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Population Statistics and Biological Traits of Endangered Kiso Horse

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The objective of this study was to clarify the current status of endangered Kiso horse, population statistics and biological traits, in order to take a step for the conservation by scientific approach. We surveyed 125 Kiso horses (86.2% of the whole breed), analyzed the construction of the population, and calculated the coefficient of inbreeding and effective population size. Moreover, we confirmed coat color variations and the traditional traits of the Kiso horse, and measured their height at the withers and chest circumference to clarify their physical characteristics. The population pyramid of the horses was stationary or contractive, suggesting a reduction of the population in the near future. The effective population size of the horse (47.9) suggested that the diversity was much less than their census size, and the high coefficient of inbreeding, 0.11 ± 0.07 on average, suggested that the horses were surely inbred. The horses had only 4 coat colors; bay, dark bay, buckskin dun, and chestnut, and 116 horses (92.8%) were bayish color, suggesting the fixation in their coat color. Moreover, the majority of them had dorsal stripe (83 horses; 66.4%), and the average heights at withers (131.9 ± 4.4 cm) and chest circumference (167.1 ± 10.1 cm) were not significantly different between males and females.

Key words: Biodiversity, Conservation, Japanese, Native horse, Kiso horse

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Domestic animals are bred exclusively to provide for human needs within a local environment, and they are deeply involved in the local culture. In the Kiso area, a mountainous region of central Japan extending from southern Nagano to eastern Gifu prefectures, the Kiso horse has played an important role in the culture of the region [3, 4, 8]. Historically, this horse had cultivated the poor highlands, and had also been used for transportation in rugged mountainous areas. Moreover, newborn foals were sold at high prices, and this income helped to alleviate the hard life of the inhabitants. And so, the horses have been treasured like real children, and have become an important part of

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their life.

So many Kiso horses were kept in the small valley historically, and maximum number of Kiso horse in the literature in 1899 was 6,823, with 58 stallions and 6,765 mares [3]. However, during the World Wars, the government had forced to mate Kiso mares with foreign breeds in order to improve the physiques of the horse for the military purposes, and ordered to castrate all the stallions. As a result, there seemed to be no remaining Kiso stallions after the World War II, but only one stallion named Shinmei dedicated to a shrine was fortunately found intact, and the Kiso horse was saved from extinction. The stallion produced an offspring, Daisan-haruyama, in 1951, and this sole stallion became the ancestor of the current Kiso horses. However, the horse was still in a season of discontent. A

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wave of modernization, motorization and mechanization, undermined the value and use of horses, and the number had gradually declined and finally became 32 in 1976. At that time, horse owners alarmed by a sense of crisis established the Kiso Horse Conservation Association in 1969 [3], and improved the infrastructure for conservation. Today, the number of horses has increased to 149 by dint of the above efforts. However, the population of Kiso horse remains small, and people are concerned about the risks of inbreeding due to the backcrossing of stallions, having mated in a small group.

In 1992, the Convention on Biological Diversity and Agenda 21 at The United Nations Conference on Environment and Development formally identified domestic animal diversity as a genuine and important component of global biodiversity [11]. However, 32 percent of these genetic resources are unfortunately at high risk of loss, and very little is known about most of the breeds involved [11]. The Kiso horse is one of such endangered horses, and is categorized as a "critical" breed that has the highest risk of extinction by The World Watch List for Domestic Animal Diversity, based on analysis of the Global Databank for Farm Animal Genetic Resources by FAO [11].

When we think about conservation of endangered species, effective management to reduce the risk of extinction and better understanding of the biological traits of the animal are essential [1, 2]. In addition, monitoring the population statistics and reporting the current status of the breed is needed in order to identify their unique qualities, and to identify any breed that may have the potential to contribute to a wider variety in the future. In this study, we therefore clarified the current status of the horse, population statistics and biological traits of the breed, for better understanding in order to take a step for their conservation by scientific approach.

Materials and Methods

Population statistics

We surveyed 125 Kiso horses (Fig. 1) confirmed by the Kiso Horse Conservation Association from July 2008 to December 2009. The number of horses registered was 149, and therefore the majority (86.2%) of the whole breed was examined in this study. They were 13 stallions, 16 geldings, and 96 mares, and aged



Fig. 1. Appearance of Kiso horse (A). The Kiso horse is midsized, long-bodied, and short-legged with a plump girth. The current number is 149, and they are categorized as "critical" by WWL-DAD, FAO. The traditional traits of the horse are a dorsal stripe (white arrow) on their back (B) and a knock-kneed appearance (black arrow) of the hind legs (C).

0–29 years. These population data were analyzed by constructing a population pyramid.

The horses were kept at 47 locations from Gunma to Fukuoka prefectures (i.e. northern to southern Japan) (Fig. 2), mainly in Nagano and Gifu prefectures; 76 (60.8%) at 24 places in Nagano and 31 (24.8%) at 13 places in Gifu. In Nagano, the horses were kept mainly in the Kiso region; 58 horses (48.4% of those surveyed, 76.3% in Nagano) at 11 places. Most of the owners had 1 horse (23 owners, 48.9% of those surveyed) or 2 horses (17 owners, 36.2% of those surveyed).

We calculated the coefficient of inbreeding according to the pedigree data based on 4 generations in order to estimate their inbreeding level, and also determined the effective population size (N_e) based on number of males (N_m) and females (N_f) ; $N_e = 4(N_m \cdot N_f) / (N_m + N_f)$ [2]. Moreover, we estimated the required N_e to



Fig. 2. Sampling locales of Kiso horses.

maintain 90% of the heterozygosity for 100 years based on generation length (*L*); Required $N_e = 475/L[2]$.

Biological traits of Kiso horse

To determine their physical characteristics, we confirmed coat color variation [12] and the presence or absence of a dorsal stripe, which is a traditional characteristic of the Kiso horse, and also measured their height at the withers, chest circumference, and cannon circumference.

Eight foals, younger than 3 years were excluded based on the results of height at the withers, chest and cannon circumferences, and dorsal stripe, because they still had the possibility of further growth and change in their physical characteristics.

Statistics

Statistical differences were determined by Student's *t* test. p<0.05 was considered to be significant.

Results

Population statistics

The population pyramid of the Kiso horse assumed a stationary or contractive shape, similar to that in countries with low fertility and low mortality (Fig. 3), and the coefficient of inbreeding was 0.11 ± 0.07 on average ranging from 0.00 to 0.32 (Fig. 4). In addition, the N_e was 45.8, and required N_e was 47.9.

Biological traits of Kiso horse

Whereas there were a variety of coat colors in 1953; bay (43.2%), dark bay (16.0%), chestnut (17.2%), dark chestnut (5%), black (4.5%), buckskin dun (2.5%), palomino (1.2%), and gray (0.2%) [9], current Kiso horses had only 4 coat colors; bay, dark bay, buckskin dun, and chestnut, and each numbered 105 (84.0%), 11 (8.8%), 5 (4.0%), and 4 (3.2%), respectively. A total of 116 horses (92.8%) were bayish, and the uniformity was distinct in their coat color. Moreover, eighty-three (66.4%) had a dorsal stripe, ranging from darker to

Table 1.	Average neight,	cnest circumference, and cannon circumference in Kiso norses	

	Males (n=25)	Females (n=91)	Total (n=116)
Height (cm)	$133.0 \pm 5.1 (122.9 \sim 143.2)$	$131.6 \pm 4.2 (123.2 \sim 140.1)$	131.9±4.4 (123.0~140.8)
Chest circumstance (cm)	168.3 ± 9.3 (149.6 ~ 186.9)	$166.7 \pm 10.3 \ (146.1 \sim 187.3)$	$167.1 \pm 10.1 (146.9 \sim 187.2)$
Cannon circumference (cm)	18.8 ± 1.1 (16.6 ~ 21.0)	18.1 ± 0.9 (16.4 ~ 19.9)	18.3 ± 1.0 (16.3 ~ 20.2)

 10^{-10}

Data are shown as mean \pm standard deviation (mean-2SD ~ mean+2SD). Geldings are included among the males.



Population pyramid of the Kiso horse

Fig. 3. Population pyramid of the Kiso horse. Shape of the pyramid suggests a reduction in the population in the near future.

Number of the horses

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lighter.

 10^{+}

5

The average in height at the withers of the horse was 131.9 ± 4.4 cm, and Kiso horse was mid-sized (Table 1). The average in chest circumference was 167.1 ± 10.1 cm, while the average in cannon circumference was 18.3 ± 1.0 cm. And they were not significantly different between males and females.

Discussion

First of all, our population statistics suggested that the future of the horse does not seem to be very optimistic, because 1) the population of Kiso horse was very small, and a smaller population loses genetic diversity and the ability to adapt to environmental change, 2) the Kiso horse showed stationary or contractive shape in the population pyramid, similar to that in countries with low fertility and low mortality, suggesting a reduction of the population in the near future, 3) the Kiso horse was an inbred, showing a high



Fig. 4. Histogram of the coefficient of inbreeding in Kiso horse. The coefficient was 0.11 ± 0.07 on average, ranging from 0.00 to 0.32.

coefficient of inbreeding (0.11) that was close to grandfather-daughter breeding (0.13) on average and more than 88.6% had a higher value than cousin breeding (0.063), and this tendency of inbreeding which decreases reproductive and survival rates, socalled inbreeding depression [2, 6, 16], gets higher with years, and 4) the aging and heirless of horse owners are concerned to accelerated reduction in the number of horse [3, 4].

Although no one knows precisely how large the population must be to avoid inbreeding depression for fitness over the long term, the N_e to avoid inbreeding depression in the short term, 5–10 generations, based on the experience of animal breeders is said to be >50, and there would be little detectable inbreeding depression when N_e was 50 [2]. Moreover, the required N_e based on generation length to maintain 90% of the heterozygosity for 100 years was estimated here as 47.9. Consequently, whereas N_e of the horse (45.8) was much smaller than the census size, this N_e was close to the criteria for avoiding the inbreeding depression ($N_e > 50$) and the required N_e (47.9), and we therefore have to at least maintain the number of horses in order to

avoid extinction.

Second, our survey suggested the fixation of their physical characteristics. Actually, the fixation is a part of being a domestic animal, because domestic animals were domesticated, developed, and fixed by their traits to provide for human needs within the environments, as human beings evolved and extended the area they lived. This selection for human need and inbreeding causing an obstacle for conserving a breed is only two sides of the same coin, and therefore inbreeding and severe fixation might be unavoidable if the population of the animals becomes so small. Moreover, since Kiso horses have been kept in one or two's in the traditional manner, the individual owners having strong attachment to the horse tend to prefer mating a typical Kiso stallion, facilitating the identification, with their mare to obtain a foal with the classical traits, and this might be accelerating selective breeding.

Also, the historical back ground, rapid reduction of the population, might have affected the fixation. The Kiso horse experienced rapid loss of the population and bottleneck in 1950–70s, and finally remained 31 horses and forefather Daisan-Haruyama. Therefore, the physical traits of current Kiso horse might be naturally affected by genetic influence of their ancestors, and this could be one of the causes of uniformity in coat color and increased rate of traditional traits [9, 14].

Genetically, the bay color is the result of allele combinations of A at the Agouti (A) locus and E at the *Extension* (E) locus, and therefore most Kiso horses in our survey have A and E alleles at such loci. Since more than 90% of the population was bayish, the A and E loci of Kiso horse may be fixed as A/A and E/E [10]. Moreover, all the stallions, approved by the committee as typical Kiso horse, were bay, and so the bay color trend would be much increasing later on.

Third, our survey also suggested current status of purification in physiques of the breed. Owners of Kiso horses backcrossed their horse in order to reconstruct the breed and to exclude genetic influences of foreign breed which were introduced during the Wars to improve Kiso horses for military purposes [3]. Today, Kiso horses become smaller than when they were in 1948, and there were no significant difference between males and females. This result suggested that genetic influence of foreign stallion in physiques is reducing, and Kiso horse might become similar to that when they were in Meiji era [9, 14]. Whereas the downsizing might be an influence of inbreeding, and now we might be achieving the purification in physiques of the breed.

The Kiso horse has definitely played an important role in our culture. However, we might have no answer but come to conclude that the extinction of the horse is obvious if we do not take any action for conservation. They lost their value as a work horse, and so we somehow need to create new ways of application and values yielding some benefit to increase their number. In this point of view, therapeutic riding [7] and other uses of the horse are suggested.

As long as the Kiso horse is a domestic animal, Kiso horse should be kept by the owners in Kiso region as they used to be. However, in a view that so many endangered animals have lost their natural habitat and are being kept in the zoo or other breeding base, the better way to conserve Kiso horse might be to conserve them by the national project, such as Japanese crested ibis. As a matter of course, we scientists need to continuously study on the horse, focusing on genetic diversity of the population and reproductive technology including cryopreservation of germ cells and embryo, and transmit such information toward the society in order to make proposals for the appropriate conservation.

We agree that the biodiversity is important, and Kiso horse is significant as a symbol of the local culture and a genetic resource. This statement sounds right, but few are willing to keep such a time- and money-consuming animal just for appearance' sake, and therefore it is difficult to find the right answer to why we should conserve a useless horse. However, a philosophical argument about the value and meaning of a native horse representing the local culture is still quite important for us, who live in a world heading toward more and more uniformity, to clarify our own identity.

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References

1. Frankham, R. 2003. Genetic and conservation biology. C. R. Biol. 326: S22–29.

- Frankham, R., Ballou, J.D., and Briscoe, D.A. 2010. Introduction to conservation genetics, 2nd ed. Cambridge University Press, Cambridge.
- 3. Ito, M. 1996. Life with Kiso Horses. Kaida Village and Kiso Horse Conservation Association, Kaida (in Japanese).
- 4. Japan Equine Affairs Association. 1984. The Kiso Horse. pp. 51–62. *In:* Japanese Native Horses — Their Preservation and Utilization, Japan Equine Affairs Association, Tokyo (in Japanese).
- Kakoi, H., Tozaki, T., and Gawahara, H. 2007. Molecular analysis using mitochondrial DNA and microsatellites to infer the formation process of Japanese native horse populations. *Biochem. Genet.* 45: 375–395.
- Liberg, O., Andrén, H., Pedersen, H.C., Sand, H., Sejberg, D., Wabakken, P., Åkesson, M., and Bensch, S. 2005. Severe inbreeding depression in a wild wolf *Canis lupus* population. *Biol. Lett.* 1: 17– 20.
- Matsuura, A., Ohta, E., Ueda, K., Nakatsuji, H., and Kondo, S. 2008. Influence of equine conformation on rider oscillation and evaluation of horses for therapeutic riding. *J. Equine Sci.* 19: 9–18.
- Mukoyama, H., Furuta, H., Yoshida, T., Tomogane, H., and Yoshimura, I. 2007. Application of a coat color gene polymorphism to evaluation of the bay monotonous phenomenon in Japanese native horse: Kiso pony. DNA Polymorphism 15: 42–50 (in Japanese).
- Okabe, T. 1953. The Kiso horse. pp. 398–405. *In:* Study on Japanese Native Horses; Especially Hokkaido, Kiso and Misaki Horses. (Okabe, T.,

Matsumoto, K., and Mimura, H. eds.), Japan Society for the Promotion of Science, Tokyo (in Japanese).

- Rieder, S., Taourit, S., Mariat, D., Langlois, B., and Guérin, G. 2001. Mutations in the agouti (ASIP), the extension (MC1R), and the brown (TYRP1) loci and their association to coat color phenotypes in horses (Equus caballus). Mamm. Genome 12: 450-455.
- 11. Scherf, B.D. 2000. World watch list of domestic animal diversity. 3rd ed., Food and Agriculture Organization of the United Nations, Rome.
- Sponenberg, P.D. 2009. Colors built from the basic colors. pp. 39–72. *In:* Equine Color Genetics, 3rd ed., Wiley-Blackwell, Ames.
- Tozaki, T., Takezaki, N., Hasegawa, T., Ishida, N., Kurosawa, M., Tomita, M., Saitou, N., and Mukoyama, H. 2003. Microsatellite variation in Japanese and Asian horses and their phylogenetic relationship using a European horse outgroup. J. Hered. 94: 374–380.
- Tsuji, H., and Yoshida, M. 1984. Body measurements of Kiso horses. *Journal of the Faculty of Agriculture SHINSHU UNIVERSITY* 21: 37–48 (in Japanese).
- 15. Tsuji, H., and Yoshida, M. 1984. The inbreeding coefficient of Kiso horses. *Journal of the Faculty of Agriculture SHINSHU UNIVERSITY* **21:** 103–110 (in Japanese).
- van Eldik, P., van der Waaij, E.H., Ducro, B., Kooper, A.W., Stout, T.A.E., and Colenbrander, B. 2006. Possible negative effects of inbreeding on semen quality in Shetland pony stallions. *Theriogenology* 65: 1159–1170.