Population Dynamics, Herd Behavior, and Genetic Analysis of the Critically Endangered Tamaraw (*Bubalus mindorensis*): A Mini Review

Shinya Ishihara and Yukio Kanai*

Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

The tamaraw (*Bubalus mindorensis*) is a wild buffalo endemic to Island of Mindoro, Philippines and one of the world's critically endangered animals. In the early 1900s the tamaraw could be seen all over the island of Mindoro, but its distribution is now restricted to only three protected areas, with a total estimated population of about 250. The main reasons for its population decline have been unrelenting habitat destruction and illegal hunting. Recent field surveys of the tamaraw have revealed that survival of the tamaraw in the Aruyan Preserve is critical, and the subpopulation in the Mount Iglit-Baco National Park seems to be the only core herd that can be subjected to a practical conservation program. Identification of the tamaraw by fecal DNA analysis would be useful in further studies to verify the animal's ecological behavior, including its existence, range, population dynamics, and genetic diversity.

Key words: tamaraw, population, behavior, fecal DNA, genetic analysis

Introduction

The tamaraw (Bubalus mindorensis) (Fig. 1) is a wild dwarf buffalo endemic to the island of Mindoro in the Philippines. This animal has been categorized as "critically endangered" on the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List (Hedges et al., 2009), and it is listed in Convention on International Trade in Endangered Species Appendix I (UNEP, 2009). The Philippine government prohibited the killing or wounding of tamaraw, or their removal from their habitat, by law as early as 1936. However, the population has remained low because of relentless habitat destruction and poaching. Effective conservation strategies are needed to save the tamaraw from extinction, but such strategies will need ecological information on the animal, including its population dynamics, herd behavior, and genetic diversity. Such information is currently extremely limited. This paper provides a general overview of changes in the tamaraw's population and habitat during the 20th Century. It also describes some of the ecological features of tamaraw, such as its population dynamics and herd behavior, from information in the literature and from recent field surveys jointly organized by the Department of Environment and Natural Resources (DENR) in the Philippines, the Philippine Carabao Center (PCC), and our group (Ishihara *et al.*, 2007, Matsubayashi *et al.*, 2009).

Changes in the Tamaraw Population and Habitat during the 20th Century

The population of the tamaraw in the early 1900s on Mindoro Island was estimated to be around 10,000 individuals, but over-hunting and exploitation of habitat due to human population increases resulted in a drastic decline in the population to less than 1000 by 1949 (Harrison, 1969). As a result, the distribution of tamaraw at the end of the 20th Century was highly fragmented and restricted to three regions of Mindoro Island: the Mount Iglit-Baco National Park (MIBNP), Mount Aruyan Preserve, and Mount Calavite Preserve; MIBNP held the largest tamaraw population. In 1979 the

Received: October 26, 2009, Accepted: December 1, 2009

^{*}Corresponding author: Professor Yukio Kanai, University of Tsukuba, Tennodai 1–1–1, Tsukuba, Ibaraki 305–8572, Japan. Tel: +81–298–53–6685, Fax: +81–298–53–6617, E-mail: kanaiy@sakura.cc.tsukuba.ac.jp



Fig. 1. Tamaraw (Bubalus mindorensis) in MIBNP.

Philippine government took action to conserve the tamaraw as part of its Tamaraw Conservation Program (TCP). As part of this program, in 1999 the DENR initiated an annual survey of the tamaraw population in MIBNP. The annual records of the TCP show that the tamaraw population in MIBNP has remained at 250 for the past decade (Ishihara et al., 2007). Only limited information is available on the other two tamaraw habitats because of the difficulty in accessing these locations and in sighting the animal there (Hedges et al., 2008). Recently, Matsubayashi et al. (2009) conducted a field survey in Mount Aruyan Preserve and found that the tamaraw habitat completely overlaps with the distribution of the local indigenous people, the Mangyan; slash-and-burn farming by the Mangyan has substantially reduced the tamaraw's habitat. Moreover, poaching by outsiders with guns has directly contributed to the severe decline in the animal's population. Although Matsubayashi et al., succeeded in identifying one male tamaraw by camera trap and some individuals by hoof prints, signs of foraging, and the presence of fresh feces, their results indicate that conservation of the tamaraw in the Mount Aruyan habitat is likely to prove extremely difficult. Whether the tamaraw has survived in the Mt. Calavite area is still unknown. Therefore, the tamaraw subpopulation in MIBNP seems to be the only one that can be subjected to a realistic conservation program.

Population Dynamics of Tamaraw in Mount Iglit-Baco National Park

Knowledge of population number is absolutely necessary for conservation biology, because this parameter is the best indicator to decide whether the species is going to the extinction or not (Primack, 1993). MIBNP (75,455 ha) consists of hilly terraces at altitudes ranging between 300 and 1000 m. The tamaraw conservation area (16,000 ha; altitude range 600 to 1,000 m) consists of natural grassland covered with perennial herbage (Fig. 2). PCC and DENR jointly conducted field surveys of the MIBNP tamaraw population in 2006. Direct observations were made simultaneously from multiple vantage points. Sixteen sites were strategically located to minimize blind areas such as trees, tall grass, hills, and valleys. Observations were made simultaneously from 5:30 AM to 7:00 AM and from 5:00 PM to 6:30 PM, because it is known that tamaraw leave their hiding places to graze on grass sprouts when it is cool, around sunrise or The tamaraw that were observed were distinguished by age and sex by the method of Kuehn (1986). The tamaraw sighted at same time and same location from more than 2 observation sites were classified as the same individuals that had been double or multiple-counted and deducted from total counted individuals. Finally, the estimated population size of tamaraw in MIBNP was decided. From this survey, approximately 250 individuals were estimated to be present in MIBNP. Thus the tamaraw population was still in the critical range. There was also a considerable deviation in the sex ratio of adult animals and in the proportion of juveniles to yearlings (Table 1; Ishihara et al., 2007). In the observed adult tamaraw, there were significantly more females than males, and the juvenile individual numbers were lower than expected based on the yearling numbers. This may be because some tamaraw such as solitary bulls or juvenile males are driven out from the family and become distributed in the outer areas of MIBNP.

Herd Behavior of Tamaraw

An understanding of the herd behavior, such as the mating system, of a species is important in both in situ and ex situ conservation. In situ, rare animals tend to suffer inbreeding depression be-



Fig. 2. Representative landscapes in MIBNP.

Table 1. Individual numbers of tamaraw in Mount Iglit-Baco National Park in 2006 (Ishihara *et al.*, 2007)

	Adult	Juvenile	Yearling	Total
Mele	53	8	2	63
Female	102	12	1	115
Unidentified	7	32	46	85
Total	162	52	49	263

Adults, estimated as more than 5 years old; juveniles, estimated between 1 and 4 years old; yearlings, estimated at less than 1 year old.

cause of their small populations. Inbreeding is associated with fitness deficit, which may further reduce the effective size of already small populations (Saccheri et al., 1999). Mating systems such as monogynous, polygynous, and polyandry systems influence relatedness levels and have large effects on effective population size, which is an

indicator of a population's ability to maintain its genetic diversity (Parker and Waite, 1997). In ex situ systems an understanding of mating systems is very important for reproductive management in captivity. Since 1982, the Philippine government has captured 20 tamaraw individuals in their habitat and has attempted to breed them in captivity. However, this first trial of captive breeding was unsuccessful because of a lack of information on the animal's ecological behavior, especially in relation to its mating system. At present, only one captured female is still alive, with her one descendant male.

Kuehn (1986) examined the social behavior of the tamaraw in MIBNP and reported that, out of 218 observations during 221 days, 172 (82%) were lone bulls. He deduced that the tamaraw was originally forest-adapted, because forest-dwelling ungulates are largely solitary or monogamous (Geist, 1974). Ishihara *et al.* (2007) also studied the herd behavior of tamaraw in MIBNP. They classified tamaraw sightings into single animals or herds.

The observed herds of tamaraw were further classified into seven types based on the ratios of bull to cows: monogamous (one bull and one cow), polygamous (one bull and two cows or one bull and three cows), and so on. Our study showed that the majority of the tamaraw (89%) lived in families ranging in size from two to seven individuals and the rest (11%) were solitary (mostly bulls). The ratio of monogamous to polygamous families was 16 to 20. These results may suggest that the tamaraw has changed its mating system from forest-dwelling type to grassland-dwelling type between 1986 and 2007 by adapting its behavior to new environments.

Genetic Analysis of Tamaraw

As techniques of molecular analysis have developed, DNA has become a useful tool in conservation biology and wildlife management in terms of acquiring valuable information on behavioral and ecological features. For example, phylogeographic analysis using molecular techniques can reveal evidence of both ancient and recent demographic events, such as population size change or dispersal (Avise, 2000). Mitochondrial DNA (mt-DNA) evolves much faster than nuclear DNA and thus contains greater sequence diversity than nuclear DNA (Brown et al., 1979). For example, mt-DNA cytochrome b has been used in species identification and phylogeographic analysis (Tanaka et al., 1996; Paxinos et al., 1997), and the D-loop has been used in studies of genetic diversity and in phylogeographic analysis (Lau et al., 1998). Microsatellites have also been used to acquire ecological information. Garnier et al. (2001) studied the mating system of the black rhinoceros by using microsatellites. However, it is difficult to collect DNA directly from rare animals like the tamaraw.

Fecal samples are very useful for collecting DNA noninvasively (Höss et al., 1992) because their surfaces contain intestinal epithelial cells. Tamaraw feces can be found easier than tamaraw individuals in MIBNP. Ishihara et al. (2010) developed a method for identifying tamaraw from feces collected in the wild by using the cytochrome b gene. This method offers promise for verifying the existence of tamaraw in the field in areas where it is hoped that they still exist. If the feces are verified as originating from the tamaraw, D-loop analysis of fecal samples can then be used as a powerful tool for

genetic diversity analysis, as has been used in some rare wild animals (Russello et al., 2004).

Conclusion

Despite worldwide concern and protection by the Philippine government, the tamaraw is still threatened by extinction, as shown by its distribution and population size. At present, the tamaraw subpopulation in MIBNP is the only one targetable by a practical conservation program, and continuous and wider ranging surveys are needed in this preserve. To make the tamaraw conservation program more effective, precise assessments of their ecological behaviors (e.g. habitat selection, mating behavior, and genetic diversity) are urgently needed.

References

- Avise, J.C., 2000. Phylogeography: the History and Formation of Species. Harvard University Press, Cambridge MA.
- Brown, W.M., M. George, and A.C. Wilson., 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy Science (USA) 76, 1967-1971.
- Garnier, J.N., Bruford, M.W. and Goossens, B., 2001. Mating system and reproductive skew in the black rhinoceros. Molecular Ecology 102031-102041.
- Geist, V., 1974. On the relationship of social evolution and ecology in ungulates. American Zoologist 14, 205-220.
- Harrison, T., 1969. The Tamaraw and its Survival. The tamaraw and its survival. International Union for the Conservation of Nature and Natural Resources (New Series), 2: 85-86.
- Hedges, S., Duckworth, J.W., de Leon, J., Custodio, C. and Gonzales, J.C., 2008. Bubalus mindorensis. In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.1. Retrieved on 06 June 2009 from http:// www.iucnredlist.org/>.
- Höss, M., Kohn, M., Pääbo, S., Knauer, F. and Schröder, W., 1992. Excrement analysis by PCR. Nature 359, 199.
- Ishihara, S., Ishida, A., Del Barrio, A.N., Lapitan, R.M., Atabay, E., Boyles, R.M., Salac, R.L., de Leon, J.L., Eduarte, M.M., Cruz, L.C. and Kanai, Y., 2007. Field survey on Tamaraw (Bubalus mindorensis) — Present population size and herd behavior in wild. Italian Journal of Animal Science 6 (suppl. 2), 1249-1251.
- Ishihara, S., Herrela, R.J., Ijiri, D., Matsubayashi, H., Hirabayashi, M., Del Barrio, A.N., Boyles, R.M., Eduarte, M.M., Salac, R.L., Cruz, L.C. and Kanai, Y., 2010. Identification of tamaraw (Bubalus mindorensis) from outdoor-derived fecal samples by PCR-RFLP analysis of cytochrome b gene. Animal Science Journal (under review).
- Kuehn, D.W., 1986. Population and social characteristics of the tamarao (Bubalus mindorensis). Biotropica 18, 263-

266.

- Lau, C.H., Drinkwater, R.D., Yusoff, K., Tan, S.G., Hetzel, D.J. and Barker, J.S., 1998. Genetic diversity of Asian water buffalo (*Bubalus bubalis*): mitochondrial DNA D-loop and cytochrome b sequence variation. Animal Genetics 29, 253–264.
- Matsubayashi, H., Boyles, R.M., Salac, R.L., Del Barrio, A. N., Cruz, L.C., Garcia, R.A., Ishihara, S. and Kanai, Y., 2009. Present status of tamaraw (*Bubalus mindorensis*) in Mt. Aruyan, Mindoro, Philippines. Tropics 18, in press.
- Parker, P.G. and Waite, T.A., 1997. Mating systems, effective population size, and conservation of natural populations. In: Clemmons JR, Buchholz R (eds), Behavioural Approaches to Conservation in the Wild, 243–261. Cambridge University Press, Cambridge.
- Paxinos, E., McIntosh, C., Ralls, K. and Fleischer, R., 1997. A noninvasive method for distinguishing among canid species: amplification and enzyme restriction of DNA from dung. Molecular Ecology 6, 483–486.
- Primack, R.B., 1993. Essentials of Conservation Biology. Sinauer Associates, Sunderland, MA.
- Russello, M.A., Gladyshev, E., Miquelle, D. and Caccone,

- A. 2004. Potential genetic consequences of a recent bottleneck in the Amur tiger of. Conservation Genetics. 5, 707–713.
- Saccheri, I.J., Wilson, I.J., Nichols, R.A., Bruford, M.W. and Brakefield, P.M., 1999. Inbreeding of bottlenecked butterfly populations: estimation using the likelihood of changes in marker allele frequencies. Genetics 151, 1053–1063.
- Tanaka, K., Solis, C.D., Masangkay, J.S., Maeda, K., Kawamoto, Y. and Namikawa, T., 1996. Phylogenetic relationship among all living species of the genus *Bubalus* based on DNA sequences of the cytochrome b gene. Biochemical Genetics 34, 443–452.
- UNEP-WCMC. "Bubalus mindorensis". UNEP-WCMC Species Database: CITES-Listed Species. United Nations Environment Programme World Conservation Monitoring Centre. Retrieved on 22 May 2009 from http://www.cites.org.
- White, P.S. and Densmore III, L.D., 1992. Mitochondrial DNA isolation. In: Hoelzel AR (ed), Molecular Genetic Analysis of Population: A Practical Approach, 2 nd edn, 29–58. IRL Press, Oxford.