Comprehensive analysis of Japanese archipelago population history by detecting ancestry-marker polymorphisms without using ancient DNA data

Yusuke Watanabe[1,2], Jun Ohashi[1*]

[1]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo 113-0033, Japan

[2]Present Adress: Genome Medical Science Project Toyama Project, National Center for Global Health and Medicine, Tokyo 162-8655, Japan.

*Correspondence: juno-tky@umin.ac.jp

Abstract

Modern Japanese have two major ancestral populations: the indigenous Jomon people and immigrants from continental East Asia. To figure out the population history in Japanese archipelago, we developed a reference-free detection method of genetic components from ancestral populations using a summary statistic, the ancestry-marker index (*AMI*). We applied the *AMI* to modern Japanese samples and identified 208,648 SNPs that were likely derived from the Jomon people (Jomon-derived SNPs). The analysis of Jomon-derived SNPs in 10,842 modern Japanese individuals recruited from all the 47 prefectures of Japan showed that the genetic differences among the prefectures were mainly caused by differences in the admixture proportion of the Jomon people and the population size of immigrants varied between regions in mainland Japan. We also estimated the migration route of the ancestral Jomon population to Japanese archipelago and their phenotype frequencies based on the haplotype structures of modern Japanese composed of Jomon-derived SNPs.

Introduction

Modern Japanese populations are divided into three main populations: the Ainu, who live mainly in Hokkaido; the Ryukyuan, who live mainly in Okinawa; and mainland Japanese, who live in Honshu, Shikoku, and Kyushu (Fig. 1). As a powerful hypothesis of the formation processes of Japanese populations, a dual structure model[1] was proposed based on morphology. This model assumes that Japanese originated through a mixture of the Jomon people, who settled in the Japanese archipelago during the Jomon period (from 16,500 YBP to 2,800 YBP)[2–4], and the immigrants came to the Japanese archipelago from continental East Asia around the beginning of the Yayoi period (around 2,800 YBP)[4]. According to this model, compared to mainland Japanese, the Ainu and the Ryukyuan were genetically less influenced by immigrants. Findings from genetical studies not only support the dual structure model, but also reveal the detailed population history of Japanese archipelago[5–11]. Whole-genome analyses extracted from the remains of the Jomon people have suggested that the Jomon were highly differentiated from other East Asians, forming a basal lineage to the East and Northeast Asians[8,10,11]. The genetic relationship between a Jomon individual and other East Asians suggested that the ancestral population of the Jomon people is one of the earliest-wave migrants who might have taken a coastal route on the way from Southeast Asia toward East Asia[11]. It was also revealed that the Jomon people were genetically closely related to the Ainu/Ryukyuan, and that 10–20% of genomic components found in mainland Japanese are derived from the Jomon people[8,10]. Altogether, though some

55 previous studies on ancient Jomon genomes have referred to the population
56 history of Japanese archipelago, these studies were based on a single Jomon
57 individual. To understand the population-level characteristics, genetic
58 information for multiple individuals is essentially required. The fact that the
59 genomes of modern Japanese have inherited Jomon-derived genomic
60 components means that the genomes of the modern Japanese contains
61 equivalent genetic information of multiple individuals of the Jomon people. With
62 such information, we can clarify, for example, how they admixed in the Japanese
63 Archipelago. From regional differences in the Jomon-derived genomic
64 components of modern Japanese, we can infer the admixture process between
65 the Jomon people and continental East Asians in Japanese archipelago. It will
66 also provide more reliable results on the migration route of the ancestral Jomon
67 population to the Japanese archipelago, which has been discussed based on
68 the genome of a single Jomon individual[10,11]. Moreover, while the previous study
69 have referred to the phenotype of a single Jomon individual[10], genomic
70 information of modern Japanese will reveal the phenotypic characteristics of the
71 Jomon people as a population (i.e. phenotype frequency). In order to achieve
72 the above objectives, the present study attempted to detect Jomon-derived
73 genomic components of modern Japanese.
74
75 In populations derived from a mixture of two source populations,
76 recombination between haplotypes from different source populations inevitably
77 occurs after the admixture event. As a result, haplotypes from two ancestral
78 populations are patchily present in the chromosomes of admixed population,

79 and the alleles in the haplotypes from each ancestral population are in linkage
80 disequilibrium (LD) with each other (Supplementary Fig. 1). Most existing
81 methods[12–16] for estimating the local ancestry of genomes using the LD state
82 require genome information of ancestral populations or of modern populations
83 as proxies of ancestral populations. In the Japanese population, only a few
84 Jomon individuals[8–11] have been sequenced so far. Furthermore, the sequence
85 depths of these samples are low, except for the Funadomari Jomon[10] people
86 excavated from Rebun Island in Hokkaido, making it difficult to obtain sufficient
87 genome information to accurately estimate the local ancestry of modern
88 Japanese (i.e., the genomes of many Jomon individuals are required to perform
89 highly accurate studies). Therefore, at present, it is not possible to estimate the
90 local ancestry of modern mainland Japanese using previous methods. In this
91 study, we developed a method using a summary statistic, the Ancestry-Marker
92 Index (*AMI*), to detect ancestry-marker SNPs derived from the Jomon people in
93 modern mainland Japanese that does not require genomes obtained from
94 Jomon skeletal specimens. The *AMI* was developed with inspiration from S*,
95 detecting archaic-hominin-derived haplotypes using the specific SNPs in the
96 out-of-Africa population which assumed to be originate from admixture of archaic
97 hominin and early Eurasians[17–20]. Since the Jomon people have been found to
98 be highly differentiated from other East Asians[8,10], they are expected to have
99 had specific variants that were not found in other East Asians. Thus, the modern
100 mainland Japanese also likely have specific variants derived from the Jomon
101 people. The *AMI* detects the Jomon-derived variants based on the LD between
102 Japanese specific variants. Based on the *AMI*, we could successfully extract the

Jomon-derived variants from real genomic data of the Japanese. Using Jomon-derived variants, we conducted comprehensive analysis to elucidate the population history of the Japanese archipelago population; for example, regional differences, the migration route to Japanese archipelago and phenotypes of the Jomon people. Based on these results, we propose a model of admixture between the Jomon people and immigrants from continental East Asia in the Japanese Archipelago.

Results

Performance of *AMI*

To confirm the usefulness of the *AMI*, we performed a coalescent simulation assuming a mixture of the Jomon people and continental East Asians (Supplementary Fig. 2). The preliminary 10 Mb simulation suggested that modern Japanese still have haplotypes of several megabases that are derived from the Jomon people (see Supplementary Fig. 3 and Supplementary Note 1).

There are three types of Japanese specific variants: (type 1) Jomon-derived variants, which appeared in the Jomon lineage before the admixture event; (type 2) variants derived from continental East Asians, which appeared in the continental East Asian populations and were moved into the Japanese lineages through the admixture, but were eventually lost in the East Asian population; and (type 3) novel variants that appeared only in Japanese lineages after the admixture (Supplementary Fig. 4). Our aim is to distinguish Jomon-derived variants (type 1) from (type 2) and (type 3). Of these Japanese specific variants, the Jomon-derived variants (type 1) are considered to be accumulated on the same haplotype or to be in strong LD with each other (Supplementary Fig. 4 (b)). In the subsequent 1 Mb simulation, Japanese specific variants (types 1, 2, and 3) were extracted from each genealogy. The distributions of *AMI* for Jomon-derived variants (type 1) and other variant types (types 2 and 3) are shown in Fig. 2 (a). *AMI* is determined by first calculating the linkage

136 disequilibrium coefficient r^2 between Japanese specific variant pairs, and then
137 counting the number of variants with $r^2 > 0.01$ for a focal Japanese specific
138 variant. It was found that Jomon-derived variants (type 1) had larger *AMI* values
139 than the other Japanese specific variants (types 2 and 3). Fig. 2 (a) indicates
140 that although variant pairs with linkage disequilibrium coefficient $r^2 > 0.01$ can
141 occur by chance, the number of variant pairs with $r^2 > 0.01$ is much larger in
142 Jomon-derived variants (type 1) than in other Japanese specific variants (type 2
143 and 3). The receiver operating characteristic (ROC) analysis showed that
144 Jomon-derived variants (type 1) could be distinguished from the other Japanese
145 specific variants (types 2 and 3) by the *AMI* (area under the curve [AUC] = 0.91;
146 Fig. 2 (b). AUC takes a value from 0 to 1, and the closer the value is to 1, the
147 better to distinguish positive from negative.). The Youden index, a measure of
148 the cutoff value, was 28.0374. We performed further simulations, varying the
149 split time between the Jomon people and continental East Asians or the effective
150 population size in simulation, to confirm robustness of *AMI* to different population
151 history. Though the value of the Youden index varied depending on the
152 population history assumed, the Jomon-derived variants could be accurately
153 detected (Supplementary Fig. 5). According to Fig. 2, we set the threshold for
154 detecting Jomon-derived variants at *AMI* > 28.0374.
155
156 We also attempted to detect the Jomon-derived genomic components by
157 S*[17,18], a reference-free method previously proposed, and found that S* was
158 unable to detect the Jomon-derived genomic components, perhaps due to an

insufficient number of Jomon-derived specific variants of mainland Japanese (Supplementary Fig. 6 and Supplementary Note 2).

Detection of Jomon variants in real data

Using the data set of 87 KPGP Koreans[21] and 26 global populations of 1KG[22], approximately 1.7 million SNPs were found to be specific to mainland Japanese (1KG JPT). Of these 1.7 million SNPs, 208,648 SNPs exceeding the threshold of *AMI* were regarded as Jomon-derived SNPs. Jomon-derived SNPs were distributed throughout the autosomal genome (Supplementary Fig. 7). Hereafter, at the Jomon-derived SNPs, an allele only found in the mainland Japanese population is called a “Jomon allele.”

To examine the detection accuracy of Jomon-derived SNPs, we calculated the *JAS* for the Ikawazu[9,11]/Funadomari[10] Jomon individuals and mainland Japanese. If Jomon-derived SNPs were properly detected by the *AMI*, the *JAS* of the Ikawazu or Funadomari Jomon were expected to be higher than those of mainland Japanese. Of the JPT mainland Japanese, NA18976 was genetically close to continental East Asians in PCA (Supplementary Fig. 8) and was expected to have a lower *JAS*. The distribution of the *JAS* is shown in Supplementary Fig. 9. The mean *JAS* of 103 mainland Japanese individuals, excluding NA18976, was 0.0164. As expected, NA18976 had the lowest *JAS*, 0.00269, which was much lower than that of the other mainland Japanese. The *JAS* in the Ikawazu/Funadomari Jomon were 0.0523 and 0.0555, respectively,

indicating that the Jomon alleles were found more frequently in Jomon people than in the modern mainland Japanese. These results suggest that the *AMI* could detect SNPs derived from the Jomon people. It should also be noted that the *JAS* values were only a few % for both Jomon individuals, which suggests that the number of Jomon-specific variants obtained from *AMI* analyses of modern Japanese were several tens of times greater than that obtained from the whole genome sequence of a single Jomon individual.

Detection of regional genetic differences in mainland Japanese by Jomon-derived SNPs

JAS by region and prefecture

Previous prefecture-scale population studies showed that the Tohoku, Kanto, and Kyushu populations (Fig. 1) are genetically more closely related to the Ryukyuans, while the Kinki and Shikoku populations are more closely related to continental East Asians[23,24]. Based on these facts, we hypothesized that the genetic regional differences among the modern mainland Japanese are caused by regional geographical differences in the admixture proportion of the Jomon and immigrants from continental East Asia. To verify this, we calculated the average *JAS* for each geographic region and prefecture from imputed genotypes of 3,917 Jomon-derived SNPs of 10,842 Japanese individuals previously used for regional population genetic analysis[24]. We removed the Hokkaido samples, which were largely affected by the immigration of Japanese after the Meiji period, for subsequent analysis and a total of 10,412 samples were used. The samples

207 of each prefecture except for Hokkaido were divided into ten regions: Tohoku,
208 Kanto, Hokuriku, Chubu, Tokai, Kinki, Chugoku, Shikoku, Kyushu, and Okinawa
209 in accordance with a previous study[25] (Fig. 1 and Supplementary Table 1). The
210 *JAS*s in these ten geographical regions are presented in Fig. 3 (a) and
211 Supplementary Table 2. We found that the *JAS* was the highest in Okinawa
212 (0.0255), followed by Tohoku (0.0189) and Kanto (0.018), and the lowest in Kinki
213 (0.0163), followed by Shikoku (0.016). In prefecture scale, the average *JAS* in
214 mainland Japan tended to be higher in prefectures located in the northernmost
215 and southernmost parts of mainland Japan (Fig. 3 (b) and Supplementary Table
216 3). The *JAS* was especially high in Aomori (0.0192), Iwate (0.0195), Fukushima
217 (0.0187), and Akita (0.0186) prefectures of the Tohoku region, as well as
218 Kagoshima Prefecture (0.0186) in Kyushu. Japanese individuals in these
219 prefectures are considered to possess more Jomon-derived genomic
220 components than those in other prefectures. Prefectures with lower *JAS*s were
221 in the Kinki and Shikoku regions, including Wakayama (0.0157), Nara (0.0156),
222 Kochi (0.016), Tokushima (0.0161), and Mie (0.0161). These populations are
223 considered to have more genomic components derived from continental East
224 Asians. The *JAS* of each prefecture and the principal component 1 (PC1) value,
225 which was obtained from the principal component analysis (PCA) of a previous
226 study by the allele frequency of autosomal 183,708 SNPs in each prefecture[24]
227 are plotted in Fig. 3 (c). The *JAS* was strongly correlated with PC1 (R = 0.91,
228 two-sided t-test $P = 2.2 \times 10^{-16}$). The geographic distribution was not changed by
229 tighter cutoff values (AMI > 100) for the detection of Jomon-derived SNPs by
230 *AMI* (Supplementary Fig. 10 and Supplementary Note 3).

231

232 To confirm the results from *JAS*, *f3* statistic[26] was calculated for a single
233 Jomon individual[10,11] (Supplementary Fig. 11 and Supplementary Table 4). The
234 distribution of *f3*(each prefecture; Jomon individual, CHB) (Supplementary Fig.
235 11) was similar to the distribution of the *JAS*s shown in Fig. 3(b). The *f3* was
236 generally small in prefectures with high *JAS*s, such as Kagoshima Prefecture in
237 Kyushu and Aomori and Iwate Prefectures in Tohoku, and was large in the
238 prefectures of regions with low *JAS*s, such as Kinki and Shikoku. Based on
239 these two findings: a correlation between the *JAS* and the PC1 of Fig. 3 (c) and
240 the concordance between the geographical distribution of *f3* and *JAS*, it is
241 strongly suggested that the genetic regional differences of modern Japanese
242 can be explained mainly by regional geographical differences in the admixture
243 proportions of the Jomon people. It should be emphasized that the admixture,
244 although to varying degrees, widely occurred throughout the Japanese
245 archipelago. Notably, prefectures in the Tohoku region showed higher *JAS*s
246 than those in the Kyushu region. However, *f3* values were lower in Kyushu than
247 in Tohoku. Since the Jomon-derived SNPs were detected in 1KG JPT
248 (Japanese living in Tokyo), the specific variants possessed by the Jomon people
249 of the Kyushu region may not have been detected, and thus the *JAS* in Kyushu
250 may have been underestimated. In other words, these results could reflect
251 differences in the genetic background of Jomon people in Tohoku and Kyushu.
252 Overall, the geographical gradient of *f3* in mainland Japan was more consistent
253 with the *JAS* than the distances from the locations (Funadomari and Ikawazu)
254 where Jomon samples were taken.

255

256 We assumed that the regional differences in the *JAS* were related to
257 regional differences in population size during the Jomon period. Therefore, we
258 examined the correlation between *JAS* and three indexes related to the Jomon
259 population size. The *JAS* of each prefecture was significantly correlated with the
260 number of archeological sites from the Jomon period (R = 0.69, two-sided t-test
261 $P = 1.27\times10^{-7}$; Fig. 4 (a)). The *JAS* of each region also correlated with the
262 population size estimated from the number of archeological sites in the Late
263 Jomon period (R = 0.7, two-sided t-test $P = 3.6\times10^{-2}$; Fig. 4 (b)). Moreover, the
264 *JAS* of each prefecture was strongly correlated with $\log_{10}$(number of
265 archeological sites in the Yayoi period/number of archeological sites in the Late
266 Jomon period) (R = -0.64, two-sided t-test $P = 2.08\times10^{-6}$; Fig. 4 (c)). It is
267 considered that Figs. 4 (a) and (b) correspond to Jomon population size in the
268 Jomon period and Fig. 4 (c) corresponds to the population growth rate occurring
269 from the Late Jomon period to the Yayoi period. The correlation between *JAS*
270 and population size in each region suggests that the smaller the population size
271 in the Jomon period, the lower *JAS* in modern mainland Japan (i.e., the higher
272 contribution of genomic components of immigrants from continental East Asia).

273

274 To summarize the above results, we can conclude that genetic differences
275 among the regions of the modern Japanese population were mainly caused by
276 differences in the admixture proportion of the Jomon people and that differences
277 in the admixture proportion were caused by differences in the population sizes in
278 each region during the Final Jomon period. Regarding these, previous

morphological analyses showed that, of several populations in Japan, the Hokkaido Ainu and contemporary Kinki populations had contrasting cranial morphologies, while other modern regional populations were intermediate, with the Tohoku population being relatively similar in morphology to the Hokkaido Ainu[1,27]. Archeological evidence suggests that immigrants from continental East Asia first reached northern Kyushu[2], which seems contradictory considering that the *JAS* was lower in the Kinki and Shikoku regions than in northern Kyushu. The reason for this could be that the Kinki and Shikoku regions had a smaller population size during the Final Jomon period (Fig. 4), and thus, the proportion of genomic components derived from the immigrants became larger than in the other regions. In this study, we could clearly evaluate the similarity of local populations to the Jomon people using the Jomon-derived SNPs, and could clarify the main cause of genetic differences among the regional populations in mainland Japan.

PCA of prefectures according to Jomon allele frequency

A PCA was conducted for 46 Japanese prefectures using Jomon allele frequencies (Supplementary Fig. 12 (a)). This PCA demonstrated that Okinawa Prefecture was separated from the other prefectures by PC1. Next, 45 prefectures in mainland Japan (Okinawa was excluded from further analyses based on Supplementary Fig. 12 (a)) were analyzed in PCA (Supplementary Fig. 12 (b)). The PC1 showed that prefectures in the Tohoku and Kanto regions, where higher *JAS*s were observed among Japanese prefectures, were greatly

differentiated from prefectures in Kinki and Shikoku, where lower *JAS*s were observed (Fig. 3). The *JAS* was strongly correlated with the PC1 of Supplementary Fig. 12 (b) (R = -0.94, $P < 2.2\times10^{-16}$; Supplementary Fig. 13), which shows that PC1 reflects the ancestry proportion of Jomon people in each prefecture. In contrast, the PC2 of Supplementary Fig. 12 (b) was strongly correlated with both the latitude and longitude of each prefecture (latitude: R = 0.78, two-sided t-test $P = 2.30\times10^{-10}$, longitude: R = 0.66, two-sided t-test $P = 9.31\times10^{-7}$; Supplementary Fig. 14 (a) and (b)). These results indicate that the PC2 is determined by the geographical location of each prefecture, which might reflect that the genetic background of the Jomon people may differ according to the geographical locations in the Japanese archipelago. Previous studies have shown regional differences in the skeletal morphology of the Jomon people in the Japanese archipelago[28–32]. For example, Kondo et al. suggested that Jomon craniofacial morphology, especially in the neurocranium, exhibit a northeast-to-southwest geographical cline across the Japanese archipelago[32]. To the best of our knowledge, including studies with ancient Jomon genomes, this is the first genome-wide study to refer to the genetic regional differences among the Jomon people in the Japanese archipelago.

Genetic relationships between the Jomon people and other East Asians

To clarify the genetic relationship between the Jomon people and other East Asian populations, we estimated allele frequencies of genome-wide SNPs in the Jomon people based on the haplotype structures composed of Jomon-derived

327 SNPs in the modern Japanese (Supplementary Fig. 15, Methods in detail). The
328 *f3*(Onge; Estimated Jomon frequencies, X) was calculated using Onge as the
329 outgroup of East Asians in line with a previous study[11]. The *f*3 values for various
330 East Asians used as X are shown in Fig. 5. The maximum value of *f3* was
331 obtained when the Ikawazu Jomon was used as X (f3=0.0656), suggesting that
332 our estimation reflects the allele frequencies of the Jomon people. In a previous
333 study, a particularly strong genetic affinity was found between the Ikawazu
334 Jomon individual and Taiwan aborigines, suggesting that the ancestral
335 population of the Jomon people migrated through the coastal areas of East Asia
336 (=coastal route). However, in this study, the *f3* values for Taiwan aborigines (Ami
337 and Atayal) were lower than Tujia and Miao. In other words, our study failed to
338 replicate a particularly strong genetic affinity between the Taiwan aborigines and
339 the Jomon people. For comparison, we calculated *f3*(Onge; Ikawazu Jomon or
340 Funadomari Jomon, X) using the Ikawazu or Funadomari Jomon individual
341 whose genome had been sequenced in previous studies (Supplementary Fig
342 16). In the case of Ikawazu Jomon, a strong genetic affinity was observed with
343 Ami and Atayal, while in the Funadomari Jomon, the affinity was similar to or
344 lower than that of Dai, Tujia and Miao. These results do not strongly support the
345 coastal route obtained in the previous study of the single ikawazu Jomon
346 genome, which seems not precisely reflect the population history of the Jomon
347 people due to a sampling bias. Although higher *f3* values were observed for Ami
348 and Atayal among East Asians in the previous study of the Funadomari Jomon
349 individual[10], the previous *f3* analysis differs from the present study in that Mbuti
350 (an African population) was used as the outgroup. The results of *f3* analysis

would vary depending on the outgroup setting. However, it is common between our estimated allele frequency of the Jomon people, Ikawazu and Funadomari that *f3* was lower when Southeast Asian populations (Burmese, Thai, and Cambodian) and Tibetan populations (Tibetan, Sherpa, and Chokhopani (an iron age individual (3.0–2.4 kya)) were used as X (Fig. 5 and Supplementary Fig. 16). The previous study showed that the ancestral population of the Jomon people migrated to East Eurasia through the southern side of the Himalayas (=southern route)[11]. A previous study of modern and ancient East Asians including the seven Jomon individuals suggest that the Jomon people have two ancestry components from ancient East Asians, i.e. the Interior South ancestry and the Coastal ancestry[33]. Considering the results of this study and previous studies, at least the ancestral population of Jomon people seem to have migrated to the Japanese archipelago from the south of East Eurasia (southern parts of the Himalayas) via the southern of China, although it cannot be concluded whether they passed through coastal or inland areas.

Haplotype structures composed of Jomon-derived SNPs in genes associated with characteristic phenotypes of East Asians

To estimate the phenotype frequencies in the Jomon people, we investigated the haplotype structures of four genes (*ABCC11*, *EDAR*, *ALDH2*, and *ADH1B*), each having a nonsynonymous SNP associated with characteristic phenotypes of East Asians[34–39]. The derived alleles of these four nonsynonymous SNPs are associated with the following phenotypes: *ABCC11*

375 rs17822931: dry ear wax[34], *EDAR* rs3827760: thicker hair[35] and shovel-shaped
376 incisors[36], and *ALDH2* rs671 and *ADH1B* rs1229984: lower alcohol tolerance[37–
377 39]. Haplotype structures composed of Jomon-derived SNPs are shown in Fig. 6.
378 The haplotypes in each region could be classified into four types according to
379 the presence or absence of the derived allele associated with the phenotype,
380 and the composition of Jomon-derived alleles. The frequency of each haplotype
381 in the Japanese population is presented in Table 1. Here, the haplotypes
382 containing the Jomon-derived SNPs are called "Jomon-derived haplotypes." For
383 *ABCC11* and *EDAR*, Jomon-derived haplotypes were observed for both
384 ancestral and derived alleles of the phenotype-associated SNPs. In *ABCC11*
385 (Fig. 6 (a)), the frequencies of the Jomon-derived haplotypes in mainland
386 Japanese were 10.6% for the ancestral allele (wet ear wax) and 24% for the
387 derived allele (dry ear wax). In *EDAR* (Fig. 6(b)), the Jomon-derived haplotype
388 frequencies were 14.9% for the ancestral allele (thinner hair and
389 non-shovel-shaped incisors) and 17.3% for the derived allele (thicker hair and
390 shovel-shaped incisors) in mainland Japanese. The haplotypes containing
391 ancestral and derived alleles of the phenotype-associated SNPs had different
392 Jomon alleles. Thus, it is unlikely that these haplotypes were generated by
393 recombination in the Japanese population after the admixture between the
394 Jomon and immigrants from continental East Asia. The present results suggest
395 that modern Japanese have derived alleles from both the Jomon people and
396 immigrants from continental East Asia in *EDAR* and *ABCC11*. Previous studies
397 examining ancient DNA of the Hokkaido Jomon population obtained from
398 archeological sites showed that the derived allele (dry ear wax) of *ABCC11* was

399 present in the Hokkaido Jomon population at a frequency of 47.6%[40,41]. As for
400 *EDAR*, although the frequency of the derived allele (shovel-shaped incisors and
401 thicker hair) in the Jomon people has not been estimated, it has been shown that
402 shovel-shaped incisors were found at a frequency of 68.9% in the Jomon
403 people[42]. The results of these previous studies are consistent with our results. In
404 *ALDH2* rs671 (Fig. 6 (c)), the Jomon-derived haplotypes containing the derived
405 allele of phenotype-associated SNPs were found to be rare (2.4%) in modern
406 Japanese, and the number of Jomon alleles per Jomon-derived haplotype
407 containing the derived allele was very small. This suggests that the Jomon
408 people had few derived alleles (lower alcohol tolerance) of rs671, and most of
409 the derived alleles found in modern Japanese originated from continental East
410 Asians. In *ADH1B* rs1229984 (Fig. 6 (d)), the total frequency of the
411 Jomon-derived haplotypes was relatively lower than that of the other three
412 genes. The frequencies of the Jomon-derived haplotype were 3.8% for the
413 ancestral allele (higher alcohol tolerance) and 4.8% for the derived allele (lower
414 alcohol tolerance). In addition, when we calculated the number of
415 Jomon-derived SNPs per 1 Mb at the genome-wide scale (Supplementary Fig.
416 17), we found that the number of Jomon-derived SNPs was especially small in
417 the region around *ADH1B* (red dashed line). Therefore, regarding *ADH1B*, it is
418 possible that the Jomon people possessed the derived allele of rs1229984 at a
419 higher frequency compared to that of *ALDH2,* but both the Jomon-derived
420 haplotypes with ancestral and derived alleles may have been lost after the
421 admixture in Japanese. Koganebuchi et al., [43] previously estimated that most of
422 the derived alleles in *ALDH2* originated from immigrants from continental East

423 Asia, which agrees with our results, while they concluded that the genetic
424 contribution of immigrants was small for *ADH1B*, which contradicts the results of
425 this study. Their study[43] assumed that, among mainland Japanese, the
426 population in northern Kyushu had a relatively large genetic contribution from
427 immigrants, but this assumption is inconsistent with the *JAS* estimated in the
428 present study (Fig. 3 (b)). Thus, it is more plausible that *ADH1B* haplotypes of
429 mainland Japanese were introduced mainly by immigrants from continental East
430 Asia, regardless of the allelic status (ancestral or derived) of rs1229984.

Discussion

In this study, we developed the *AMI* as a summary statistic to detect the Jomon-derived variants in modern Japanese without requiring any genomic sequences from the former. The computer simulation showed that *AMI* can detect ancestral variants with high accuracy, even in an admixed population whose source populations diverged tens of thousands of years ago. Since we were able to detect Jomon-derived SNPs by the *AMI* even changing the population history in the simulations, the present approach using the *AMI* is likely to be applied to other admixed populations which source population whose source populations diverged relatively recently. Potential applications include the population of Madagascar[44,45] and the current Polynesian population[46,47], which were formed around hundreds to thousands years ago by population admixture. As exemplified by these cases, the genetic diversity of modern humans has been greatly influenced by population admixture events[48,49]. The *AMI* will be a powerful tool for clarifying the population history of not only the Japanese but also other admixed populations. It should be noted that the threshold of the *AMI* was determined by the Youden index calculated based on coalescent simulations in this study, but one may set the threshold according to one's own research purpose; if one wants to reduce false positives (i.e., variants derived from ancestral admixture), one can set the threshold strictly; if one wants to reduce false negatives, one can just set the threshold loosely. Practically, the *AMI* threshold does not necessarily have to be set based on simulations that assume a population history. In this study, our main aim is to extract

455 Jomon-derived variants from the whole-genome to determine the prevalence of
456 each prefecture, so the threshold was set to pick up as many Jomon-derived
457 SNPs as possible in order to grasp the trend of the entire genome in each
458 Japanese prefectural population.
459
460 As for the process of population formation in the Japanese archipelago from
461 the Late Jomon period to the present, we propose a model, which is shown in
462 Fig. 7. From the Late to Final Jomon period, the Jomon people settled down in
463 mainland Japan, and the population size or the population density of the Jomon
464 people varied among regions. According to Koyama 1979[25], based on the
465 number of archeological sites, it was estimated that the population sizes in the
466 Tohoku and Kanto regions were relatively large at 43,800 and 52,100,
467 respectively, while those in the Kinki and Shikoku regions were relatively small at
468 4,400 and 2,700, respectively. Thus, in the Kinki and Shikoku regions, modern
469 Japanese have lower degrees of genomic components derived from the Jomon
470 people. In the Final Jomon period, continental East Asians arrived in northern
471 Kyushu and started to admix with the Jomon people in all regions of mainland
472 Japan. During the Yayoi period, the population size of immigrants was relatively
473 increased in the Kinki and Shikoku regions, where the populations were small at
474 the end of the Jomon period. Further analyses of ancient human DNA from the
475 Final Jomon period to the Yayoi period will allow the verification of the Japanese
476 population history model proposed in this study.
477

478 We proposed a method to estimate allele frequencies in ancestral populations
479 by classifying haplotypes of the current population according to their origin using
480 SNPs derived from the ancestral population as markers. Even for admixed
481 populations for which ancient DNA analysis cannot be performed, the same
482 approach as in this study will help to infer the population history reflecting the
483 genetic information of a large part of individuals (namely, allele frequency) of the
484 ancestral population and to clarify their phenotype frequencies from the
485 estimated genotype frequencies. For example, by calculating the allele
486 frequencies of ancestral population of modern Ryukyuans, which seemed to
487 have the same roots as the Jomon people in mainland Japanese, and/or of the
488 Jomon people in each region in the mainland Japanese as this study, we can
489 clarify genetic regional differences among the ancient Jomon populations.
490 Ryukyu islands have an extremely hot and humid environment, and the amount
491 of DNA from human remains is small, making ancient DNA analysis difficult[50].
492 This method allows us to obtain genetic information equivalent to multiple
493 ancient individuals in such hot and humid regions. The use of modern human
494 populations from various regions of the Japanese archipelago will further clarify
495 the migration history of the Jomon people in future studies.

Methods

Coalescent simulation by Msprime

To investigate the characteristics of the Jomon-derived autosomal genomic components of mainland Japanese, we conducted a coalescent simulation assuming the admixture of the Jomon and continental East Asians using msprime[51] (Supplementary Fig. 2). A remarkable feature of the msprime program is that it specifies the time and population where the mutation and coalescence events occurred. Our simulation code was made with reference to a previous study[52]. The split between the Jomon ancestors and continental East Asians was set to 1,200 generations ago (30,000 YBP), according to the divergence time estimated in Kanzawa-Kiriyama et al.,[10] (between 18,000 YBP and 38,000 YBP) and the beginning of the Jomon period (around 16,000 YBP)[2]. Migration from continental East Asia to mainland Japan was set between 120 and 80 generations ago, with reference to the beginning of the Yayoi period, around 2,800 years ago[4]. The total admixture proportion of the Jomon people in the modern mainland Japanese was set to 12%[8]. The effective population size was set to 5,000 for both populations. The mutation rate and recombination rate were set to 1.2×10^{-8} per bp per generation and 1.3×10^{-8} per bp per generation, respectively[53–56].

This study aimed to detect Jomon-derived variants based on LD among Japanese specific variants. There are three types of Japanese specific variants:

520 (type 1) Jomon-derived variants; (type 2) variants derived from continental East
521 Asians; and (type 3) novel variants (Supplementary Fig. 4 (a) and (b)). It should
522 be noted that Japanese specific variants generated earlier than the split time of
523 the Jomon people and the continental East Asians were classified as
524 Jomon-derived variants (type 1). We compared the LD status of three types of
525 Japanese specific variants in the coalescent simulations. The origin of each
526 haplotype of mainland Japanese can be estimated from coalescent time to the
527 haplotypes of the Jomon or continental East Asians. That is, if a haplotype of a
528 mainland Japanese sample coalesced with haplotypes of Jomon samples earlier
529 than the admixture of the Jomon people and continental East Asians, the
530 haplotype is inferred to be derived from Jomon. To extract the three types of
531 Japanese specific variants (i.e., variants not found in samples from continental
532 East Asians), 3,000 replicates of 1 Mb simulations were performed. We sampled
533 200 haplotypes from each of the four populations (modern mainland Japanese,
534 modern continental East Asians, Jomon people 120 generations ago, and
535 continental East Asians 120 generations ago) to detect variants observed in
536 modern mainland Japanese but not seen in continental East Asians. Each
537 Japanese specific variant was classified into (type 1) the Jomon-derived variant,
538 (type 2) the continental East Asian-derived variant, and (type 3) the novel variant
539 based on when and in which lineage the mutation occurred (Supplementary Fig.
540 4 (a)). To calculate the ancestry marker index (*AMI*), we first calculate the
541 linkage disequilibrium coefficient r^2 between Japanese specific variant pairs
542 within each 1Mb bin. For each type of the Japanese specific variant, the *AMI*
543 was calculated as:

$$AMI = \frac{\{Number\ of\ variants\ with\ linkage\ disequilibrium\ coefficients\ (r^2)\ > 0.01\}}{(Number\ of\ Japanese\ specific\ variants\ per\ KB)}$$

Jomon-derived variants are expected to have higher *AMI* values. The performance of the *AMI* was verified by receiver operating characteristic (ROC) analysis using the ROCR package in R. The threshold to detect Jomon-derived variants was determined based on the Youden Index.

Detection and verification of Jomon-derived SNPs on autosomes using real data

Detection of Jomon-derived variants in real data

Jomon-derived SNPs were inferred from the whole genome sequence data from 26 populations from different parts of the world, including mainland Japanese (JPT) and four continental East Asian populations (CHB, CHS, CDX, and KHV), obtained from the 1000 Genomes Project Phase III (1KG)[22], and 87 individuals from the Korean Personal Genome Project[21]. In this study, only biallelic SNPs were used. Prior to extracting the Jomon-derived SNPs, we performed a principal component analysis (PCA) in PLINK (version 1.9)[57] using 1KG mainland Japanese (JPT) and Han Chinese (CHB) data. During this analysis, we found that one JPT individual (NA18976) was close to the continental East Asians (Supplementary Fig. 8), so NA18976 was excluded from subsequent analyses. First, 1,784,634 SNPs specific to 1KG JPT were detected using VCFtools v0.1.13[58]. Next, LD coefficients (r^2) were calculated between the Japanese specific SNPs located within 1 Mb from each other with the --hap-r2

option of VCFtools in combination with the --ld-window-bp option. The number of SNPs with $r^2 > 0.01$ was counted for each Japanese specific SNP. The density of Japanese specific variants per 1 kb of each chromosome was calculated using the --SNPdensity option of VCFtools, and the *AMI* was calculated for each Japanese specific SNP. To eliminate the possibility of sequence errors, regions with a density of Japanese specific variants per kb below a mean of - 1sd of each chromosome were excluded from the analysis. In this analysis, we assumed that the number of Japanese specific variants per kb, which is the denominator of the *AMI*, is constant for each chromosome (i.e., the numerator of the *AMI* was normalized for each chromosome). Based on the threshold set by the ROC analysis of simulated Japanese specific variants, we inferred variants originating from the Jomon people.

Verification of Jomon-derived SNPs based on whole-genome sequence data from Jomon remains

For the verification of Jomon-derived SNPs based on the whole genome sequence data, the "Jomon allele score" (*JAS*) was calculated for the Ikawazu[9,11] and Funadomari[10] Jomon, as well as for 104 individuals from the 1KG JPT. The *JAS* was calculated using the following formula:

$$JAS = \frac{(Jomon\ derived\ allele\ count)}{2*(\text{Number of total Jomon}-\text{derived SNPs})}.$$

The BAM file of the Ikawazu Jomon was provided by Hiroki Ota of Tokyo University, Tokyo, Japan, and Takashi Gakuhari of Kanazawa University, Ishikawa, Japan. The BAM file of the Funadomari Jomon was provided by Naruya Saito from the National Institute of Genetics, Shizuoka, Japan, and

Hideaki Kanzawa-Kiriyama from the National Museum of Nature and Science, Tokyo, Japan. The genotypes of Ikawazu Jomon and Funadomari Jomon samples were called by the UnifiedGenotyper tool in the GenomeAnalysisToolkit version 3.6[59]. For the Ikawazu Jomon, the --mbq 30 --ploidy 2 --output_mode EMIT_ALL_CONFIDENT_SITES options were specified. For the Funadomari Jomon, the options described in the original paper were specified. Jomon SNPs were subjected to LD pruning by the --indep-pairwise command of PLINK (--indep-pairwise 1000 200 0.8). In addition, only the Jomon-derived SNPs of depth ≥ 6 in the Ikawazu and Funadomari Jomon were used for the calculation of the *JAS*. As a result, 4,458 SNPs were used to calculate *JAS*.

Detection of regional genetic differences in mainland Japanese by Jomon-derived SNPs

Sample data

We used 183,708 SNPs from 10,842 individuals from the Japanese archipelago published by Watanabe et al.[24]. All the individuals investigated in this study were customers of the Japanese Direct to Consumer (DTC) genetic-testing service, HealthData Lab (Yahoo! Japan Corporation, Tokyo, Japan). They were provided an agreement, and informed consent was obtained for their data to be used for research. In this study, the Japanese archipelago was divided into eleven regions (Fig. 1 and Supplementary Table 1): Hokkaido (430 individuals), Tohoku (746 individuals), Kanto (3,990 individuals), Hokuriku (431 individuals), Chubu (410 individuals), Tokai (933 individuals), Kinki (1,861

individuals), Chugoku (600 individuals), Shikoku (314 individuals), Kyushu (1,016 individuals), and Okinawa (111 individuals). All statistical analyses were conducted at the Yahoo! Japan Corporation, with personal information of the customers completely hidden. We obtained approval from the Ethics Committee of the Yahoo! Japan Corporation.

Imputation of genotypes of Jomon-derived SNPs

Haplotype phasing and genotype imputation were performed using EAGLE2[60] and Minimac3[61], respectively, with whole genome sequence data of 413 mainland Japanese[62] phased by SHAPEIT2[63]. After the imputation, Jomon-derived SNPs with high imputation quality ($R^2 > 0.8$) were extracted. Also, LD pruning was performed with PLINK (--indep-pairwise 1000 200 0.1), and a total of 3,917 Jomon-derived SNPs were used for the analysis.

Geographical distribution of the Jomon allele score

In subsequent analyses, individuals from Hokkaido that were largely affected by immigration after the Meiji period were excluded. Using 3,917 Jomon-derived SNPs, we calculated the *JAS* for individuals of each prefecture and compared them between regions and prefectures.

***f3*-testing of prefectural populations in Japan**

The *f3*-test[26] was carried out in order to examine the relatedness between contemporary populations of each prefecture in Japan and the Funadomari Jomon or Ikawazu Jomon. Each Jomon sample and the 1KG CHB were set as

the source populations of admixture, and each prefecture of Japan was set as the target population, (described as *f3*(each prefecture; Jomon, CHB)). LD pruning was carried out on whole genome SNPs common in the Japanese, 1KG CHB, Funadomari Jomon, and Ikawazu Jomon by PLINK (--indep-pairwise 1000 200 0.1), with 17,492 SNPs being used for subsequent analyses. For the Funadomari and Ikawazu Jomon people, VCF files were converted to PED files using VCFtools and combined with the PED file of the Japanese. The PED files were converted to eigenstrat format with the Admixtools[26] convertf command, and then the *f3*-test was conducted with the Admixtools qp3Pop command.

Examination of correlations between population size during the Jomon period and *JAS* in each prefecture

We compared the population size estimated from the number of archeological sites in each prefecture, assuming that the population size per archeological site was constant in each prefecture during the same period. We examined the correlations between (a) the average *JAS* in each prefecture and the number of archeological sites from the Jomon period (obtained from the Statistical report of buried cultural properties, Agency of Cultural Affairs, Japan; https://www.bunka.go.jp/seisaku/bunkazai/shokai/pdf/h29_03_maizotokei.pdf), (b) the average *JAS* in each region and the population size estimated from the number of archeological sites in the Late Jomon period[25], and (c) the average *JAS* in each prefecture and the $\log_{10}$(number of archeological sites in the Yayoi period/number of archeological sites in the Late Jomon period)[25]. Finally, (a) and (c) were plotted for each prefecture, while (b) was plotted for each region

because data for each prefecture were not available. Correlation test was conducted by R cor.test function (df = 43).

PCA based on the Jomon allele frequencies of each prefecture

The Jomon allele frequency was calculated for 50 randomly sampled individuals from each prefecture using VCFtools version 0.1.13. A PCA was performed based on the Jomon allele frequency using R version 3.6.0. Correlation test between PC1 and JAS, and PC2 and longitude/latitude were conducted by R cor.test function (df = 43).

Estimation of the allele frequencies of genome-wide SNPs of the Jomon people based on the haplotype structure composed of the Jomon-derived SNPs

We calculated the allele frequencies of genome-wide SNPs in Jomon people based on the haplotype structure composed of the Jomon-derived SNPs (Supplementary Fig. 15). First, haplotypes surrounding a focal bi-allelic SNP with alleles 1 and 2 are classified into Jomon-derived haplotypes and Continental-East-Asian-derived haplotype, based on the presence or absence of Jomon alleles in the 500 kb upstream and downstream (1Mb in total) of the focal SNP. The frequency of the allele 1 of the focal SNP in Jomon people is expressed as follows.

$$Frequency\ of\ the\ allele\ 1\ in\ the\ Jomon\ people = \frac{Number\ of\ Jomon\ derived\ haplotypes\ containing\ the\ allele\ 1\ of\ the\ focal\ SNP}{Total\ number\ of\ Jomon\ derived\ haplotypes\ surrounding\ the\ focal\ SNP}$$

In the example of Supplementary Fig. 15, the frequency of the allele 1 in the Jomon people is 1/3. We calculated the allele frequency in the Jomon people

685 based on the phased-genotypes of 104 modern Japanese in 1KG for 5,316,769
686 SNPs in the whole genome. The --IMPUTE option of VCFtools was used to
687 extract haplotypes composed of Jomon-derived SNPs in regions 500 kb
688 upstream and downstream (1 Mb in total) of focal SNPs. Based on the estimated
689 frequencies of genome-wide SNPs, we estimated the genetic relationship
690 between the Jomon people and other East Asians. The population genotype
691 data set (Panel 2240K) used in Gakuhari et al 2020[11] was kindly provided by
692 Takashi Gakuhari of Kanazawa University, Ishikawa, Japan, and 81 individuals
693 including the Ikawazu Jomon[9,11] of 38 East Asian populations were extracted.
694 Nivkh and Ulchi were excluded from this analysis because it has been
695 suggested that they were recently admixed with Jomon lineage population (likely
696 Ainu, who have a genetic background of the Jomon people)[10,11]. Among the
697 genome-wide SNPs for which we estimated the Jomon allele frequencies, we
698 used 20,053 SNPs with genotype information was available in all East Asian
699 individuals of 2240K. We then calculated *f3*(Onge ; Estimated Jomon
700 frequencies, X) for testing the genetic relationship between the Jomon people
701 and each test population X, using Onge as the outgroup of East Asians[11]. In
702 addition, we focused on the haplotype structures composed of Jomon-derived
703 SNPs in the regions surrounding SNPs associated with characteristic
704 phenotypes of East Asians (*ABCC11*: rs17822931[34], *EDAR*: rs3827760[35,36],
705 *ALDH2*: rs671[37,43,64–66], and *ADH1B*: rs1229984[38,67–70]), and estimated the
706 frequencies of these phenotypes in the Jomon people.

Statistics and reproducibility.

Coalescent simulations of this study using msprime can be reproduced by specifying seeds in our code described in supplementary note 4. Statistical analyses were done using publicly available packages, so reproducibility can be accomplished using parameters described in Methods sections.

Data and code availability

The individual genotypes of 10,842 Japanese analyzed in this study are not available to avoid personal identification. The list of Jomon-derived SNPs detected in this study, and the allele frequencies of Jomon-derived SNPs in each Japanese prefecture are available from the corresponding author upon request. Our custom code for msprime simulation was described in Supplementary Note 4.

References

1. Hanihara, K. Dual structure model for the population history of the Japanese. *Japan Rev.* **2**, 1–33 (1991).
2. Habu, J. *Ancient Jomon of Japan*. (Cambridge University Press, 2004).
3. Mizoguchi, K. *The archaeology of Japan : from the earliest rice farming villages to the rise of the state*. (Cambrige University Press, 2013).
4. Fujio, S. *History of Yayoi Period*. (Kodansha, 2015).
5. Jinam, T. *et al.* 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 (2012).
6. Jinam, T. A. *et al.* Unique characteristics of the Ainu population in Northern Japan. *J. Hum. Genet.* **60**, 565–571 (2015).
7. Nakagome, S. *et al.* Model-based verification of hypotheses on the origin of modern Japanese revisited by Bayesian inference based on genome-wide SNP data. *Mol. Biol. Evol.* **32**, 1533–1543 (2015).
8. Kanzawa-Kiriyama, H. *et al.* A partial nuclear genome of the Jomons who lived 3000 years ago in Fukushima, Japan. *J. Hum. Genet.* **62**, 213–221 (2017).
9. Mccoll, H. *et al.* The prehistoric peopling of Southeast Asia. *Science (80-.).* **361**, 88–92 (2018).

10. Kanzawa-Kiriyama, H. *et al.* Late Jomon male and female genome sequences from the Funadomari site in Hokkaido , Japan. *Anthropol. Sci.* **127**, 83–108 (2019).

11. Gakuhari, T. *et al.* Ancient Jomon genome sequence analysis sheds light on migration patterns of early East Asian. *Commun. Biol.* **3,** 437, (2020). https://doi.org/10.1038/s42003-020-01162-2

12. Price, A. L. *et al.* Sensitive detection of chromosomal segments of distinct ancestry in admixed populations. *PLoS Genet.* **5**, e1000519 (2009).

13. Baran, Y. *et al.* Fast and accurate inference of local ancestry in Latino populations. *Bioinformatics* **28**, 1359–1367 (2012).

14. Churchhouse, C. & Marchini, J. Multiway Admixture Deconvolution Using Phased or Unphased Ancestral Panels. *Genet. Epidemiol.* **37**, 1–12 (2013).

15. Guan, Y. Detecting structure of haplotypes and local ancestry. *Genetics* **196**, 625–642 (2014).

16. Hui, D. *et al.* LAIT: A local ancestry inference toolkit. *BMC Genet.* **18**, 1–5 (2017).

17. Plagnol, V. & Wall, J. D. Possible ancestral structure in human populations. *PLoS Genet.* **2**, e105 (2006).

18. Vernot, B. & Akey, J. M. Resurrecting Surviving Neandeltal Linages from Modern Human Genomes. *Science (80-.).* **343**, 1017–1021 (2014).

19. Vernot, B. *et al.* Excavating Neandertal and Denisovan DNA from the genomes of Melanesian individuals. *Science (80-.).* **352**, 235–239 (2016).

769 20. Jacobs, G. S. *et al.* Multiple Deeply Divergent Denisovan Ancestries in
770 Papuans. *Cell* **177**, 1010-1021.e1-e24 (2019).
771 21. Zhang, W. *et al.* Whole genome sequencing of 35 individuals provides
772 insights into the genetic architecture of Korean population. *BMC*
773 *Bioinformatics* **15**, 1–13 (2014).
774 22. Auton, A. *et al.* A global reference for human genetic variation. *Nature* **526**,
775 68–74 (2015).
776 23. Yamaguchi-Kabata, Y. *et al.* Japanese Population Structure, Based on
777 SNP Genotypes from 7003 Individuals Compared to Other Ethnic Groups:
778 Effects on Population-Based Association Studies. *Am. J. Hum. Genet.* **83**,
779 445–456 (2008).
780 24. Watanabe, Y., Isshiki, M. & Ohashi, J. Prefecture-level population
781 structure of the Japanese based on SNP genotypes of 11,069 individuals.
782 *J. Hum. Genet.* **In press**, (2020).
783 25. Koyama, S. Jomon Subsistence and Population. *Senri Ethnol. Stud.* **2**, 1–
784 65 (1979).
785 26. Patterson, N. *et al.* Ancient admixture in human history. *Genetics* **192**,
786 1065–1093 (2012).
787 27. Hanihara, K. Geographic variation of modern Japanese crania and its
788 relationship to the origin of Japanese. *Homo* **36**, 1–10 (1985).
789 28. Kaifu, Y. Regional variation in mandibular morphology of the Jomon
790 people. *Bull Natl Sci Mus* **21**, 37–50 (1995).

29. Maeda, T. Mandibular ramus morphology of the Jomon people from Hokkaido. Anthropological. *Anthropol. Sci. (Japanese Ser.* **110**, 27–40 (2002).

30. Takigawa, W. Inter-regional variation of metric traits of limb bones in Jomon and modern Japanese. Anthropological. *Anthropol. Sci. (Japanese Ser.* **114**, 101–129 (2006).

31. Fukase, H. *et al.* Geographic variation in body form of prehistoric Jomon males in the Japanese archipelago: Its ecogeographic implication. *Am. J. Phys. Anthropol.* **149**, 125–135 (2012).

32. Kondo, O., Fukase, H. & Fukumoto, T. Regional variations in the Jomon population revisited on craniofacial morphology. *Anthropol. Sci.* **125**, 85–100 (2017).

33. Wang, C.-C. *et al.* Genomic Insights into the Formation of Human Populations in East Asia. *Nature* (2021). doi:https://doi.org/10.1038/s41586-021-03336-2

34. Yoshiura, K. I. *et al.* A SNP in the ABCC11 gene is the determinant of human earwax type. *Nat. Genet.* **38**, 324–330 (2006).

35. Fujimoto, A. *et al.* A scan for genetic determinants of human hair morphology: EDAR is associated with Asian hair thickness. *Hum. Mol. Genet.* **17**, 835–843 (2008).

36. Kimura, R. *et al.* A Common Variation in EDAR Is a Genetic Determinant of Shovel-Shaped Incisors. *Am. J. Hum. Genet.* **85**, 528–535 (2009).

37. Harada, S., Agarwal, D. P. & Goedde, H. W. Aldehyde Dehydrogenase Deficiency As Cause of Facial Flushing Reaction To Alcohol in Japanese. *Lancet* **318**, 982 (1981).

38. Edenberg H.J. & Bosron W.F. *Alcohol dehydrogenases. In: Comprehensive Toxicology, Vol. 3: Biotransformation*. (Pergamon Elsevier Science, 1997).

39. Edenberg, H. J. & McClintick, J. N. Alcohol dehydrogenases, aldehyde dehydrogenases and alcohol use disorders: a critical review. *Alcohol Clin Exp Res* **42**, 2281–2297 (2018).

40. Sato, T. *et al.* Allele frequencies of the ABCC11 gene for earwax phenotypes among ancient populations of Hokkaido, Japan. *J. Hum. Genet.* **54**, 409–413 (2009).

41. Kazuta, H. *et al.* Genotype frequencies of the ABCC11 gene in 2000-3000-year-old human bones from the Epi-Jomon and Jomon sites in Hokkaido, Japan. *Anthropol. Sci.* **119**, 81–86 (2011).

42. Matsumura, H. A Microevolutional History of the Japanese People from a Dental Characteristics Perspective. *Anthropol. Sci.* **102**, 93–118 (1994).

43. Koganebuchi, K. *et al.* The allele frequency of ALDH2*Glu504Lys and ADH1B*Arg47His for the Ryukyu islanders and their history of expansion among East Asians. *Am. J. Hum. Biol.* **29**, 1–14 (2017).

44. Pierron, D. *et al.* Genomic landscape of human diversity across Madagascar. *Proc. Natl. Acad. Sci. U. S. A.* **114**, E6498–E6506 (2017).

835 45. Pierron, D. *et al.* Strong selection during the last millennium for African
836 ancestry in the admixed population of Madagascar. *Nat. Commun.* **9**, 1–9
837 (2018).
838 46. Skoglund, P. *et al.* Genomic insights into the peopling of the Southwest
839 Pacific. *Nature* **538**, 510–513 (2016).
840 47. Pugach, I. *et al.* The gateway from near into remote oceania: New insights
841 from genome-wide data. *Mol. Biol. Evol.* **35**, 871–886 (2018).
842 48. Lazaridis, I. *et al.* Ancient human genomes suggest three ancestral
843 populations for present-day Europeans. *Nature* **513**, 409–413 (2014).
844 49. Nielsen, R. *et al.* Tracing the peopling of the world through genomics.
845 *Nature* **541**, 302–310 (2017).
846 50. Hofreiter, M. *et al.* The future of ancient DNA: Technical advances and
847 conceptual shifts. *BioEssays* **37**, 284–293 (2015).
848 51. Kelleher, J., Etheridge, A. M. & McVean, G. Efficient Coalescent
849 Simulation and Genealogical Analysis for Large Sample Sizes. *PLoS*
850 *Comput. Biol.* **12**, 1–22 (2016).
851 52. Browning, S. R., Browning, B. L., Zhou, Y., Tucci, S. & Akey, J. M.
852 Analysis of Human Sequence Data Reveals Two Pulses of Archaic
853 Denisovan Admixture. *Cell* **173**, 1–9 (2018).
854 53. International Human Genome Sequencing Consortium. Initial sequencing
855 and analysis of the human genome International Human Genome. *Nature*
856 **409**, 860–921 (2001).
857 54. Altshuler, D. L. *et al.* A map of human genome variation from
858 population-scale sequencing. *Nature* **467**, 1061–1073 (2010).

859 55. Scally, A. & Durbin, R. Revising the human mutation rate: Implications for
860 understand ing human evolution. *Nat. Rev. Genet.* **13**, 745–753 (2012).
861 56. Kong, A. *et al.* Rate of de novo mutations and the importance of father-s
862 age to disease risk. *Nature* **488**, 471–475 (2012).
863 57. Purcell, S. *et al.* PLINK: A tool set for whole-genome association and
864 population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575
865 (2007).
866 58. Danecek, P. *et al.* The variant call format and VCFtools. *Bioinformatics* **27**,
867 2156–2158 (2011).
868 59. McKenna, A. *et al.* The genome analysis toolkit: A MapReduce framework
869 for analyzing next-generation DNA sequencing data. *Genome Res.* **20**,
870 1297–1303 (2010).
871 60. Loh, P. R. *et al.* Reference-based phasing using the Haplotype Reference
872 Consortium panel. *Nat. Genet.* **48**, 1443–1448 (2016).
873 61. Das, S. *et al.* Next-generation genotype imputation service and methods.
874 *Nat. Genet.* **48**, 1284–1287 (2016).
875 62. Watanabe, Y. *et al.* Analysis of whole Y-chromosome sequences reveals
876 the Japanese population history in the Jomon period. *Sci. Rep.* **9**, 8556
877 (2019).
878 63. Delaneau, O., Zagury, J. F. & Marchini, J. Improved whole-chromosome
879 phasing for disease and population genetic studies. *Nat. Methods* **10**, 5–6
880 (2013).

881 64. Oota, H. *et al.* The evolution and population genetics of the ALDH2 locus:
882 Random genetic drift, selection, and low levels of recombination. *Ann.*
883 *Hum. Genet.* **68**, 93–109 (2004).
884 65. Li, H. *et al.* Refined geographic distribution of the oriental ALDH2* 504Lys
885 (nee 487Lys) variant. *Ann. Hum. Genet.* **73**, 335–345 (2009).
886 66. Luo, H. R. *et al.* Origin and dispersal of atypical aldehyde dehydrogenase
887 ALDH2*487Lys. *Gene* **435**, 96–103 (2009).
888 67. Osier, M. *et al.* Linkage disequilibrium at the ADH2 and ADH3 loci and risk
889 of alcoholism. *Am. J. Hum. Genet.* **64**, 1147–1157 (1999).
890 68. Osier, M. V. *et al.* A global perspective on genetic variation at the ADH
891 genes reveals unusual patterns of linkage disequilibrium and diversity. *Am.*
892 *J. Hum. Genet.* **71**, 84–99 (2002).
893 69. Han, Y. *et al.* Evidence of positive selection on a class I ADH locus. *Am. J.*
894 *Hum. Genet.* **80**, 441–456 (2007).
895 70. Li, H. *et al.* Geographically separate increases in the frequency of the
896 derived ADH1B*47His allele in eastern and western Asia. *Am. J. Hum.*
897 *Genet.* **81**, 842–846 (2007).
898

Acknowledgments

We are grateful to the individuals who participated in the study. We would like to express our deepest gratitude to Mr. Masahiro Inoue, Shota Arichi, and Akito Tabira who obtained the genotype data and provided the technical environment for analyzing them. We would like to thank Dr. Hiroki Ota of Tokyo University, Tokyo, Japan, and Dr. Takashi Gakuhari of Kanazawa University, Ishikawa, Japan, for providing us the BAM file of the IKawazu Jomon. Dr. Takashi Gakuhari also gave us the population genotype data set (Panel 2240K) used in Gakuhari et al 2020. We also thank Dr. Naruya Saito from the National Institute of Genetics, Shizuoka, Japan, and Dr. Hideaki Kanzawa-Kiriyama from the National Museum of Nature and Science, Tokyo, Japan for providing us the BAM file of the Funadomari Jomon. This study was partly supported by Grant-in-Aid for Scientific Research (B) (18H02514) and Grant-in-Aid for Scientific Research on Innovative Areas (19H05341) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. Computations were partially performed on the NIG supercomputer at ROIS National Institute of Genetics.

Author contributions

Y.W. and J.O. conceived the study. Y.W. designed and conducted the data analyses. Y.W. performed the computer simulations. Y.W. wrote the manuscript with support from J.O. J.O. supervised the project. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Fig. legends

Fig. 1 Map of the Japanese prefectures. The prefectures of Japan are divided into eleven regions. The prefecture numbers in Supplementary Table 1 are indicated (the corresponding prefecture names are given in Supplementary Table 1). In this study, “mainland Japanese” means the Japanese people except for individuals from Hokkaido and Okinawa.

Fig. 2 The performance of the *AMI* for the detection of the Jomon-derived SNPs

(a) Distribution of *AMI* simulated by msprime. The histogram of *AMI* for the Jomon-derived variants (type 1) and the other variants (types 2 and 3) are shown. The red dashed line indicates the threshold of *AMI* (28.0374) obtained from ROC analysis for the detection of the Jomon-derived variants (type 1).

(b) ROC curve illustrating the performance of the *AMI* for the detection of the Jomon-derived SNPs. The ROC curve was drawn based on the simulated data shown in Fig. 2 (a). The *AMI* showed high accuracy (AUC = 0.91) for discriminating the Jomon-derived variants (type 1) from the other variants (types 2 and 3).

Fig. 3 JAS of each Japanese region.

(a) Distribution of *JAS* in ten regions. The boxplot of the *JAS* is presented for each of the ten regions, excluding Hokkaido.

(b) *JAS* of each prefecture in Mainland Japan. The average *JAS* by prefecture was calculated. Hokkaido and Okinawa prefectures are not illustrated. The prefecture with the higher average *JAS* is illustrated by the darker color.

(c) Relationship between the PC1 of the PCA performed in a previous study by the allele frequency of autosomal 183,708 SNPs in each prefecture and average *JAS*. Each prefecture was colored according to the region of Japan in Fig. 1. Horizontal axe: PC1, vertical axe: average *JAS*. Pearson's correlation coefficients (*R*), *P* values, regression lines and 95% CI are shown.

Fig. 4 Relationship between the *JAS* and values associated with the population size of prefectures in the Jomon to Yayoi periods.

The horizontal axis shows the average *JAS* and the vertical axis shows the (a) number of archaeological sites of the whole Jomon period, (b) population size in the Late Jomon period, and (c) $\log_{10}$ (number of archaeological sites in the Yayoi period/number of archaeological sites in the Late Jomon period). Pearson's correlation coefficients (*R*), *P* values, regression lines and 95% CI are shown in each figure. Each prefecture is colored according to the region in Fig. 1.

Fig. 5 f3-statistics between East Asians and estimated allele frequencies of the Jomon people.

f3(Onge ; Estimated Jomon frequencies, X), where X are the East Asians including 37 present day populations and one ancient population (Chokhopani, an iron age individual). Darker plots (higher *f3* value) indicates higher genetic affinity with the Jomon people.

Fig. 6 Haplotype structures composed of Jomon-derived SNPs in four genes associated with the characteristic phenotypes of East Asians.

The haplotype structures surrounding four nonsynonymous SNPs, (a) rs17822931 in *ABCC11* (associated with ear wax type), (b) rs3827760 in *EDAR* (associated with hair thickness and shovel-shaped incisors), (c) rs671 in *ALDH2* (associated with alcohol tolerance), and (d) rs1229984 in *ADH1B* (associated with alcohol tolerance) are illustrated. Each horizontal line represents each haplotype, and each vertical line represents each of the Jomon-derived SNP or phenotype-associated SNP. The derived alleles of the SNPs associated with phenotypes are shown in orange, and the ancestral alleles are shown in blue. The red color represents the Jomon allele, i.e., horizontal lines containing the red colored grid indicate the Jomon-derived haplotypes.

Fig. 7 The formation process of the Japanese population from the Late Jomon period to the present.

From the Late to the Final Jomon period, the Jomon people settled down in Mainland Japan, and the population size varied between regions. In the Final Jomon period, the continental East Asians arrived in northern Kyushu and then admixed with Jomon people in all the regions of Mainland Japan. In regions such as Kinki and Shikoku, where the population size was smaller at the end of the Jomon period, modern Japanese have lower degrees of genome components derived from the Jomon people.

Table 1 Frequencies of the Jomon-derived haplotypes specified by phenotypes associated SNPs in *ABCC11*, *EDAR*, *ALDH2*, and *ADH1B.*

(a) *ABCC11*	rs17822931		
	Ancestral	Derived	Total
Jomon-derived haplotype	0.106	0.240	0.346
Non-Jomon–derived haplotype	0.014	0.639	0.653
Total	0.120	0.879	

(b) *EDAR*	rs3827760		
	Ancestral	Derived	Total
Jomon-derived haplotype	0.149	0.173	0.322
Non-Jomon–derived haplotype	0.048	0.630	0.678
Total	0.197	0.803	

(c) *ALDH2*	rs671		
	Ancestral	Derived	Total
Jomon-derived haplotype	0.125	0.024	0.149
Non-Jomon–derived haplotype	0.635	0.216	0.851
Total	0.760	0.240	

(d) *ADH1B*	rs1229984		
	Ancestral	Derived	Total
Jomon-derived haplotype	0.038	0.048	0.086
Non-Jomon–derived haplotype	0.231	0.683	0.914
Total	0.269	0.731	

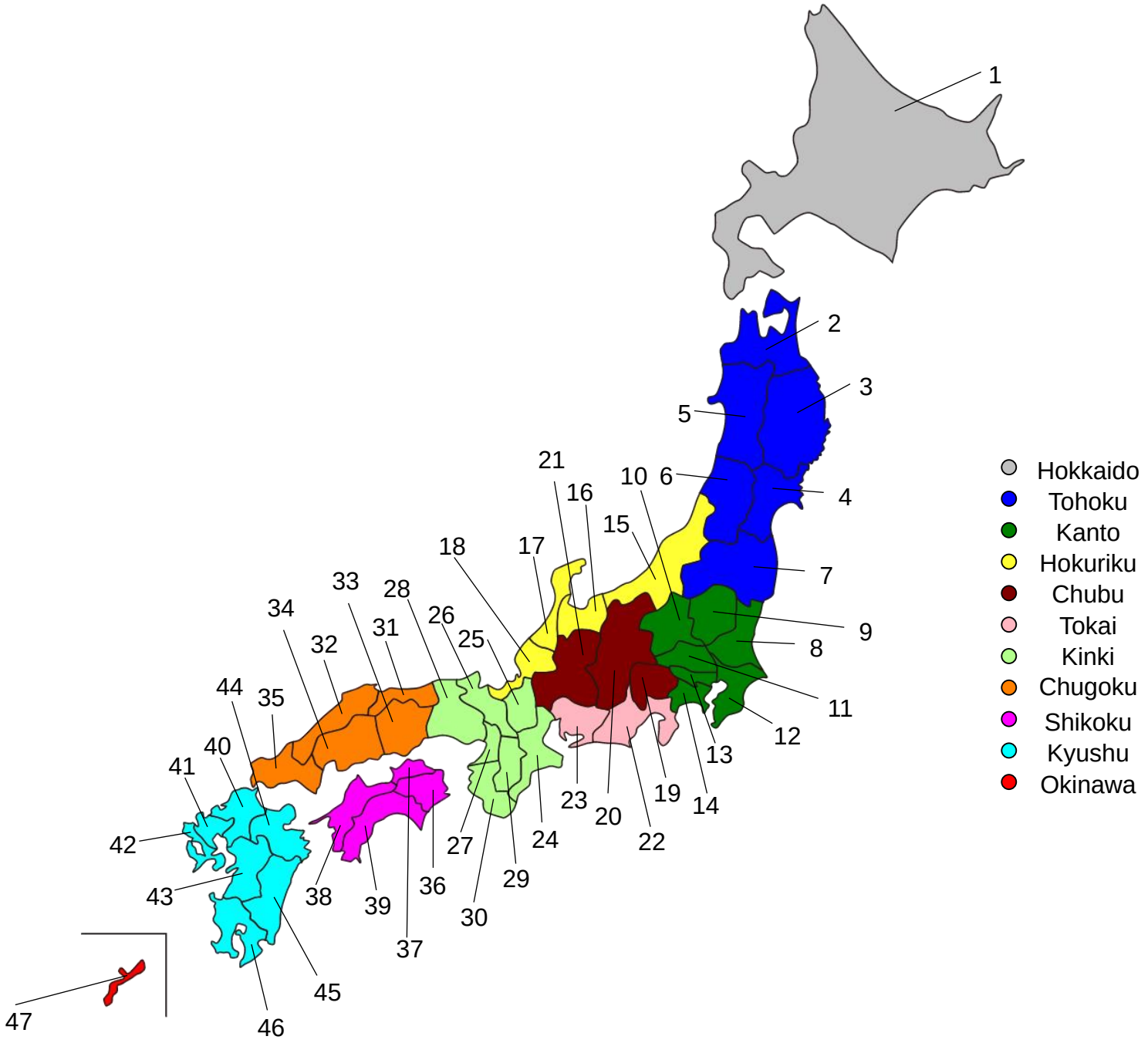
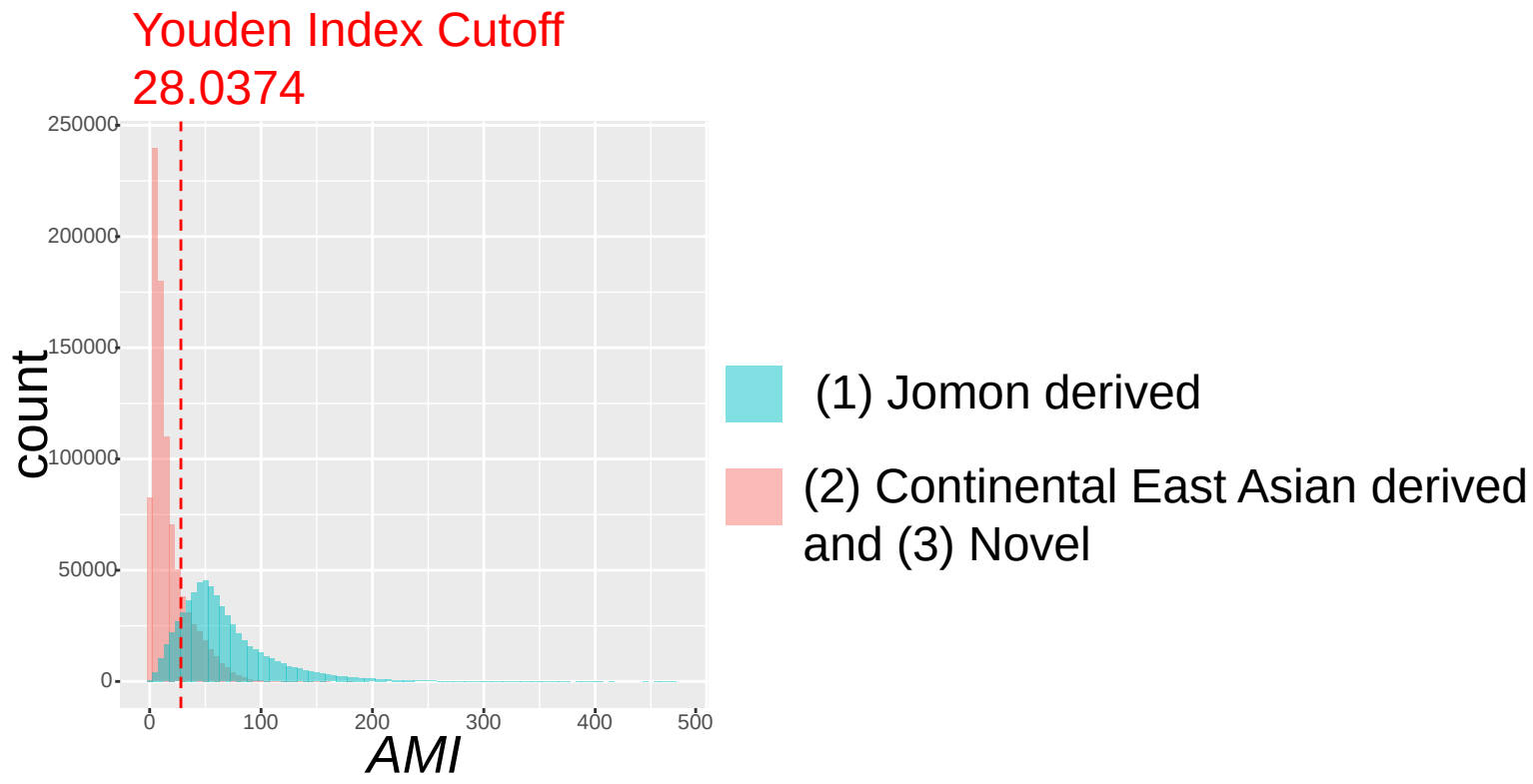

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
Hokkaido
Tohoku
Kanto
Hokuriku
Chubu
Tokai
Kinki
Chugoku
Shikoku
Kyushu
Okinawa

Fig. 1

(a)


Youden Index Cutoff
28.0374
250000
200000
150000
100000
50000
0
count
0
100
200
300
400
500
AMI
(1) Jomon derived
(2) Continental East Asian derived and (3) Novel

(b)

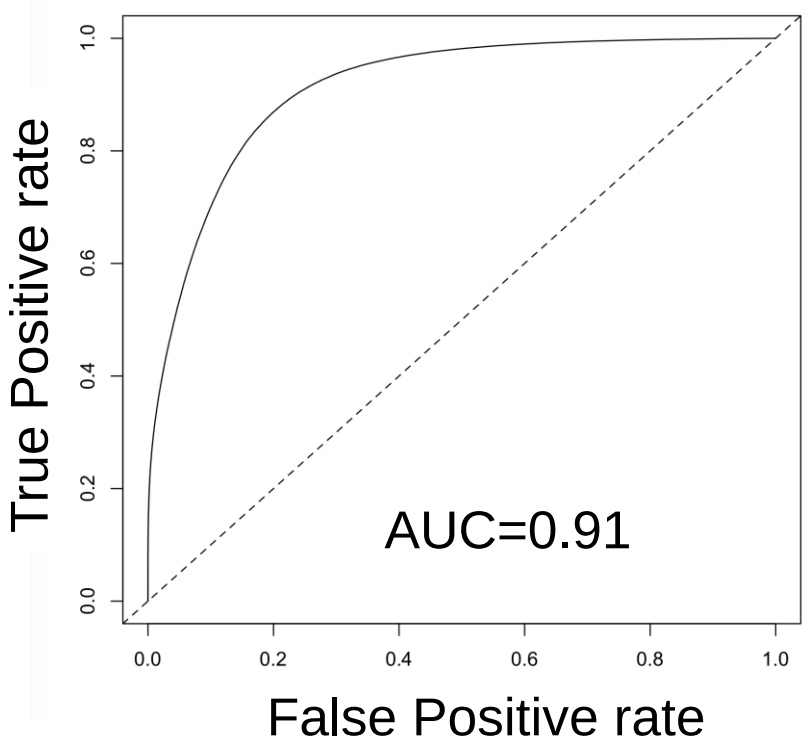
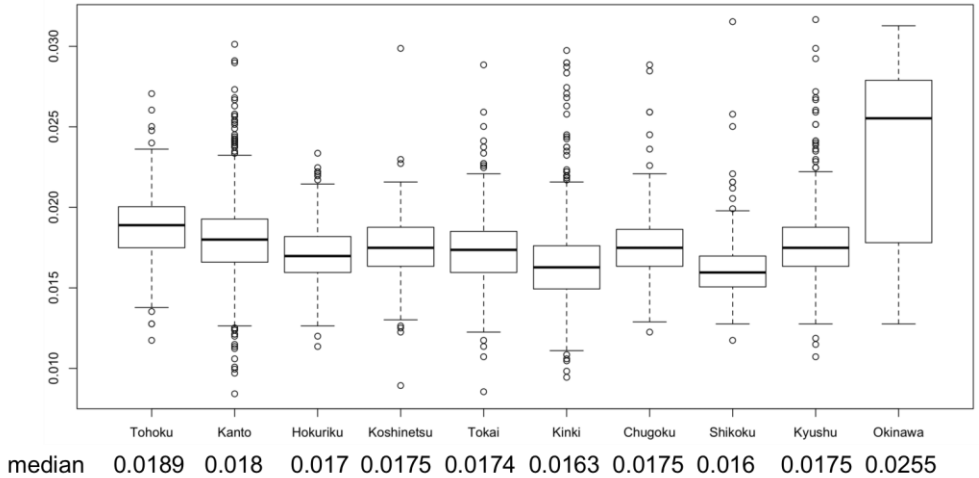
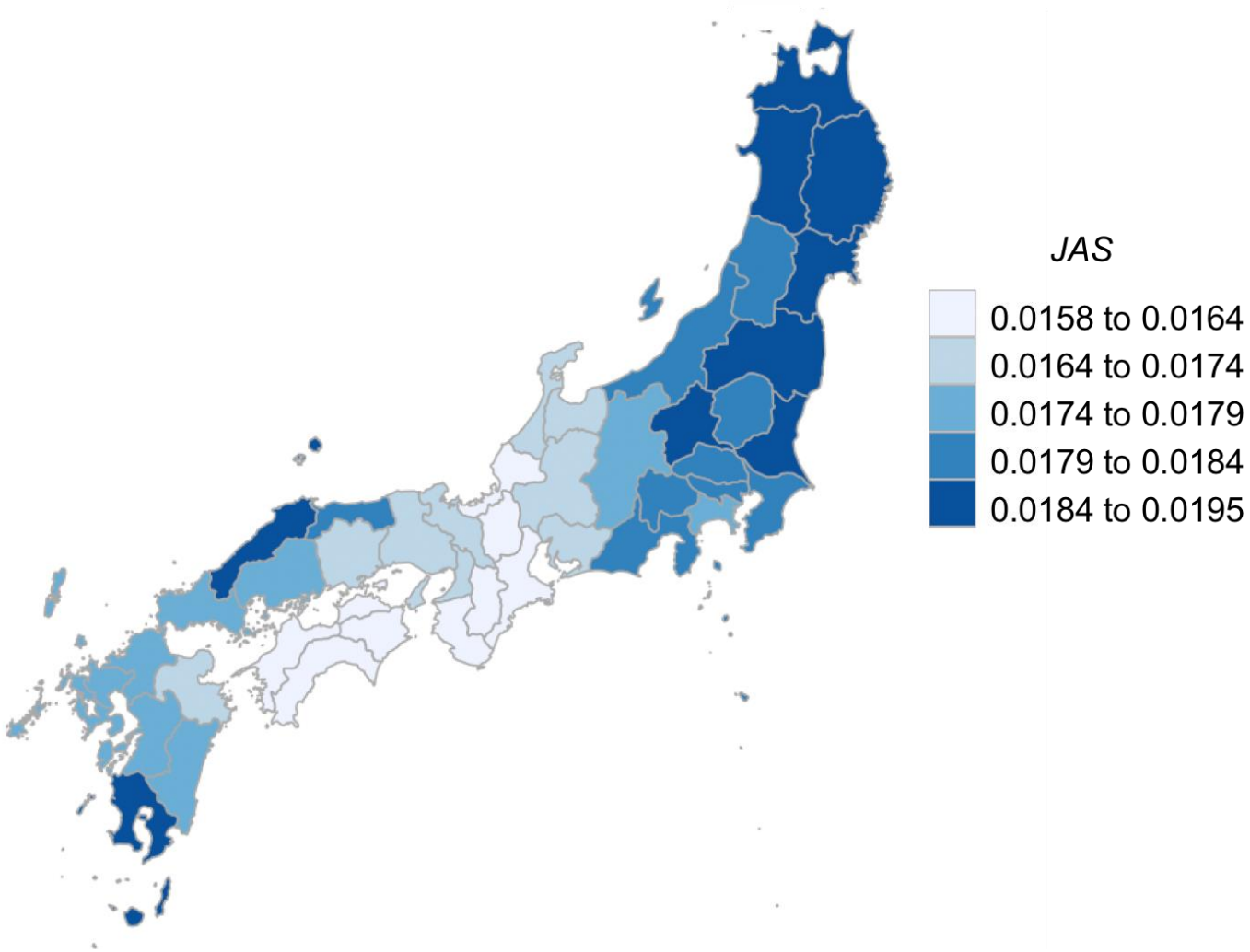

True Positive rate
False Positive rate
0.0
0.2
0.4
0.6
0.8
1.0
AUC=0.91

Fig. 2

(a)


0.030
0.025
0.020
0.015
0.010
Tohoku
Kanto
Hokuriku
Koshinetsu
Tokai
Kinki
Chugoku
Shikoku
Kyushu
Okinawa
median 0.0189 0.018 0.017 0.0175 0.0174 0.0163 0.0175 0.016 0.0175 0.0255

(b)


JAS
0.0158 to 0.0164
0.0164 to 0.0174
0.0174 to 0.0179
0.0179 to 0.0184
0.0184 to 0.0195

(c)

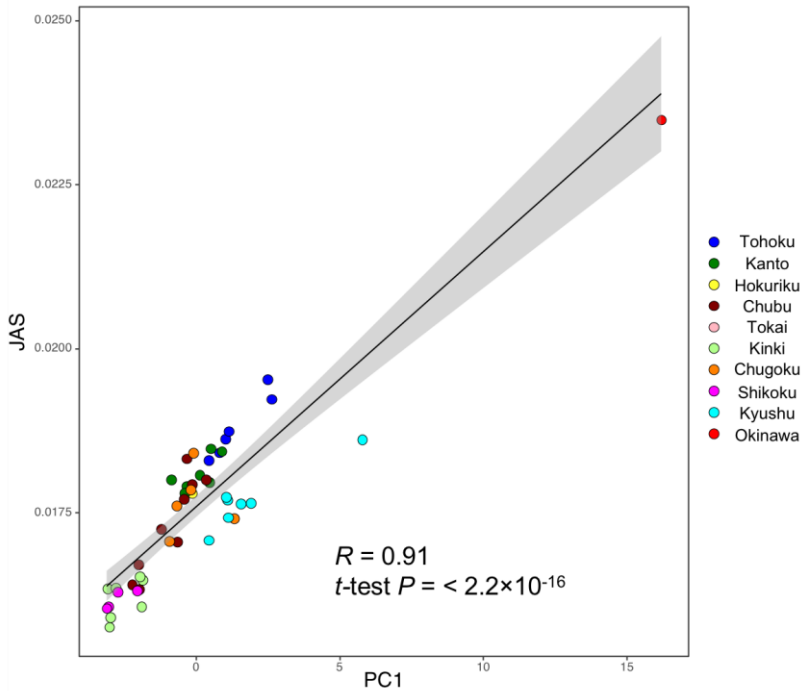

0.0250
0.0225
0.0200
0.0175
JAS
0
5
10
15
PC1
R = 0.91
t-test P = < 2.2×10-16
Tohoku
Kanto
Hokuriku
Chubu
Tokai
Kinki
Chugoku
Shikoku
Kyushu
Okinawa

Fig. 3

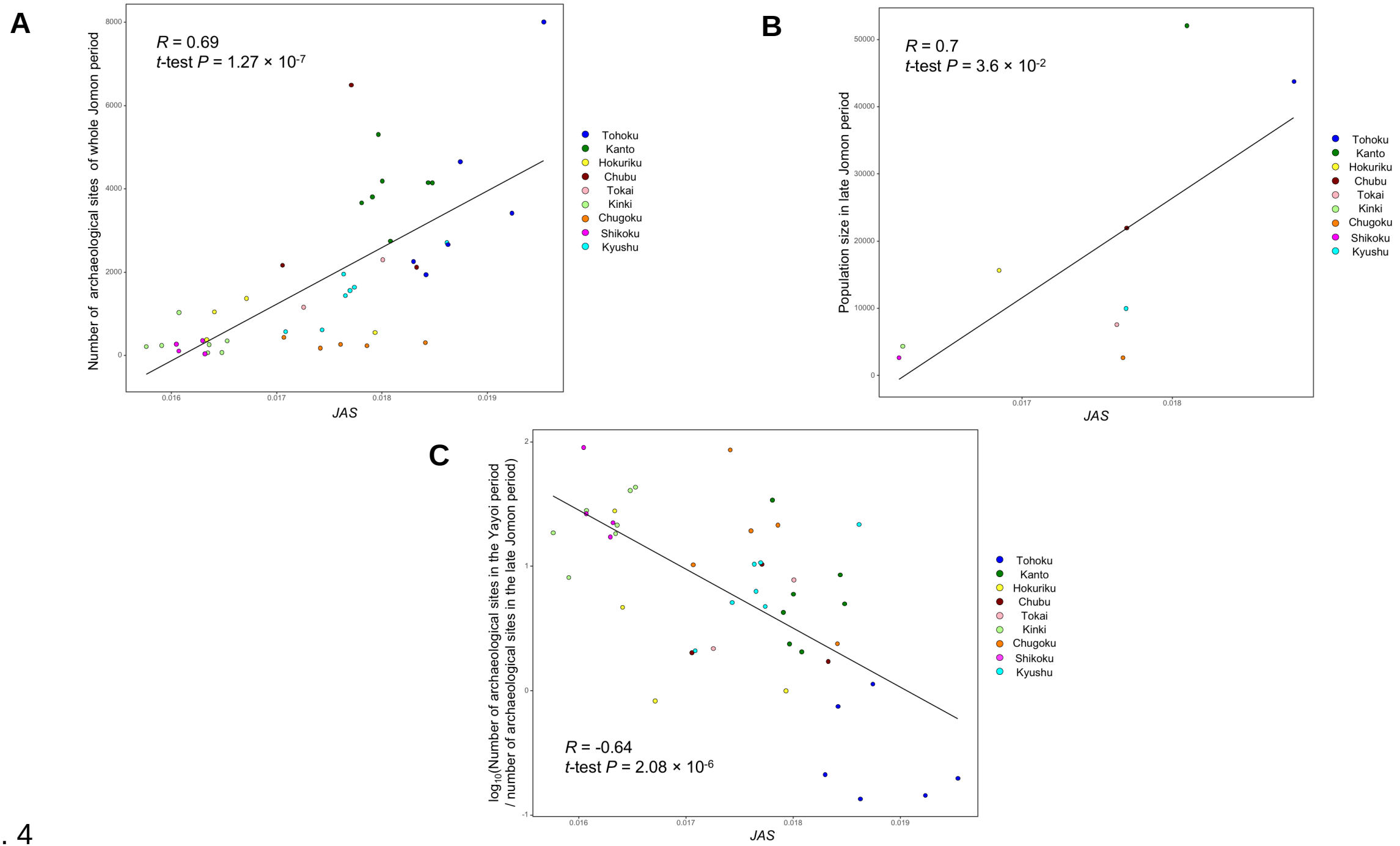

A
R = 0.69
t-test P = 1.27 × 10-7
Number of archaeological sites of whole Jomon period
JAS
B
R = 0.7
t-test P = 3.6 × 10-2
Population size in late Jomon period
JAS
C
R = -0.64
t-test P = 2.08 × 10-6
log10(Number of archaeological sites in the Yayoi period / number of archaeological sites in the late Jomon period)
JAS
Tohoku
Kanto
Hokuriku
Chubu
Tokai
Kinki
Chugoku
Shikoku
Kyushu

Fig. 4

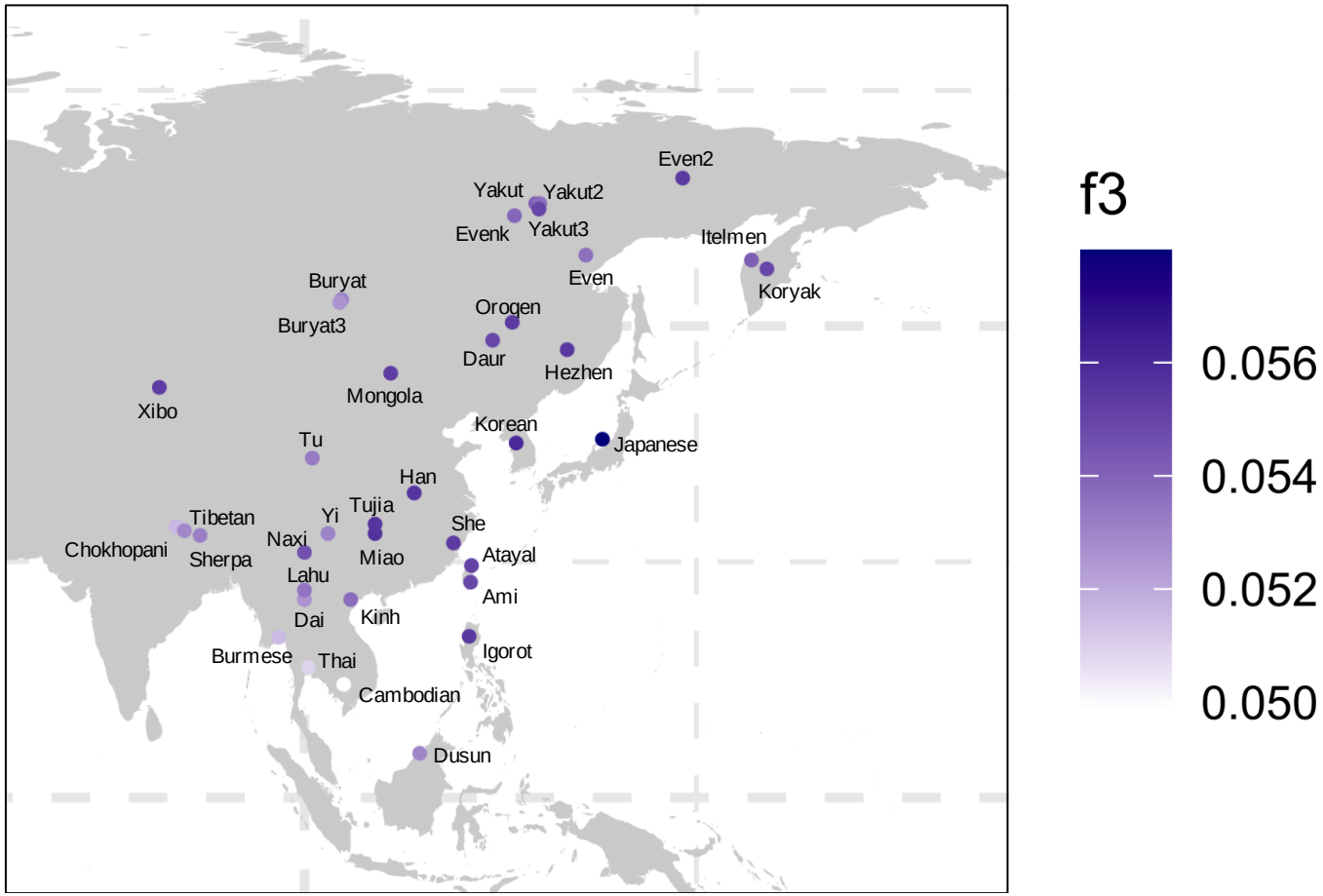

Even2
Yakut
Yakut2
Evenk
Yakut3
Itelmen
Even
Koryak
Buryat
Buryat3
Orogen
Daur
Hezhen
Mongola
Xibo
Korean
Japanese
Tu
Han
Tibetan
Yi
Tujia
She
Chokhopani
Naxi
Sherpa
Miao
Atayal
Lahu
Ami
Dai
Kinh
Igorot
Burmese
Thai
Cambodian
Dusun
f3
0.056
0.054
0.052
0.050

Fig. 5

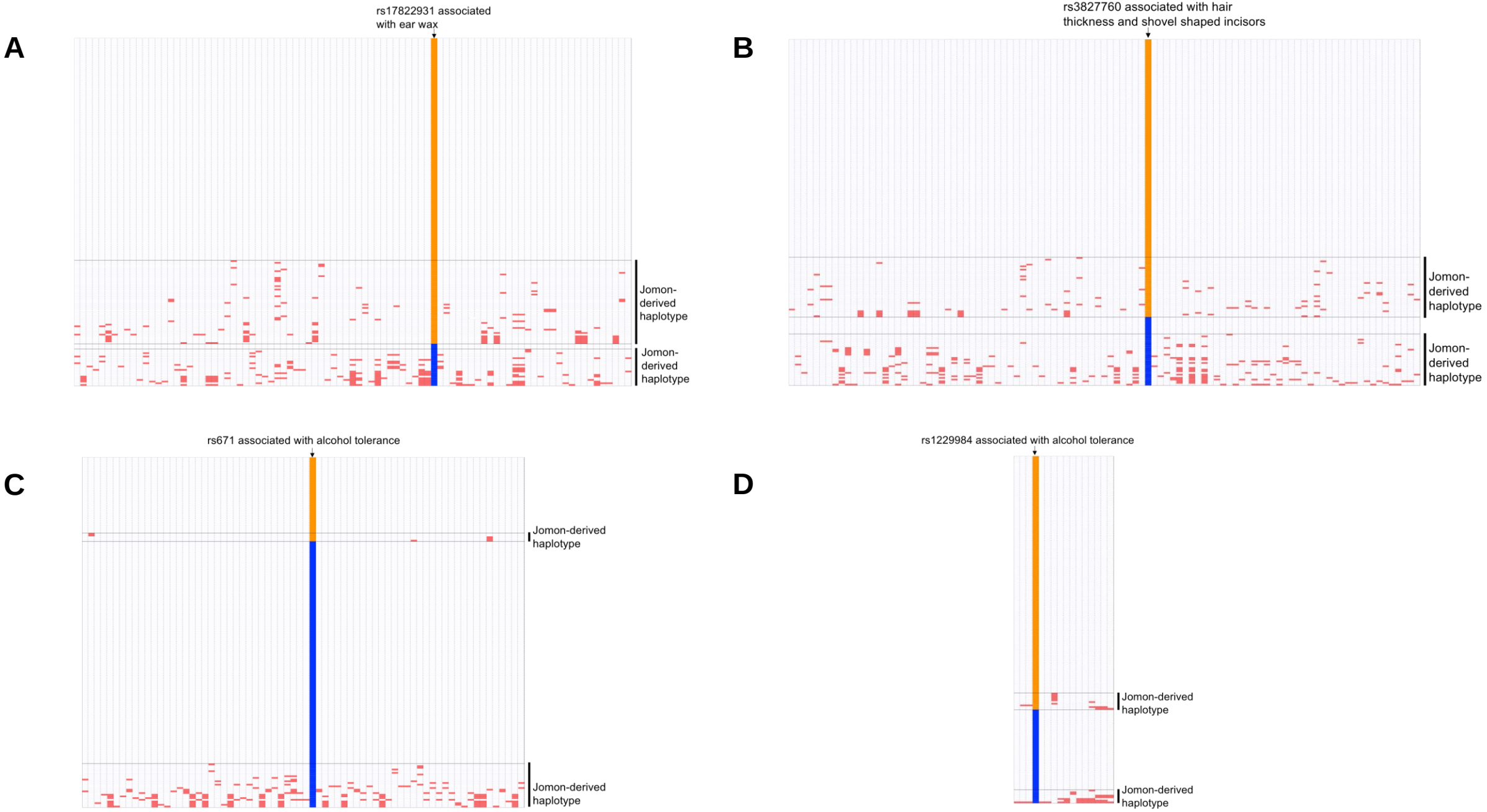

A
rs17822931 associated with ear wax
Jomon-derived haplotype
Jomon-derived haplotype
B
rs3827760 associated with hair thickness and shovel shaped incisors
Jomon-derived haplotype
Jomon-derived haplotype
C
rs671 associated with alcohol tolerance
Jomon-derived haplotype
Jomon-derived haplotype
D
rs1229984 associated with alcohol tolerance
Jomon-derived haplotype
Jomon-derived haplotype

Fig. 6

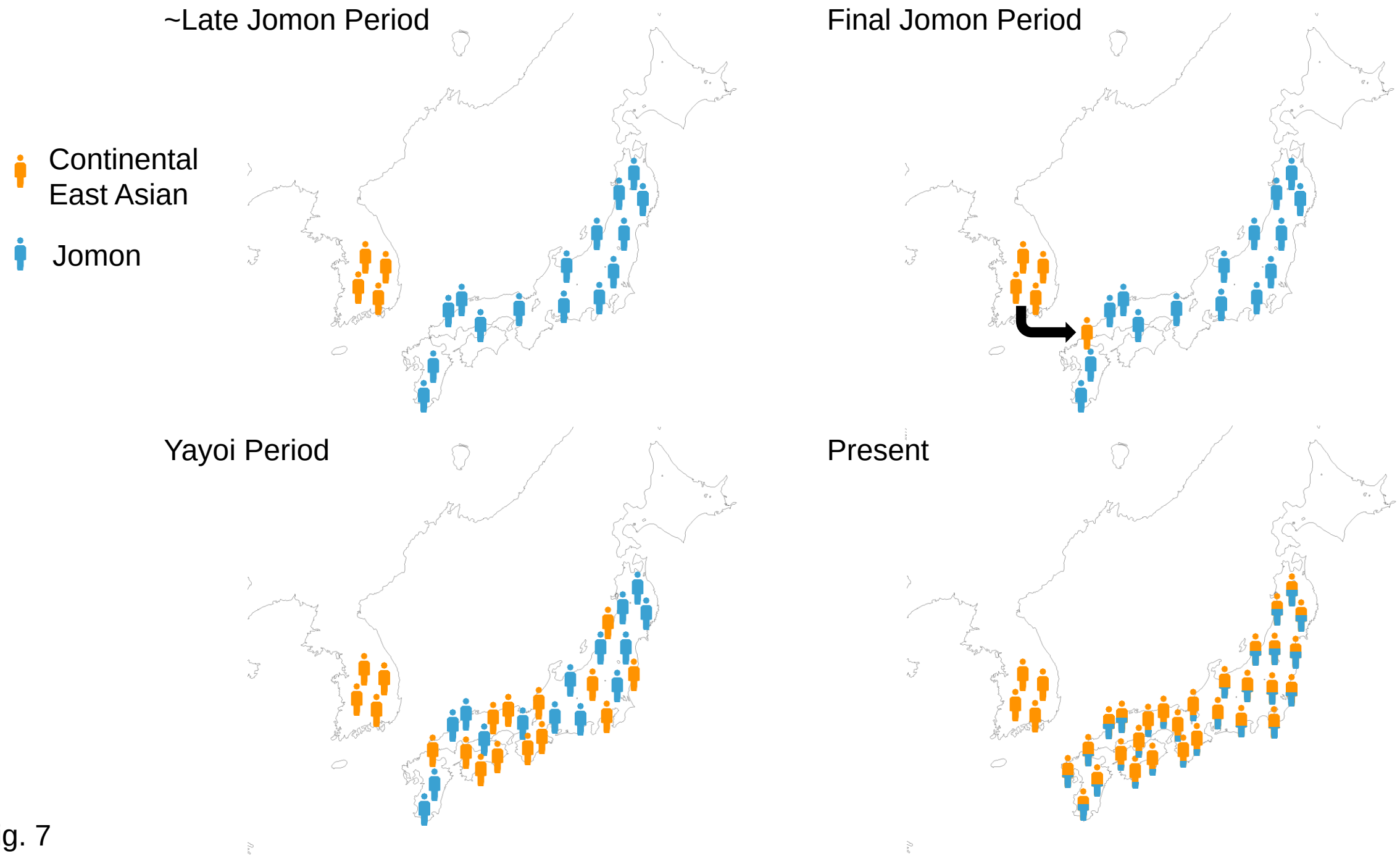

~Late Jomon Period
Final Jomon Period
Continental East Asian
Jomon
Yayoi Period
Present

Fig. 7