

## Distribution of Y chromosomal haplotypes in Japanese native horse populations

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*The distribution of Y chromosomal haplotypes in Japanese native horse populations was investigated to obtain genetic information on these populations. Here, 159 male/gelded horses from eight local populations were investigated, and three Y haplotypes (JHT-1, JHT-2, and JHT-3) were identified by analyzing five Y-linked loci. Five populations had only JHT-1, whereas two populations had only JHT-2. One population had JHT-1 and JHT-3. Based on the geographical distribution of these haplotypes and previously reported haplotypes for other Asian horses, JHT-1 is considered to be a major haplotype in ancestral native horses. The fixation of each haplotype suggests the influence of independent breeding and genetic drift in each population. These findings complement the results from previous genetic studies of Japanese native horses.*

**Key words:** Japanese native horse, Y chromosome, haplotype, variation

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According to historical literature, Japanese indigenous horses were distributed throughout Japan and they formed many local populations. The size of native horse populations has decreased significantly since the latter half of the 19th century, with motorization and changes in military policy promoting their hybridization with European breeds [3]. Currently, descendants of Japanese native horses are found in only eight local areas and are generally classified into the following populations: Hokkaido, Kiso, Noma, Taishu, Misaki, Tokara, Miyako, and Yonaguni. The present classification is defined by the Japan Equine Affairs Association (JEAA; <https://www.bajikyo.or.jp/>). Each local area is geographically separate and the horse populations have been raised in isolation. Currently, the population sizes of these horses range from 39 to 150 horses, except for the Hokkaido population, which includes 1,106 horses, as reported by the JEAA in 2016. Local organizations make efforts to conserve the unique characteristics and reproductive resources within

each of the respective populations.

To date, analyses of protein polymorphisms, short tandem repeats (STRs), and mitochondrial DNA (mtDNA) variation have been carried out in Japanese native horses to characterize their genetic information and relatedness within and between populations [4, 13–15, 17, 18, 20]. Phylogenetic studies based on protein and STR polymorphisms concluded that Japanese native horses likely originated from Mongolian-type horses that subsequently spread across the country [12, 13, 20]. In addition, genetic composition data from STRs and mtDNA indicated the influence of independent breeding and genetic drift recently in each population [4, 20], which is well accounted for, in the breeding history of the native horses. This information was obtained from autosomal and matrilineal DNA markers, but no study has been developed using patrilineal information.

Limited variability in the Y chromosome of domestic horses has been reported in previous studies. This was attributed to the influence of limited patrilines in horse domestication processes and an extremely low effective population size of males [1, 10, 11, 21]. However, single nucleotide polymorphisms (SNPs) and indel variations of the Y chromosome have been identified for breeds worldwide [22], and clustering modern horse lineages is accomplished using haplotype data [23]. Several haplotypes have also been found in Chinese indigenous horses [2].

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**Table 1.** Distribution of observed haplotypes in each Japanese native horse population and the corresponding haplotypes in previous reports

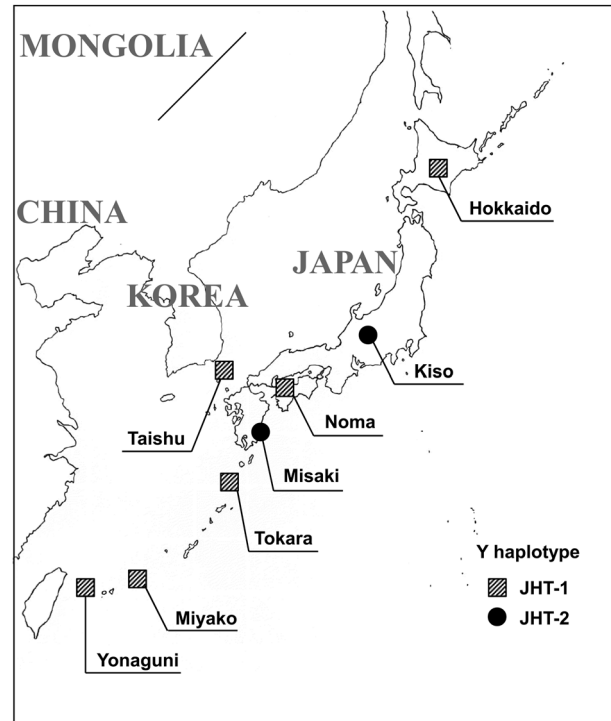
Haplotype	Locus					Local population										Other haplotype nomenclature	
	25345	45288	45701/997	50869	90117	Hokkaido	Kiso	Noma	Taishu	Misaki	Tokara	Miyako	Yonaguni	Wallner	Han <i>et al.</i>		
	N	13	37	7	11	38	16	17	20	<i>et al.</i> [22]	[2]						
JHT-1	G	T	Normal	T	G	13		7	11		16	17	19	HT1	CHT1		
JHT-2	G	T	Normal	A	G		37			38				HT2	CHT3		
JHT-3	G	-	Normal	T	G								1		CHT2		

Therefore, the distribution of Y chromosomal haplotypes in the Japanese native populations was investigated in the present study to complement genetic information obtained in previous studies.

In the present study, blood and hair samples were obtained from 159 male/gelded horses from eight populations: 13 from Hokkaido, 37 from Kiso, 38 from Misaki, 7 from Noma, 11 from Taishu, 16 from Tokara, 17 from Miyako, and 20 from Yonaguni. Sampling was conducted in accordance with the ethical consideration of the research and the welfare of the horses. Genomic DNA was extracted from whole blood using an MFX-2000 MagExtractor System (Toyobo, Osaka, Japan) and from hair follicles using previously reported methods [5]. Haplotyping was carried out using five Y chromosomal loci (Y-25345, Y-45288, Y-45701/997, Y-50869, and Y-90117) reported in previous studies [2, 22]. PCR for the five loci was performed under standard protocols with the reported primers. The SNPs or indels of these loci were analyzed by direct sequencing of the PCR amplicons using an ABI PRISM 3130xl Genetic Analyzer and Sequencing Analysis Software 6 (Thermo Fisher Scientific, Waltham, MA, U.S.A.).

As shown in Table 1, SNP variations were found at only two loci, Y-45288 and Y-50869. The analyzed Y chromosomes were classified into three haplotypes, namely JHT-1, JHT-2, and JHT-3. These were identical to the previously reported haplotypes in breeds worldwide [2, 22]. The Hokkaido, Noma, Taishu, Tokara, and Miyako populations had only JHT-1. In contrast, both the Kiso and Misaki populations had only JHT-2. Although JHT-1 and JHT-3 were observed in the Yonaguni population, parentage verification using the STR panels [6, 19] confirmed that JHT-3 appeared because of a *de novo* mutation of JHT-1. Thus, it is clear that JHT-3 did not originate from the CHT2 haplotype observed in China [2]. At the least, the present data suggests that the ancestral native horses possessed both JHT-1 and JHT-2.

A previous study concluded that HT1 was the ancestral haplotype as inferred by comparison with the Przewalski horse [22] and that Chinese indigenous horses exhibit the CHT1 haplotype frequently (72.04%) [2]. JHT-1 is identical to HT1 and CHT1 (Table 1), and most of the Japanese native populations have the JHT-1 haplotype, which

**Fig. 1.** Geographical distribution of Y chromosome haplotypes, JHT-1 and JHT-2, in the Japanese native horse populations.

is widely distributed throughout Japan (Fig. 1). Thus, it can be assumed that JHT-1 was a major haplotype in the ancestral native horses and became widely distributed and integrated into most of the modern horse populations. This scenario complements the hypothesis from phylogenetic studies based on protein and STR polymorphisms that the Mongolian-type horses were first imported into Japan and that subsequently the ancestral native horses spread across the country [12, 13, 20].

On the other hand, fixation of either JHT-1 or JHT-2 was observed in each population. By increasing the sample size, slight intrapopulation diversity may be uncovered. However, the present observation suggests that each population is strongly biased to integrate into a single haplotype. This result suggests the influence of a significant bottleneck

in the breeding history of each population; genetic drift was traced by STR and mtDNA analyses that showed high inter-population differentiation and low intrapopulation diversity in Japanese native horses [4, 20].

Interestingly, the Kiso and Misaki populations had only JHT-2 in spite of their separate geographic distribution (Fig. 1). In this study, since we could obtain samples from most of the male horses in both populations, the haplotypes of both the Kiso and Misaki populations may be completely fixed into JHT-2. The Misaki horse has been maintained and bred under conditions similar to those in the wild, although its population has decreased [7, 8]. Thus, the fixation of a single haplotype is likely due to random genetic drift. According to a previous report [9], a stallion named *Komatsu-go* that descended from a foreign Trotter breed was introduced into the Misaki population once, in 1913, and was used reproductively for only one year. Such introgression of a different patriline is noteworthy, but those horses that paternally influenced the current population could not be identified. On the other hand, the breeding history of the Kiso horse suggested the influence of particular patrilines. The Kiso horse experienced a rapid population decline and bottleneck in the 1950s–1970s, with only 31 horses remaining, along with the forefather *Daisan Haruyama-go* [16]. The current Kiso horse has likely descended from these ancestral horses. Therefore, it can be assumed that the current population is affected by the genetic influence of the stallion that possessed JHT-2 by chance.

Recently, next-generation sequencing-based analysis for the male-specific region of the equine Y chromosome was developed, and further haplotypes with novel single nucleotide variants and indels were identified [23]. Such technology may help in elucidating the origin of the fixed patriline in each population. Furthermore, not only JHT-2 but also the major JHT-1 haplotype may be classified into more detailed haplotypes, which could lead to an interpretation of the distribution of the populations of ancient Japanese native horses.

In conclusion, this study complements the hypothesis from previous studies that the genetic composition in each local population was derived from independent breeding and genetic drift. In addition, the present haplotype data would be useful for a conservation program based on classification and identification of each population.

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