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Ancient Mitochondrial DNA Reveals the Origin of *Sus scrofa* from Rebun Island, Japan

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Abstract. The Kabukai A site (5 to 8C A.D.) of the Okhotsk cultural area is on Rebun Island, a small island near the coast, north–northwest of Hokkaido, Japan. Specimens of *Sus scrofa,* called the Sakhalin pig, were discovered in five cultural layers at the Kabukai A site. Ancient DNA was extracted from the remains of 42 Sakhalin pig bones. Thirty-nine nucleotide sequences of the 574-bp mitochondrial DNA control region, estimated to have originated from at least 21 individuals, were amplified and analyzed phylogenetically. Nine distinct haplotypes (A1, A2, A3, B1, B2, C1, C2, D1, and D2) from this site were classified into four haplotype groups (A, B, C, and D) by parsimonious network analysis. Phylogenetic analysis of 9 ancient and 55 modern haplotypes indicated that the population of Sakhalin pigs at the Kabukai A site belonged to two distinct clusters; haplotype groups A and B formed a cluster comprised only of themselves, and haplotype groups C and D belonged to the cluster of one of the two genetic groups of Japanese wild boars uniquely distributed in the western part of Japan, including one northeast Mongolian wild boar. Analysis of the haplotype distribution among three archaeological sites and their historical transitions among the five layers reflecting the cultural periods at the Kabukai A site suggests that the Sakhalin pig populations were introduced from Sakhalin island and the Amur River basin in the northeastern Eurasian continent together with some cultural influences.

Key words: *Sus scrofa* — Ancient DNA — Mitochondrial DNA — Control region — Population structure — Molecular phylogeny

Introduction

From the fifth to the tenth century, the Okhotsk Culture spread from Sakhalin, along the Okhotsk sea coast of the northernmost main island of Japan, Hokkaido, to southern Kamchatka (Yamamura and Ushiro 1999). This culture is distinct from almost all contemporary cultures in ancient Hokkaido, such as the latter Epi-Jomon and early Satsumon Cultures, which received direct influences from Honshu island of Japan (Imamura 1996). Remains from the Okhotsk cultural sites are characterized by unique pottery shards, various tools used to hunt or fish, and bones of dogs and pigs called Sakhalin pigs (Naora 1937; Nishimoto 1981). Especially, the Kabukai A site on Rebun, a small island near Hokkaido, is a typical archaeological site of the early Okhotsk Culture. A large number of Sakhalin pig bones, together with many fish bones, were recovered from thick cultural layers at the

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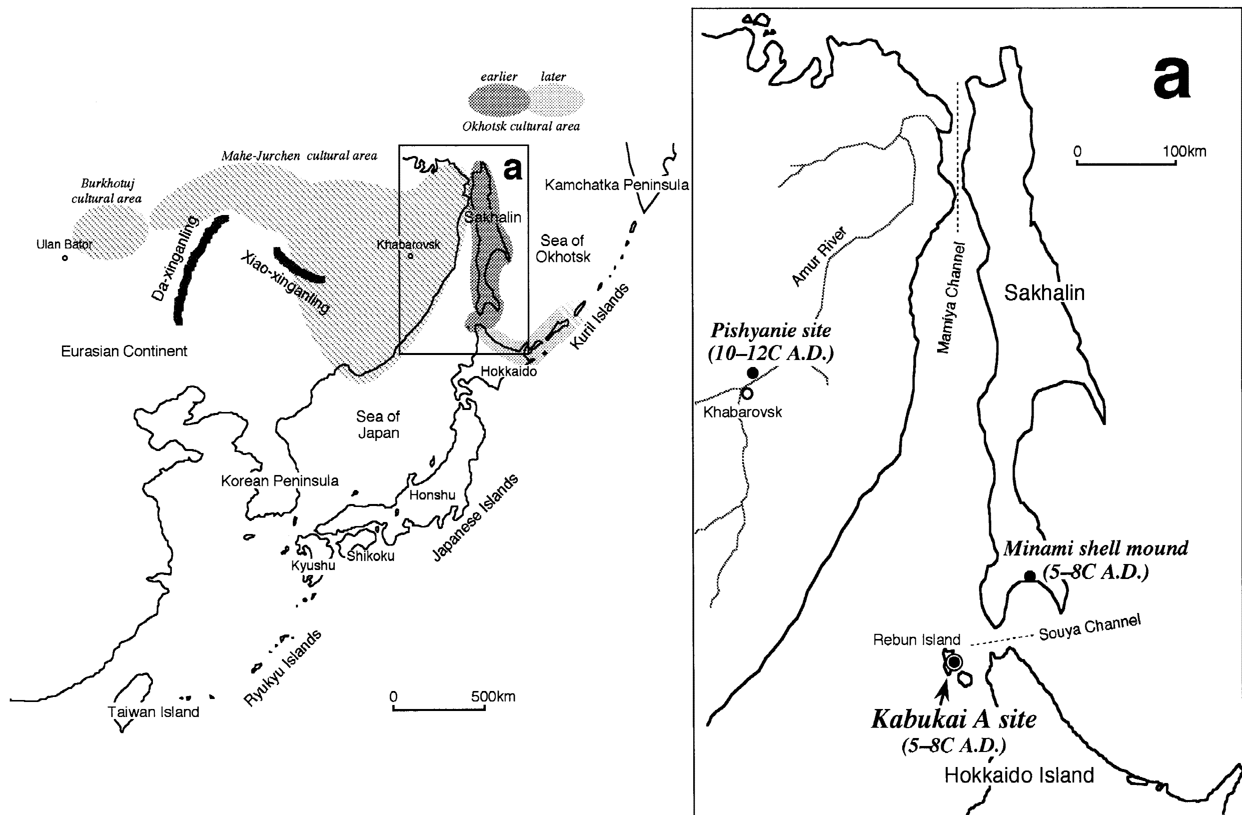

Fig. 1. Archaeological sites of ancient *Sus scrofa* specimens examined. The *shaded* areas represent the approximate areas of the earlier (5–8C A.D.) and the later (7–9C A.D.) Okhotsk Culture, and the *hatched* areas represent the areas of the Mohe (4–8C A.D.)–Jurchen Culture (10–12C A.D.) and the Burkhotuj Culture (2–8C A.D.).

Kabukai A site, suggesting that the pigs were kept as domesticated herds at this site (Nishimoto 1981). The Sakhalin pig has been designated a new species, *Sus inoi,* or a new subspecies, *Sus scrofa inoi,* on the basis of its morphology (Naora 1937; Nishimoto 1981). However, in Sakhalin, Hokkaido, and on Rebun Island, where the Okhotsk Culture developed in the past, wild boar populations do not exist today. The genetic background of the Sakhalin pig remains unknown.

In Japan, the Japanese wild boar (*S. scrofa leucomystax*) inhabits the main Japanese islands Honshu, Shikoku, and Kyushu and the Ryukyu wild boar (*S. scrofa riukiuanus*) inhabits the Ryukyu islands. We previously examined the genetic relationship between these two wild boar populations and the East Asian domestic pig by comparing their mitochondrial DNA (mtDNA) control region and *cyt b* gene sequences, and we showed that the Japanese wild boar is genetically closer to the East Asian domestic pig than to the Ryukyu wild boar (Watanobe et al. 1999). Advances in DNA techniques in recent decades allow phylogenetic analyses of extant species (Thomas et al. 1989; Cooper et al. 1992; Krings et al. 1997; Ozawa et al. 1997) and molecular genetic analyses of ancient animal and human populations (Horai et al. 1991; Stone and Stoneking 1993; Hardy et al. 1995; Oota et al. 1999). These molecular studies indicate that direct genetic information from ancient remains is very useful for understanding the evolution and population history of modern animals. It is of interest to know what genetic relationships exist among the ancient Sakhalin pig, the modern Asian wild boar, and the domestic pig, as well as the geographical region from which these ancient Sakhalin pigs were introduced into the Kabukai A site.

To investigate the genetic background of the ancient pig maintained in the Okhotsk cultural area, we extracted ancient DNA from Sakhalin pig bones excavated at the Kabukai A site and we determined the nucleotide sequences of the mtDNA control region using polymerase chain reaction (PCR) techniques. We examined the phylogenetic relationships between the Sakhalin pig and modern wild boars and domestic pigs, and also, we investigated the genetic transition of the ancient pig kept in the Okhotsk cultural area.

Materials and Methods

Archaeological Sites and Samples

The Kabukai A site is on Rebun, a small island in Hokkaido prefecture, Japan (Fig. 1). This site belongs to the earlier part of the Okhotsk cultural period [fifth to eighth centuries (5 to 8C) A.D.] and is thought to have been continuously occupied by the Okhotsk peoples during this

Table 1. Characteristics of archaeological specimens and the corresponding results of PCR amplification

				PCR results[b]				PCR[d]	
Site	Layer	Specimen[a]	ID	fA	fB	fC	Haplotype[c]	Ordinary	Seminested
Kabukai A	I	Humerus (R)	269	++	++	++	C1	fA, fB, fC	
	I	Humerus (L)	272	+−−	+−−	+−−	ND	fA	fB, fC
	I	Pelvis (R)	243	++	++	++	C1		fA, fB, fC
	I	Pelvis (R)	270	++	++	++	D1	fA	fB, fC
	I	Pelvis (R)	273	++	++	+++	D1	fA, fB, fC	
	I	Pelvis (L)	239	++	++	++	D1	fA, fC	fB
	I	Scapula (L)	271	++	++	++	C1		fA, fB, fC
	I	Ulna (R)	238	++	+++	++	C1		fA, fB, fC
	II	Atlas vertebra	246	++	++	++	D1	fA, fB	fC
	II	Femur (L)	268	+−−	+−−	+−−	ND		fA, fB, fC
	II	Mandible (R)	264	++	++	++	D1	fA, fC	fB
	II	Pelvis (R)	234	++	+++	++	D1		fA, fB, fC
	II	Pelvis (R)	265	++	++	++	A1		fA, fB, fC
	II	Pelvis (R)	267	++	++	++	C1	fA, fC	fB
	II	Pelvis (L)	235	++	++	++	D1	fC	fA, fB
	II	Pelvis (L)	251	++	++	++	A1	fA, fB	fC
	II	Scapula (R)	242	++	++	++	D1	fA, fC	fB
	II	Scapula (R)	248	++	+++	++	C1	fA, fC	fB
	II	Scapula (R)	266	++	++	+++	A1	fA, fB, fC	
	II	Ulna (R)	233	++	+++	++	D1	fA, fB, fC	
	II	Ulna (L)	236	++	++	++	D1		fA, fB, fC
	III0	Humerus (L)	260	++	++	++	B2	fA, fC	fB
	III0	Humerus (L)	261	++	++	++	C2		fA, fB, fC
	III0	Mandible (R)	249	++	+++	++	A1		fA, fB, fC
	III0	Scapula (R)	240	++	++	++	B1	fA, fC	fB
	III0	Scapula (R)	263	++	++	++	A3	fA	fB, fC
	III0	Scapula (L)	247	++	++	++	D1	fA, fC	fB
	III0	Tibia (R)	259	++	++	++	C1		fA, fB, fC
	III0	Tibia (R)	262	++	++	++	C1	fA, fB, fC	
	III	Humerus (R)	244	++	++	++	D2	fA, fB	fC
	III	Mandible (R)	257	+++	++	+++	D1	fA	fB, fC
	III	Pelvis (R)	250	++	++	++	A1		fA, fB, fC
	III	Radius (R)	256	++	++	++	D1	fA, fB, fC	
	III	Radius (R)	258	++	++	++	D1	fA, fC	fB
	III	Scapula (R)	255	+−−	+−−	++	ND	fA	fB, fC
	III	Tibia (L)	254	++	++	++	A1	fA, fB, fC	
	III	Ulna (R)	237	++	++	++	D1	fC	fA, fB
	III	Ulna (L)	232	++	++	++	B1	fA, fB, fC	
	IV	Mandible (R)	245	++	+++	++	A2	fA, fC	fB
	IV	Pelvis (R)	241	++	+++	++	A1	fA, fB, fC	
	IV	Radius (L)	252	++	++	++	A1	fA, fB, fC	
	IV	Ulna (L)	253	++	++	++	A1	fA, fB, fC	
Minami shell mound		Teeth		++	++	++	A1	fA, fB, fC	
		Teeth		++	++	++	A1	fA, fB, fC	
Pishyanie		Teeth		++	++	++	C1	fA, fB, fC	

[a] R, right; L, left.
[b] fA, fB, and fC indicate the DNA fragment amplified by the set A, B, and C primers, respectively. –, not amplified; +, amplified once; ++, amplified twice independently; +++, amplified three times.
[c] ND, not determined.
[d] Ordinary or seminested PCR was performed to amplify the ancient DNA.

period (Ushiro 1995). This site has seven cultural layers in which mainly fish bones and sea urchin shells were found (Ohyi 1981). A total of 42 bone samples (11 pelvises, 8 scapulae, 6 ulnae, 5 humeri, 4 mandibles, 3 radii, 3 tibiae, 1 femur, and 1 atlas vertebra) was collected from the upper five layers (layers I, II, III, III$_0$, and IV). Detailed information on these bone samples is provided in Table 1. Two teeth samples from the Minami shell mound in southern Sakhalin and one sample from the Pishyanie site in the middle Amur River region, Russia, were also examined. The Minami shell mound belongs to the earlier Okhotsk cultural period (5 to 8C A.D.) and the Pishyanie site belongs to the Amur Jurchen cultural period (10 to 12C A.D.) (Fig. 1).

DNA Extraction

To avoid possible contamination from the surfaces of archaeological remains, the soil and outer layers of the bone and tooth samples were removed by scraping with a sterile razor blade. Bone powder (0.5 to 1.0 g) was collected using an electric drill, suspended in 10 ml of 0.5 *M* ethylenediaminetetraacetate (EDTA), and decalcified by rotating the sample for a few days. Thereafter, the sample was centrifuged at 3000 rpm for 10 min, the supernatant was removed, and the pellet of bone powder was repeatedly decalcified by treatment with 10 ml of 0.5 *M*

EDTA. After decalcification, the bone powder was treated overnight in 5 ml of a mixture of 0.5 *M* EDTA, proteinase K (300 μg/ml), and *N*-lauroyl sarcosine (0.5%) (Hardy et al. 1995, with slight modifications). The sample was centrifuged at 3000 rpm for 10 min, and then the supernatant containing the ancient DNA was extracted twice with phenol, once with a mixture of chloroform:phenol (1:1), and once with chloroform to remove the protein. The supernatant was concentrated using a Centricon 30 microconcentrator (Amicon) and washed with distilled water. By these treatments the DNA samples were concentrated to a final volume of about 20–100 μl. The extracted ancient DNA samples were directly used as PCR templates. Precautions were taken to prevent contamination from other nonancient DNA as described by Okumura et al. (1999). To verify that no contamination had occurred during DNA extraction, we also examined extracts prepared in the same manner without bone powder as blank controls.

PCR and Direct Sequencing of mtDNA

To amplify the ancient DNA, we used three primer sets (A, B, and C) designed to correspond to sequences within the mtDNA control region. Primers mitL76 (5′-AATATGCGACCCCAAAAATTTAACCATT^{130}) and mitH62 (5′-CCTGCCAAGCGGGTTGCTGG^{351}) as set A, primers mitL119 (5′-CAGTCAACATGCGTATCACC^{301}) and mitH124 (5′-ATGGCTGAGTCCAAGCATCC^{567}) as set B, and primers mitL104 (5′-TGGACTAGTGACTAATCAGCCCAT^{518}) and mitH106 (5′-ACGTGTACGCACGTGTACGC^{704}) as set C were designed to amplify 258, 305, and 229 bp of the control region, respectively. The numbers shown at the right end of the primers denote the nucleotide positions in the control region of pig mtDNA (Okumura et al. 1996) corresponding to the 3′-terminal nucleotide of the primers; L and H are light and heavy strands, respectively. PCR was performed under the following conditions: one cycle of DNA denaturation and activation of AmpliTaq Gold (Perkin Elmer) at 95°C for 10 min, annealing at 57°C (with primer set A, 60°C) for 1 min, extension at 72°C for 1 min, followed by 50 cycles of denaturation at 94°C for 30 s, annealing at 57°C (60°C) for 30 s, and extension at 72°C for 1 min. When little or no PCR product was detected in the 50 cycles of PCR, a seminested PCR strategy was usually used. The second seminested PCR step was performed with primers mitL76 and mitH61 (5′-CTGGTTTCACGCGGCATGG^{336}) as set A, primers mitL120 (5′-ACCGCCATTAGATCACGAGC^{318}) and mitH124 as set B, and primers mitL105 (5′-CCATGCTCACACATAACTGAGGTT^{537}) and mitH106 as set C, and 1 μl of the products of the first PCR step, for 30 cycles under the same PCR conditions. To verify the reliability of the results, we repeated the amplification at least twice independently for each sample. PCR was performed with blank extracts as controls in each instance.

DNAs amplified by PCR were purified using a Centricon 100 microconcentrator (Amicon) and used as sequencing templates. The nucleotide sequences of both strands were determined using an Applied Biosystems 377 DNA sequencer with a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). When nucleotide sequences obtained from two independent PCR products were discrepant, PCR amplification a third time and subsequent direct sequencings were performed to verify the sequences. The 574-bp nucleotide sequence was constructed by connecting three DNA fragments amplified by means of primer sets A, B, and C.

Modern Wild Boars and Domestic Pigs

In addition to 59 Japanese wild boars, 13 Ryukyu wild boars, 12 Asian domestic pigs, 7 European domestic pigs, and 3 European wild boars reported by Watanobe et al. (1999), 63 Japanese wild boars, 3 Northeast Asian wild boars (1 from near Ulan Bator in northern Mongolia, 1 from near Da-xinganling in southern Mongolia, and 1 from near Xiaoxinganling, China), 65 Asian domestic pigs, and 66 European domestic pigs were newly examined using the procedure described by Okumura et al. (1996) (accession Nos. AB041464–AB041499). In total, 291 individual samples were examined. Fifty-five haplotypes were obtained for the 574-bp mtDNA control region and were used in this study (Fig. 2).

Phylogenetic Analysis

Parsimonious network analysis was conducted by the split decomposition method (Dopazo et al. 1993). Neighbor-joining analysis (Saitou and Nei 1987) was performed using the PHYLIP program package, version 3.572 (Felsenstein 1995). The distance matrix for the neighbor-joining tree was constructed from distance values using the two-parameter method (Kimura 1980). Bootstrap treatment was performed 1000 times.

Results

The results of PCR and sequencing are summarized in Table 1. All negative controls for PCR, i.e., the results obtained with the blank extracts, were negative, and there was no disagreement in the nucleotide sequences in overlapping regions comparing the three DNA fragments from regions A to C in all samples. In the case of 42 of 45 ancient samples, three DNA fragments were amplified (that is, all except for 3 samples from the Kabukai A site; Table 1), and 574-bp nucleotide sequences of the mtDNA control region were constructed. These 42 DNA sequences have been deposited in the DDBJ/EMBL/GenBank database (accession Nos. AB041422–AB041463). Figure 2 shows the nucleotide polymorphic sites of the 574-bp ancient and modern haplotypes (including two gaps) in the control region. Among the 42 ancient DNA sequences, 11 polymorphic sites were found, among which 6 polymorphic sites were newly found in this study, and the other 5 sites were shared with the modern haplotypes. Nine distinct haplotypes were obtained from 39 ancient Kabukai A samples.

Figure 3 shows a parsimonious network of nine distinct haplotypes. In this phylogenetic network, we defined four haplotype groups, A, B, C, and D, based on two or more nucleotide substitutions existing between neighboring haplotypes, and the nine haplotypes were designated A1, A2, A3, B1, B2, C1, C2, D1, and D2. The nucleotide sequences derived from two samples obtained from the Minami shell mound were identical to haplotype A1 and one derived from the sample obtained from the Pishyanie site was identical to haplotype C1. Haplotype C1 was also identical to modern haplotype 1, uniquely found in Japanese wild boars collected from western Honshu, Shikoku, and Kyushu in Japan (Okumura et al. 1996; Watanobe et al. 1999).

To estimate the genetic relationship between the ancient DNAs from the Kabukai A site and the modern DNA haplotypes, a neighbor-joining tree was constructed for 9 ancient and 55 modern haplotypes (Fig. 4). This showed that the haplotypes can be divided into two

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Nucleotide positions

           11111111222222222333333333444444455556666666667
           33345588144677889000012889004566704670135667990
Haplotypes 16864926529178095123734891674327623162188398033   Origins

1          A--TTGCTTTGCTACAGCCGTTTAATTTATGCCGATGCTGCCATTAG   Japanese wild boar
2          .--..................................C..........A
3          .--..........................C.........A.......
4          .--.........................G..................
5          .--.....................G..........C...........
6*         .--................................C...........
7          .--.......................C..G.................
8          .--.....................................T.....A
9          .--..A.....T.......AC............A......T.....A
10         .--..A.............AC............A......T.....A
11         .--................A.........................GA
12         .--................AC........C.....C..........A
13         .--....C......T....A...............C..........A
14         .--...........T....A..............GC.....T....A
15         .--...........T....A...............C.....T....A

16         .--...T.......T....AC..G....GC...A.C.........G.   Ryukyu wild boar
17         .--...T.......T...TAC..G....GC...A.C.........G.
18         .--...T.C.....T...TAC..G....GC...A.C........C..
19         .--...T.C.....T...TA...G....GC...A.C........C..
20         .--...T.......T....AC..G....GC...A.C........C..

21         .--..............T.A.............A.C...........   Northeast Asian wild boar
22         .--..............T.A.........C...A.............

23         .--......CA...T....A.........C...A...........G.   East Asian domestic pig
24         .--......C....T....A.........C...A...........G.
25         .--...T..C....T....A.........C...A...........G.
26         .--...T..C....T....A.........CA..A...........G.
27         .--......C.........A........G....A...........G.
28         .--................A........G....A...........G.
29         .--......C.........A........G....A.C.........G.
30         .--...........T....A.........C...............G.
31         .--......C.........A......C......A...........G.
32         .--......C....T....A.........C..TA...........G.
33         .--...........T....A.........C...A...........G.
34         .--...............TA.............A.C...........
35         .--................A.......CG....A...........G.
36         .--...............TA.............A.C.........G.
37         .--...T..C.........A......C......A...........G.
38         .--......C....T....A.........C...A.............
39         .--...........T....A.........C.....C.........G.

40         GC-CCAT.........A..AC.C..C...C...A..A........G.   European domestic pig
41         GC-CCAT.........A..AC.C..C...C...............G.
42         GC-CCAT.........A..A..C..C...C...A..A........G.
43         GC-CCA.C..A.....A..AC....C...C...A..A........G.
44         GC-CCA........TGA..AC.C..C...C...A..A..........
45         GC-CCAT.........A..AC.C..C..GC...A..A..........
46         GC-CCA..........A..AC.C..C...C...A..A........G.
47         GC-CCAT.........A..AC.C..CC..C...A..A........G.
48         GC-CCA......C.T.A..AC.C..C.C.C...A..A..........
49         GCACCAT.........A..AC.C..C...C...A..A........G.
50         GC-CCAT.........A..AC....C...C...A..A........G.
51         GC-CCAT.........A..AC.C..C...C...A..A.........A
52         GC-CCAT.......T.A..AC.C..C...C...A..A........G.
53         GC-CCAT.........A..AC.C..C...C...A..A..........

54         GC-C..T.....C.T....AC....C..GC...A.C..G...G.C..   European wild boar
55         GC-C..T.....C.T....AC.C..C..GC...A.C..G...G.C..

A1*        .--.....C..........A.......................C...   Kabukai A site
A2         .--.....C........T.A.......................C...
A3         .--.....C..........A.........C.............C...
B1         .--...........T....A.......................C...
B2         .--...........T....A...........T...........C...
C1*        .--............................................
C2         .--...................................T........
D1         .--..........G........C........................
D2         .--..........G.......TC........................
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Fig. 2. Variability of the partial mitochondrial DNA control region (574 bp) for 55 haplotypes from 291 modern wild and domestic animals and 9 haplotypes from 42 ancient *Sus scrofa* specimens. Nucleotide position 1 corresponds to the first position of the complete DNA sequences of control regions described by Okumura et al. (1996). *Dots* indicate nucleotide identity to the Japanese wild boar haplotype 1. Haplotypes marked with an *asterisk* were isolated from two or more animal groups or archaeological sites: haplotype 6 from the Japanese wild boar and Northeast Asian wild boar (around Ulan Bator, northeastern Mongolia), haplotype A1 from the Kabukai A site and Minami shell mound, and haplotype C1 from the Kabukai A and Pishyanie sites and Japanese wild boar.

distinct lineages, Asian (haplotypes 1 to 39) and European (haplotypes 40 to 55). The Asian lineage was subdivided into two sublineages, a Japanese, Northeast Asian wild boar and an East Asian domestic pig sublineage (haplotypes 1 to 15 and 21 to 39), and a Ryukyu wild boar sublineage (haplotypes 16 to 20). Ancient haplotypes from the Kabukai A site belonging to the former sublineage and formed a monophyletic group with Japanese (including one Northeast Mongolian) wild boar haplotypes (Fig. 4). In this phylogenetic tree, haplotype groups A and B formed a cluster independent of the other haplotype groups, C and D, of the Kabukai A site, though the bootstrap value for this cluster was not so high (46%), and this independent cluster included no modern haplotype previously detected. Haplotype groups C and D belonged to the cluster of Northeast Mongolian and Japanese wild boars distributed widely in western Japan. This phylogenetic analysis indicated that the genetic background of the Sakhalin pigs found at the Kabukai A site was more similar to that of modern Northeast Mon-

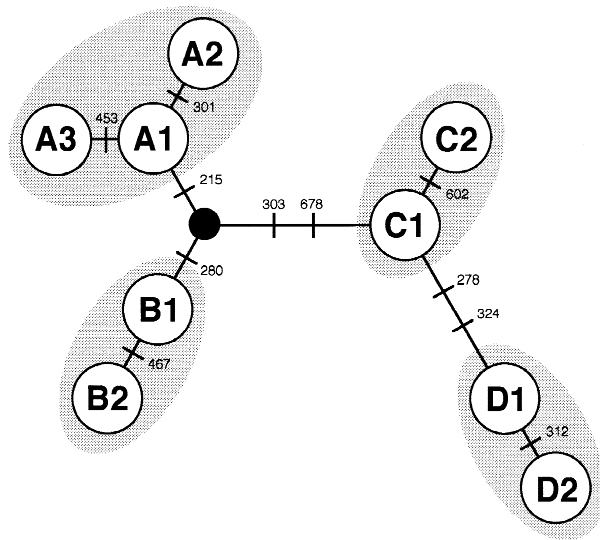

Fig. 3. Parsimonious network among nine ancient haplotypes. *Open circles* indicate identified haplotypes, and the *filled circle* indicates a hypothetical haplotype. The four *shaded* haplotype groups have two or more nucleotide substitutions between them. Each *bar* with a nucleotide position number on the branch indicates a nucleotide substitution.

golian and Japanese wild boars (haplotypes 1 to 15) than to Chinese domestic pigs such as the Meishan pig (haplotypes 23 and 24), the Jinhua pig (haplotypes 25 and 26), and the Yontsuan pig (haplotype 28) and the Northeast Asian wild boars from the southern area of Da- and Xiao-xinganling (haplotypes 21 and 22).

Table 2 shows the haplotype distribution at the three archaeological sites (Kabukai A site, Minami shell mound, and Pishyanie site) and among the five layers of the Kabukai A site. Based on the information about the specimens distributed among the cultural layers and their haplotypes that we detected, we conclude that the archaeological specimens examined in this study originated from 21 individuals, as the minimum estimation (Tables 1 and 2). Our minimum number of individuals as estimated by DNA analysis exceeded the number of crania and mandibles in layer II (Nishimoto 1981). Thus, the number of individuals estimated on the basis of the number of partial bones found might be an underestimate, due to losses of crania or mandibles or both. Haplotype A1 found at the Minami shell mound was detected in the oldest layer IV and continued to layer II. Both haplotype B1 and haplotype D1 appeared in layer III, and haplotype B1 continued to layer III_0, while haplotype D1 continued to the latest layer I. Haplotype C1, found at the Pishyanie site, appeared in layer III_0 and continued to the latest layer I. Subtypes A2, A3, B2, C2, and D2 occasionally appeared as genetic variants with their major types A1, B1, C1, and D1. The results regarding the haplotype distribution at the archaeological sites and their historical transitions among the layers at the Kabukai A site indicate the coexistence of multiple haplotype groups in the periods corresponding to at least cultural layer III and the upper layer. The continuous detection of haplotypes A1, B1, C1, and D1 in some cultural layers indicated that the Sakhalin pigs having these haplotypes had continuously existed at this site.

Discussion

The Kabukai A site is a typical Okhotsk cultural archaeological site and is thought to have been continuously used by the Okhotsk peoples throughout the earlier part of the Okhotsk Culture in Japan. The site consists of seven continuous cultural layers containing numerous artifacts and faunal remains, such as characteristic pottery shards of the Okhotsk Culture, various tools for sea mammal hunting and deepwater fishing, and bones of dogs and so-called Sakhalin pigs (Naora 1937; Nishimoto 1981). Especially, many Sakhalin pig bones in layer IV and the upper layers are rather unusual for a maritime hunting society and are unique to the Okhotsk Culture. In this study, we found that some mitochondrial DNA haplotypes had been maintained in the Sakhalin pig population of the Kabukai A site for hundreds of years. This finding implies that the Sakhalin pigs having these haplotypes had been maintained by repetitive introduction to this site or by artificial propagation at this site or both.

Two of nine haplotypes detected at the Kabukai A site were independently found at other archaeological sites. Haplotype A1, which was detected from the oldest layer IV to layer II, dated previously at approximately 5 to 8C A.D. by ^{14}C analysis (Ushiro 1995), was found in two ancient pigs from the Minami shell mound on Sakhalin. Although this archaeological site was not exactly dated, it is known as a site belonging to the earlier Okhotsk cultural period (5 to 8C A.D.), like the Kabukai A site. Some archaeological evidence indicates that the earlier part of the Okhotsk cultural area expanded from the lower Amur River and northern Sakhalin to southern Sakhalin and ultimately to the northern tip of Hokkaido including Rebun Island (Ushiro 1995). According to this direction of ancient cultural movement, individuals having haplotype A1 might have been transported from the Sakhalin pig population maintained in the Okhotsk cultural area on Sakhalin Island to this site. This speculation could be confirmed by further investigations of Sakhalin pig specimens from an older archaeological site of the Okhotsk Culture in Sakhalin. However, the original habitat of *Sus scrofa* having this haplotype remains unclear. Haplotype C1 was detected in an ancient *Sus scrofa* from the Pishyanie site of the Amur Jurchen Culture in the Amur River basin. However, haplotype C1 was also found in western populations of Japanese wild boar as modern haplotype 1. Two possibilities may explain the origin of Sakhalin pigs having haplotype C1 at the Kabukai A site. First, the pigs may have been transported to this site from the western part of Honshu Island. Modern

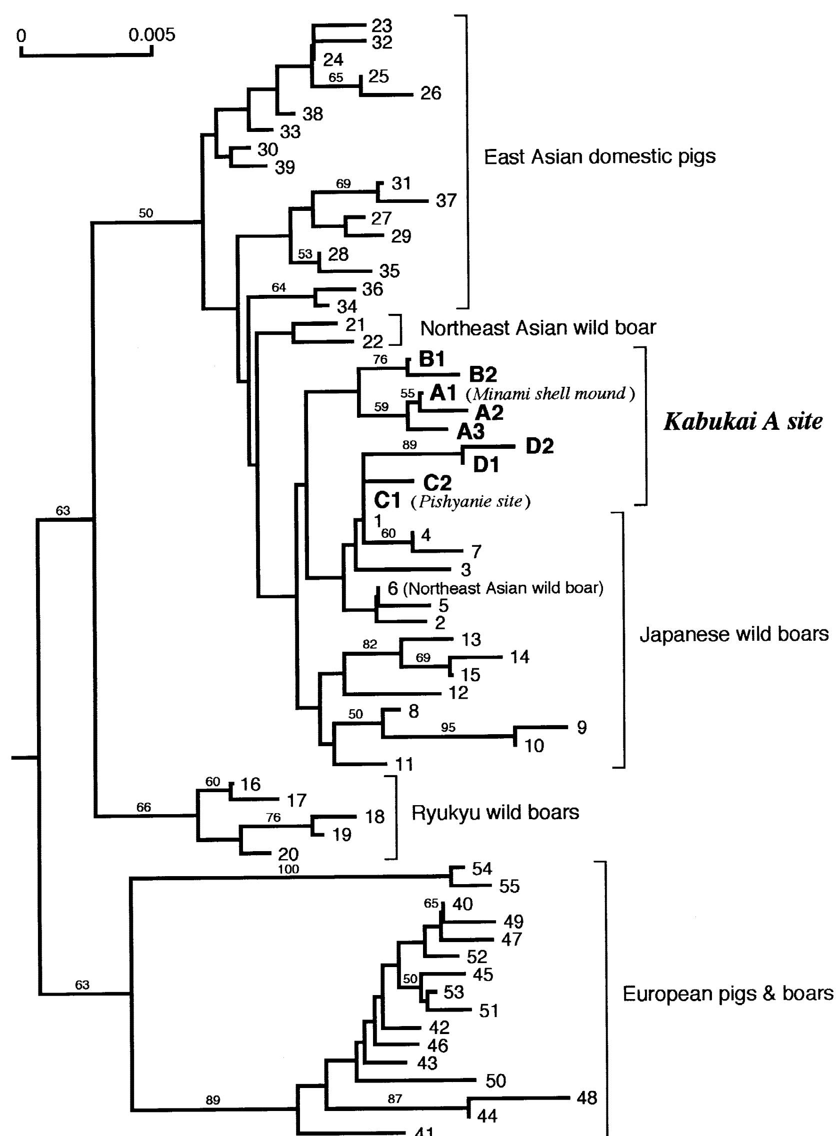

Fig. 4. Phylogenetic tree constructed from 9 ancient and 55 modern partial sequences (574 bp) of the mitochondrial DNA control region by the neighbor-joining analysis method. Haplotype numbers are the same as in Fig. 2. Bootstrap resampling was done 1000 times, and resulting bootstrap probabilities of more than 50% are shown on the corresponding branches.

Table 2. Haplotype distribution in archaeological sites and their transitions among cultural layers

				Number of haplotypes (minimum number of individuals)[b]								
				A			B		C		D	
Site	Period	Layer	n^a	1	2	3	1	2	1	2	1	2
Kabukai A site	8C A.D.	I	7						4 (1)		3 (1)	
	8C A.D.	II	20	3 (1)					2 (1)		7 (1)	
	7C A.D.	III_0	6	1 (1)		1 (1)	1 (1)	1 (1)	2 (2)	1 (1)	1 (1)	
	7C A.D.	III	13	2 (1)			1 (1)				4 (2)	1 (1)
	5C A.D.	IV	8	3 (1)	1 (1)							
Minami shell mound	5–8C A.D.			2								
Pishyanie site	10–12C A.D.								1			

[a] The minimum number of individuals estimated on the basis of the numbers of crania and mandibles in the layers (Nishimoto 1981).
[b] Numbers in parentheses are the minimum numbers of individuals estimated on the basis of the types of bone specimens and their haplotypes recovered in this study.

haplotype 1 and its closely related haplotypes 2 to 7 have not been found in eastern specimens of the Japanese wild boar (Okumura et al. 1996; Watanobe et al. 1999). Archaeological findings indicating cultural exchanges between the Okhotsk area and Honshu Island are few (Kikuchi 1995). Also, eastern Japanese haplotype 15, but not western haplotypes 1 to 7, was detected in analysis of an ancient pig bone excavated from an archaeological

site of the Epi-Jomon Culture in southern Hokkaido (data not shown). Thus, no evidence exists to support the possibility that the Japanese wild boar, which inhabited especially western Japan, was transported to Hokkaido Island. The second possibility is that pigs were introduced from the northeast to the Eurasian continent, such as Northeast Mongolia or the Amur River basin and the Maritime Province of the Russian Far East, to the Kabukai A site. Haplotype C1 and its closely related haplotype 6 were found in an ancient *Sus scrofa* from the Pishyanie site on the Amur River basin and in a modern wild boar from Northeast Mongolia, respectively. This is supported by the following archaeological evidence: (i) among artificial remains excavated from the Kabukai A site, most metal, glass, and stone objects, for example, halberds, small swords with curved handles, jasper ornaments, and glass beads, are thought to be continental-type objects (Yamada et al. 1995); (ii) the same pattern (Kokumon) of pottery was also seen among continental pottery shards from the Mohe Culture (4 to 8C A.D.) in the Maritime Province of the Russian Far East and the Amur River basin and from the Burkhotuj Culture (2 to 8C A.D.) in northeastern Mongolia and the neighboring region of Russia (Kikuchi 1995; Yamada et al. 1995); and (iii) a continuous pig farming culture in the Amur River basin is suggested by the findings of pig bones in the Mohe cultural sites and in earlier cultural sites (Pol'tse, 8–7C B.C. to 3–4C, A.D.; and Yankovskij, 2000 to 6–5C B.C.) (Kikuchi 1995). Thus, the second possibility seems more plausible than the first one. In other words, these findings suggest that the Japanese wild boar population may be from the Northeast Eurasian continent. As the sample size of ancient and modern *Sus scrofa* from the Northeast Eurasian continent was small, we detected only haplotypes C1 (identical to modern haplotype 1) and 6 among the Kabukai A haplotypes and the Japanese wild boar haplotypes in this study. When a comprehensive investigation of ancient and modern *Sus scrofa* animals, especially in the far north of the mountainous Da- and Xiao-xinganling regions of Northeast Eurasia, is performed, other ancient and modern haplotypes found in Sakhalin pigs and Japanese wild boars, for example, haplotypes A1, B1, and D1 and haplotype 1, may be detected in those areas.

At the Kabukai A site, the coexistence of multiple haplotype groups in cultural layer III and the upper layers III_0, II, and I was indicated in this study (Table 2). The number of Sakhalin pig bones suddenly increased at layer III and gradually increased to the latest layer I (Ohyi 1981). Continental-type remains, such as small swords with curved handles, jasper decorations, and glassy beads, began to appear in layers III and III_0, dated approximately 7C A.D., and continued until the upper layers II and I, dated 8C A.D. (Kikuchi 1995; Ushiro 1995). The proportion of Kokumon pattern pottery found in the Mohe and Burkhotuj Cultures gradually increased from layer III to the upper layers (Ohyi 1981; Kikuchi 1995). This archaeological evidence indicates that cultural exchange between the Kabukai A site and the Northeast Eurasian continent was active through trade during these periods. This archaeological information and our results on the historical transition of haplotype distribution among cultural layers lead us to propose the hypothesis that the increase in Sakhalin pig bones was due to the introduction of new *Sus scrofa* animals belonging to genetic groups B, C, and D from the Northeast Eurasian continent through trade (Table 2). The quality of the bone specimens prevented us from examining sufficient ancient individuals in this study. Because the sample size of individuals was relatively smaller than the minimum number of individuals estimated on the basis of the number of crania and mandibles (21 of 54 individuals), some sampling errors may be included in the haplotype distribution among the cultural layers (Table 2). However, this hypothesis is also supported by the result indicating that haplotype C1, detected in the cultural layers formed in the active trading periods (layers III_0, II, and I), was found at the continental site of the Amur Jurchen Culture following the Mohe Culture at the same place. This is the most plausible explanation for the increase in Sakhalin pig bones at the Kabukai A site, and it could be tested by obtaining more genetic information on ancient pig specimens excavated from other Okhotsk cultural sites from various periods on Hokkaido and Sakhalin Island. Further genetic studies of ancient and modern pig specimens from the Amur River basin and other parts of Northeast Eurasia will also clarify the ancient pig movement, and even cultural movements, in ancient times.

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