—Review Article—

Genetic relationship between Miyako and Yonaguni horses native to Okinawa based on polymorphisms of microsatellites

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The Miyako and Yonaguni horses are native horses in Okinawa. Here, we evaluated their genetic relationship using microsatellite data and Kiso horses, which have four subpopulations, as a reference population for evaluating this relationship. Microsatellite data from 35 Miyako, 78 Yonaguni, and 172 Kiso horses were evaluated using the STRUCTURE software for analyzing multilocus genotype data to investigate the population structures and their underlying relationship. The results of the STRUCTURE analysis were stable when ΔK was 2, suggesting that the Okinawan horses are different from the Kiso horses. Moreover, the results were also stable when ΔK was 6; the sample was then divided into four subpopulations of the Kiso horses and two Okinawan horse breeds. However, the diagrams from the STRUCTURE analysis were unstable when ΔK was 3. These results suggest that the genetic relationship of the Okinawan horse breeds may be close, similar to that among the subpopulations of the Kiso horses.

Key words: *genetic relationship, microsatellite, Miyako horse, Yonaguni horse*

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Miyako and Yonaguni horses are two closely-related Okinawan horse breeds, which originated during the reign of the Ryuku Kingdom that ruled Okinawa from 1429 to 1879 C.E. (Fig. 1) [12]. Although ancestors of the Okinawan horses have not been conclusively identified, these two breeds share considerable similarity in terms of physique. However, no genetic data concerning their relationship exists.

The Okinawan horses are considered as unique genetic resources of the islands and are to be conserved [8–11]. The numbers of surviving Miyako and Yonaguni horses in the world at present are around 40 and 120, respectively, and they are classified as "endangered" by the World Watch List for Domestic Animal Diversity, Food and Agricultural Organization [7]. Microsatellite data analysis has revealed that these are inbred breeds, and their genetic diversity is not very high [8, 9]. To prevent possible extinction of these breeds owing to their very small population sizes, conservation strategies must be devised. One such possible strategy could be importing exogenous genes into a population [2]. However, the Okinawan horses might lose their unique characteristics if exogenous genes were imported from horse breeds that are genetically more diverse. Therefore, possible exchange of genes between the Miyako and Yonaguni horses themselves is a more practical approach.

In this study, we evaluated the genetic relationship between the Miyako and Yonaguni horses based on analysis of microsatellite polymorphisms using the STRUCTURE software, which uses multilocus genotype data to investigate population structures, infer the presence of subpopulations, and assign individuals to specific populations [6, 8, 9, 13]. We used the Kiso horse, which is also a native Japanese horse and is known to have four subpopulations [13], as a reference population for the relationship between the two Okinawan horse breeds.

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Fig. 1. Geographic distribution of the Miyako (A), Yonaguni (B), and Kiso horses (C).

Microsatellite Analysis

We analyzed previously acquired microsatellite data from 35 Miyako, 78 Yonaguni, and 172 Kiso horses [8, 9, 13]. In total, 30 microsatellites were genotyped: AHT4, AHT5, ASB2, ASB17, ASB23, CA425, HMS3, HMS6, HMS7, HTG4, HTG10, LEX33, TKY19, TKY28, TKY321, VHL20, TKY279, TKY287, TKY294, TKY297, TKY301, TKY312, TKY325, TKY333, TKY337, TKY341, TKY343, TKY344, TKY374, and TKY394 [4, 8, 9, 14]. Allele discriminations were based on the consensus of the Equine Genetics and Thoroughbred Parentage Testing Workshop, International Society for Animal Genetics (ISAG).

STRUCTURE Analyses for Evaluating the Relationship between the Okinawan Horse Breeds

STRUCTURE analysis of 285 horses, including the Miyako, Yonaguni, and Kiso horses, was performed to evaluate the relationship between the two Okinawan horse breeds. The analysis was performed using STRUCTURE, ver 2.3.4 [6], performing 20 independent runs with 100,000 steps of burn-in and a 100,000-step Markov chain Monte Carlo simulation for each potential number of clusters (K) from K=1 to K=10. The ΔK was calculated using STRUCTURE HARVESTER, ver. 0.6.94 [1]. The procedure was repeated five times to confirm the reliability of the results.

The average ΔKs from K=2 to K=9 were 343.5 ± 64.1, 0.9 ± 0.4, 1.0 ± 0.9, 0.6 ± 0.5, 9.7 ± 5.6, 2.1 ± 1.4, 4.1 ± 3.3, and 0.2 ± 0.1, respectively. ΔK was the highest at K=2; this conclusively proved that the Kiso horses are genetically distinct from Okinawan horses (Fig. 2). When K=6, ΔK was 9.7 ± 5.6, which was the second-highest value; in all the 20 diagrams generated during STRUCTURE analysis with five replications, the total sample of horses was always shown to be divided into the Miyako horses, Yonaguni horses, and four subpopulations of Kiso horses (Fig. 3). However, when K=3, STRUCTURE analysis showed unstable results: among the 20 diagrams generated per analysis, 9.6 ± 2.8 diagrams showed that the horses were divided into two subpopulations within the Kiso horses and the Okinawan horses, and 10.4 ± 2.8 diagrams showed division of the sample into the Kiso, Miyako, and Yonaguni breeds (Fig. 4). These results suggest that the genetic relationship of the Okinawan horse breeds may be close, similar to that among the subpopulations of the Kiso horses.

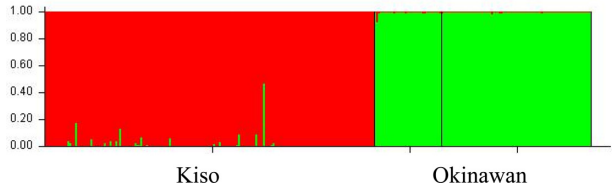

Fig. 2. Classification of the populations of the Miyako, Yonaguni, and Kiso horses using STRUCTURE (K=2). The Okinawan horses (green) are genetically different from the Kiso horses (red).

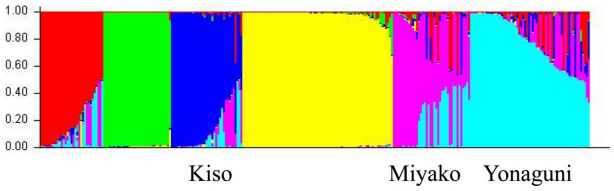

Fig. 3. Classification of the populations of the Miyako, Yonaguni, and Kiso horses using STRUCTURE (K=6). The STRUCTURE analysis showed stable results. The analyzed horses were divided into six subpopulations: four subpopulations of the Kiso horses (red, green, blue, and yellow), of the Miyako horses (pink), and the Yonaguni horses (light blue).

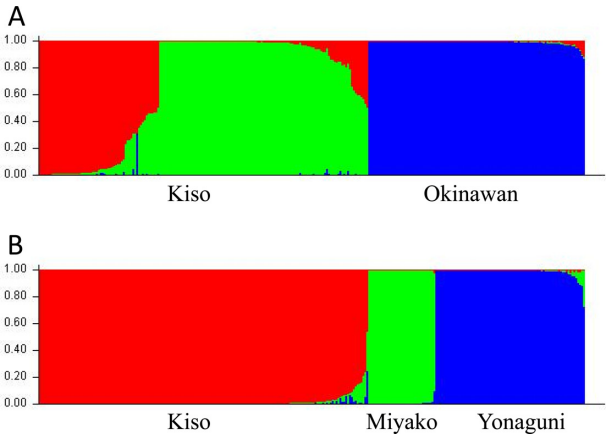

Fig. 4. Classification of the populations of the Miyako, Yonaguni, and Kiso horses using STRUCTURE (K=3). The STRUCTURE analysis showed unstable results. Among the 20 diagrams obtained from the analysis, 9.6 ± 2.8 diagrams divided the sample into two within the Kiso horses (red and green) and the Okinawan horses (blue) (A), while 10.4 ± 2.8 diagrams divided the sample into the Kiso (red), Miyako (green), and Yonaguni (blue) horses (B).

Close Relationship between the Two Okinawan Horse Breeds

The two Okinawan horse breeds, known to have originated during the reign of the Ryukyu Kingdom, have traditionally been known to have a close relationship; however, this relationship remained scientifically unestablished to date. Our data suggested that the two Okinawan horse breeds are genetically close, with a relationship comparable to the relationship among the different subpopulations of the Kiso horses. However, as we only used microsatellite data for genetic indexing, further study of the relationship between the two Okinawan horse breeds using other genetic markers and other indexes, through exhaustive gene analyses for multiple specimens using SNP and full genome sequencing, is needed.

Application of the Findings for Conservation

Microsatellite data analysis suggested a close relationship between the two Okinawan horse breeds, the Miyako and Yonaguni. Although these horses are currently considered to belong to two distinct breeds, grouping them into a single Okinawan horse breed may help in effective conservation, in case the dwindling numbers of these breeds create the threat of extinction. In other words, in the case of an extinction emergency, exchanging genes that impart minimum genetic influence on a population may be a practical approach for effective conservation. This idea to reduce the inbreeding coefficient may also be relatively acceptable for people involved.

Okinawan Horses as Japanese Native Horses

The relationship between the Tokara horses and the Okinawan horses is interesting [3, 5, 15]. Tokara horses are also an island breed of horses, originating in the Satsunan Islands, which are geographically close to Okinawa. Therefore, it is possible that Tokara horses might be genetically close to the Okinawan horses. However, the Tokara and Okinawan horses have different historical and political backgrounds: the Okinawans originated during the reign of the Ryukyu Kingdom, whereas the Tokara horse originated during the time of the Satsuma Domain in Japan [3]. Therefore, it is also possible that the genetic backgrounds of the Tokara and Okinawan horses are completely different. This is an interesting point from the perspective of conservation of native horses that not only have varied biological backgrounds but also distinct socio-histories.

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