Amami and Okinawa Archipelagos), Japan. J. Isl. Coast. Archaeol. 14, 375–393. https://doi.org/10.1080/15564894.2018.1501443

Takanashi, O., 2009. Doki dōtai kara kangaeru “Nihon bunka no nanzen”, in: Takanashi, O., Abe, M., Nakamoto, K., Yoshinari, N, Okinawa bunka wa doko kara kitaka: Gusuku jidai to iu kakki. Shinwasha, Tokyo, pp. 47–132. (in Japanese)

570 Takanashi, O., 2011. Nampō sekai eno hirogari: Ritsuryō kokka to Ryukyu-ko, in:
571 Arano, Y., Ishii, M., Murai, S. (Eds.), Ritsuryō kokka to Higashi Ajia.
572 Yoshikawakobunkan, Tokyo, pp. 319–333. (in Japanese)
573 Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions
574 in the control region of mitochondrial DNA in humans and chimpanzees. Mol.
575 Biol. Evol. 10, 512–526.
576 https://doi.org/10.1093/oxfordjournals.molbev.a040023
577 Tan, J., Yang, Y., Tang, K., Sabeti, P.C., Jin, L., Wang, S., 2013. The adaptive
578 variant EDARV370A is associated with straight hair in East Asians. Hum.
579 Genet. 132, 1187–1191. https://doi.org/10.1007/s00439-013-1324-1
580 Tanaka, M., Cabrera, V.M., González, A.M., Larruga, J.M., Takeyasu, T., Fuku, N.,
581 Guo, L.J., Hirose, R., Fujita, Y., Kurata, M., Shinoda, K.I., Umetsu, K.,
582 Yamada, Y., Oshida, Y., Sato, Y., Hattori, N., Mizuno, Y., Arai, Y., Hirose, N.,
583 Ohta, S., Ogawa, O., Tanaka, Y., Kawamori, R., Shamoto-Nagai, M.,
584 Maruyama, W., Shimokata, H., Suzuki, R., Shimodaira, H., 2004.
585 Mitochondrial genome variation in Eastern Asia and the peopling of Japan.
586 Genome Res. 14, 1832–1850. https://doi.org/10.1101/gr.2286304
587 Tsuha, T., 2012. Okinawa gawa kara mita Amami no bunka henyō. Daiichi Shobo,
588 Tokyo. (in Japanese)
589 Uchihi, R., Yamamoto, T., Usuda, K., Yoshimoto, T., Tanaka, M., Tokunaga, S.,
590 Kurihara, R., Tokunaga, K., Katsumata, Y., 2003. Haplotype analysis with 14
591 Y-STR loci using 2 multiplex amplification and typing systems in 2 regional
592 populations in Japan. Int. J. Legal Med. 117, 34–38.
593 https://doi.org/10.1007/s00414-002-0319-6
594 Vigilant, L., Pennington, R., Harpending, H., Kocher, T.D., Wilson, A.C., 1989.
595 Mitochondrial DNA sequences in single hairs from a southern African

population. Proc. Natl. Acad. Sci. U. S. A. 86, 9350–9354. https://doi.org/10.1073/pnas.86.23.9350

Xue, Y., Zhang, X., Huang, N., Daly, A., Gillson, C.J., MacArthur, D.G., Yngvadottir, B., Nica, A.C., Woodwark, C., Chen, Y., Conrad, D.F., Ayub, Q., Mehdi, S.Q., Li, P., Tyler-Smith, C., 2009. Population Differentiation as an Indicator of Recent Positive Selection in Humans: An Empirical Evaluation. Genetics 183, 1065 LP – 1077. https://doi.org/10.1534/genetics.109.107722

Yamaguchi-Kabata, Y., Nakazono, K., Takahashi, A., Saito, S., Hosono, N., Kubo, M., Nakamura, Y., Kamatani, N., 2008. Japanese Population Structure, Based on SNP Genotypes from 7003 Individuals Compared to Other Ethnic Groups: Effects on Population-Based Association Studies. Am. J. Hum. Genet. 83, 445–456. https://doi.org/10.1016/j.ajhg.2008.08.019

Yamaguchi-Kabata, Y., Tsunoda, T., Kumasaka, N., Takahashi, A., Hosono, N., Kubo, M., Nakamura, Y., Kamatani, N., 2012. Genetic differences in the two main groups of the Japanese population based on autosomal SNPs and haplotypes. J. Hum. Genet. 57, 326–334. https://doi.org/10.1038/jhg.2012.26

Yoshinari, N., 2011. Ryukyu no seiritsu: Ijū to kōeki no rekishi. Nanpou Shinsha, Kagoshima. (in Japanese)

Yoshinari, N., Fuku, H., 2007. Ryukyu ōkoku tanjō: Amami shotō shi kara. Shinwasha, Tokyo. (in Japanese)

Yoshiura, K., Kinoshita, A., Ishida, T., Ninokata, A., Ishikawa, T., Kaname, T., Bannai, M., Tokunaga, K., Sonoda, S., Komaki, R., Ihara, M., Saenko, V.A., Alipov, G.K., Sekine, I., Komatsu, K., Takahashi, H., Nakashima, M., Sosonkina, N., Mapendano, C.K., Ghadami, M., Nomura, M., Liang, D.-S., Miwa, N., Kim, D.-K., Garidkhuu, A., Natsume, N., Ohta, T., Tomita, H., Kaneko, A., Kikuchi, M., Russomando, G., Hirayama, K., Ishibashi, M.,

622 Takahashi, A., Saitou, N., Murray, J.C., Saito, S., Nakamura, Y., Niikawa, N.,
623 2006. A SNP in the ABCC11 gene is the determinant of human earwax type.
624 Nat. Genet. 38, 324–330. https://doi.org/10.1038/ng1733

Table 1. Nucleotide diversities and Tajima's D values of mtDNA

	Size	Nucleotide diversity	Tajima's *D*	*P* value	Ref.
Amami islands	102	0.019 ± 0.010	-1.613	0.024	Present study
Amami-Oshima island	78	0.019 ± 0.010	-1.516	0.042	Present study
Kikai island	24	0.020 ± 0.011	-1.494	0.050	Present study
Main-island Okinawa	95	0.019 ± 0.010	-1.972	0.004	Matsukusa et al., 2010
Miyako island	66	0.015 ± 0.008	-1.916	0.008	Matsukusa et al., 2010
Ishigaki island	63	0.016 ± 0.008	-1.582	0.039	Matsukusa et al., 2010
Honshu	89	0.015 ± 0.008	-2.050	0.002	Oota et al., 2002

For the analyses of mtDNA, one subject from Kikai island whose maternal grandmother was born in Amami-Oshima island was excluded from Kikai island samples and included in Amami-Oshima island samples.

Table 2. Pairwise F_{ST} values of mtDNA

	Amami-Oshima island	Kikai island	Main-island Okinawa	Miyako island	Ishigaki island	Kyushu	Honshu	Ainu	Korea	Taiwan Han	Taiwan aborigines
Amami-Oshima island (n=78)	0										
Kikai island (n=24)	-0.007	0									
Main-island Okinawa (n=95)	**0.026**	0.003	0								
Miyako island (n=66)	0.004	-0.001	**0.034**	0							
Ishigaki island (n=63)	**0.044**	**0.028**	**0.013**	**0.043**	0						
Kyushu (n=104)	-0.001	-0.004	**0.036**	**0.013**	**0.055**	0					
Honshu (n=89)	**0.009**	0.010	**0.043**	**0.012**	**0.045**	**0.008**	0				
Ainu (n=51)	**0.043**	**0.027**	**0.043**	**0.062**	**0.068**	**0.042**	**0.074**	0			
Korea (n=64)	0.004	-0.002	**0.036**	**0.014**	**0.048**	-0.001	0.004	**0.040**	0		
Taiwan Han (n=52)	**0.033**	**0.037**	**0.081**	**0.053**	**0.102**	**0.030**	**0.033**	**0.078**	**0.039**	0	
Taiwan aborigines (n=180)	**0.074**	**0.057**	**0.097**	**0.103**	**0.120**	**0.060**	**0.092**	**0.080**	**0.069**	**0.084**	0

Data of Miyako, Ishigaki and Main-island Okinawa were taken from Matsukusa et al. (2010). Data of Honshu were taken from Oot a et al. (2002) 2002). Data of Ainu, Korea and Taiwan Han were taken from Horai et al. (1996). Data of Taiwan aborigines were taken from Tajima et al. (2003). Significant differences are in bold ($p < 0.05$).

Table 3. Pairwise R_{ST} values of Y-STR

		Amami-Oshima island	Kikai island	Okinawa	Kyushu		Honshu		
					Fukuoka	Osaka	Tottori	Yamagata	Miyagi
Amami-Oshima island (n=48)		0							
Kikai island (n=10)		**0.113**	0						
Okinawa (n=83)		0.027	0.079	0					
Kyushu	Fukuoka (n=212)	-0.002	**0.102**	0.010	0				
Honshu	Osaka (n=131)	-0.010	**0.140**	**0.031**	0.001	0			
	Tottori (n=243)	-0.008	**0.117**	**0.017**	-0.003	-0.003	0		
	Yamagata (n=201)	-0.011	**0.161**	**0.047**	**0.010**	-0.004	0.002	0	
	Miyagi (n=296)	-0.004	**0.120**	**0.031**	0.001	-0.004	0.000	0.002	0

Data of the regions except Amami-Oshima island and Kikai island were taken from Hashiyada et al. (2008). Significant differences are in bold ($p < 0.05$).

Table 4. Allele frequencies of four autosamal SNPs

SNP	Gene	Allele frequencies								*P* value					
		Amami·Oshima island		Kikai island		Ryukyu		Hondo		Between Amami·Oshima and Kikai	Between Amami·Oshima and Ryukyu	Between Amami·Oshima and Hondo	Between Kikai and Ryukyu	Between Kikai and Hondo	Between Ryukyu and Hondo
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2						
rs3827760	*EDAR*	0.385	0.615	0.647	0.353	0.398	0.602	0.222	0.778	0.005	0.426	< 0.001	0.004	< 0.001	< 0.001
rs17822931	*ABCC11*	0.311	0.689	0.118	0.882	0.258	0.742	0.121	0.879	0.015	0.123	< 0.001	0.044	0.612	< 0.001
rs2070235	*MYBL2*	0.324	0.676	0.265	0.735	0.291	0.709	0.185	0.815	0.324	0.228	< 0.001	0.456	0.167	< 0.001
rs14103	*C1orf212*	0.791	0.209	0.853	0.147	0.753	0.247	0.855	0.145	0.287	0.187	0.022	0.126	0.562	< 0.001

Data of Ryukyu and Hondo clusters were taken from Yamaguchi-Kabata et al. (2008) for rs3827760 and rs17822931, and Yamaguchi-Kabata et al. (2012) for rs2070235 and rs14103.

Table 5. Mean of pairwise F_{ST} values of mtDNA and pairwise R_{ST} values of Y-STR

	mtDNA	Y-STR
	F_{ST}	R_{ST}
Amami islands	-0.007	0.113
Ryukyu Archipelago	0.019	0.073
Ryukyu and Kyushu	0.021	0.055
Japan	0.020	0.034

Data used for calculation were taken from Matsukusa et al. (2010) and Hashiyada et al. (2008) except those of the Amami islands.

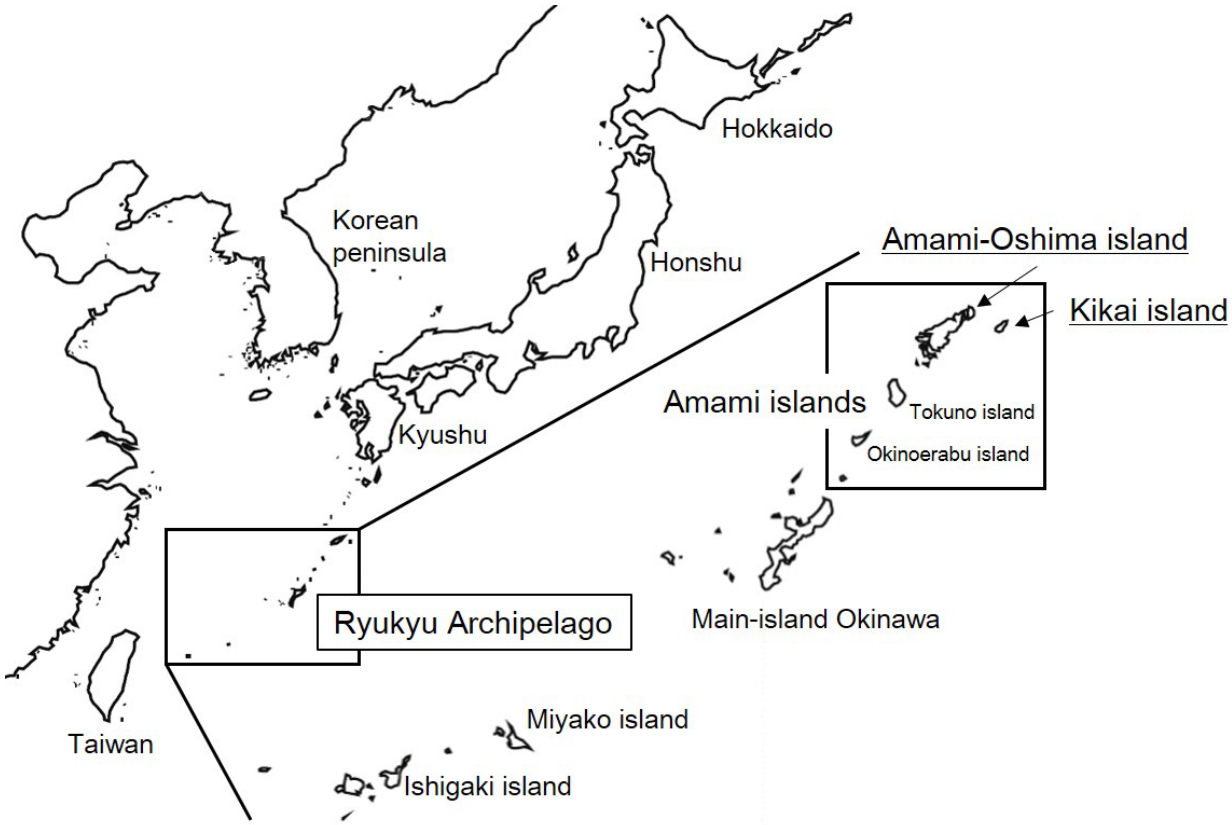

Fig. 1. Map of East Asia. Amami-Oshima island and Kikai island are placed on the northern edge of the Ryukyu Archipelago. Main-island Okinawa, Miyako island and Ishigaki island are included in Okinawa. Hokkaido, Honshu and Kyushu are included in mainland Japan (Hondo).

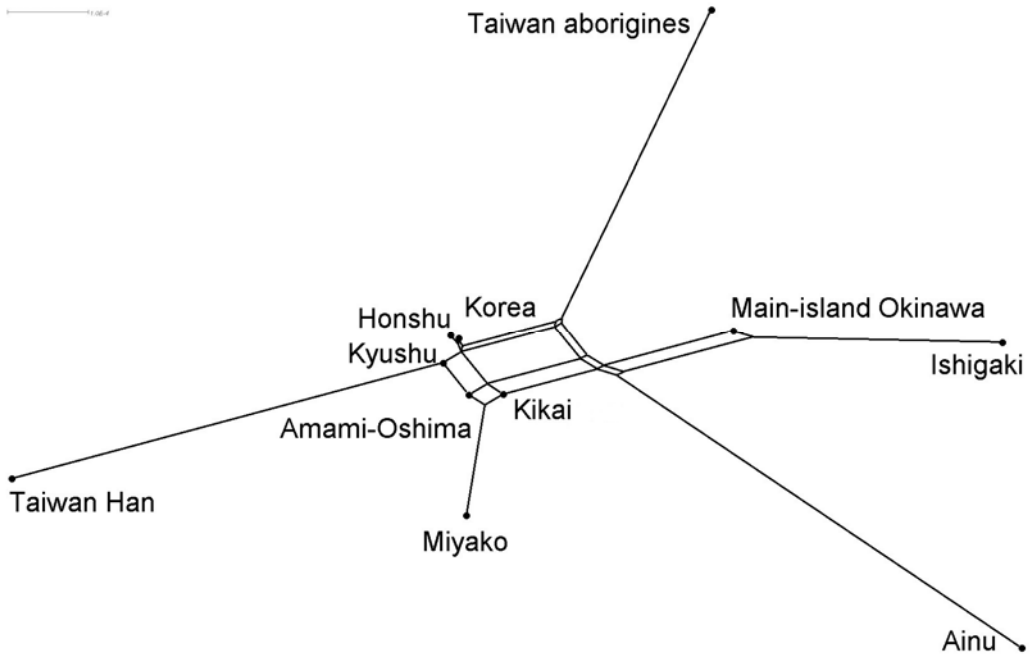

Fig. 2. Phylogenetic network between populations based on d_A distances calculated from 337 bp of D-loop region. Data of Miyako, Ishigaki and Main-island Okinawa were taken from Matsukusa et al. (2010). Data of Honshu were taken from Oota et al. (2002). Data of Ainu, Korea and Taiwan Han were taken from Horai et al. (1996). Data of Taiwan aborigines were taken from Tajima et al. (2003).