Genetic diversity and estimated geographical origin of captive critically endangered black rhinoceros in Japan: Implication for future conservation and breeding

Mohamed M.S. Rijja[1,2,3]*, Yu Sato[3], Sherif I. Ramadan[4] & Miho Inoue-Murayama[3]
[1]Graduate School of Science, Kyoto University, Japan
[2] Wildlife DNA Forensic Laboratory, Government Chemist Laboratory Authority, Tanzania
[3]Wildlife Research Center, Kyoto University, Japan
[4]Faculty of Veterinary Medicine, Benha University, Egypt
*mohamedrijja@gmail.com

Introduction

Black rhinoceros (*Diceros birconis*) are on the verge of extinction (Lacy, 2019).

- Currently listed as critically endangered due to fewer remaining individuals caused by poaching and habitat loss.
- Captive breeding programs play a vital role as insurance for wild populations (McGowan et al., 2017).

However, the success of captivity depends on maintaining genetically healthy individuals (Gaines et al., 2010)

Purpose: To assess the genetic diversity and potential geographical origin of almost all captive individuals in Japan to propose future conservation and management interventions.

Materials and Methods

- **Target species**

Black rhinoceros in 10 Japanese zoos. (9 males, 12 females)

- **DNA extraction**
  - Blood & Muscle tissue (*n* =12) [DNeasy Blood & Tissue Kit (QIAGEN)]
  - Feces (*n* = 9) [QIAMP Fast Stool Kit (QIAGEN)]
- **Genotyping**
- **Mitochondrial control region (D-loop) [477 bp].**

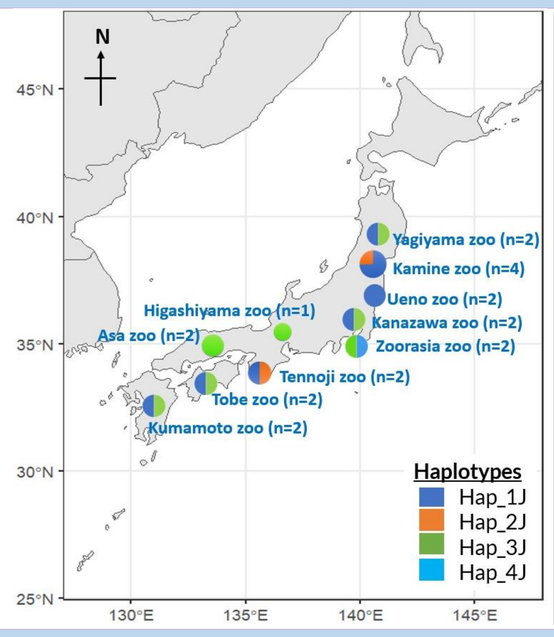
→ mt15996L (F primer): 5' TCCACCATCAGCACCCAAAGC 3'
→ mt16502H (R primer): 3' TTTGATGGCCCTGAAGTAAGAACCA 5' (Moodley et al., 2017)

- **Microsatellite markers**

→ 11 microsatellite loci were analyzed (Moodley et al., 2017)

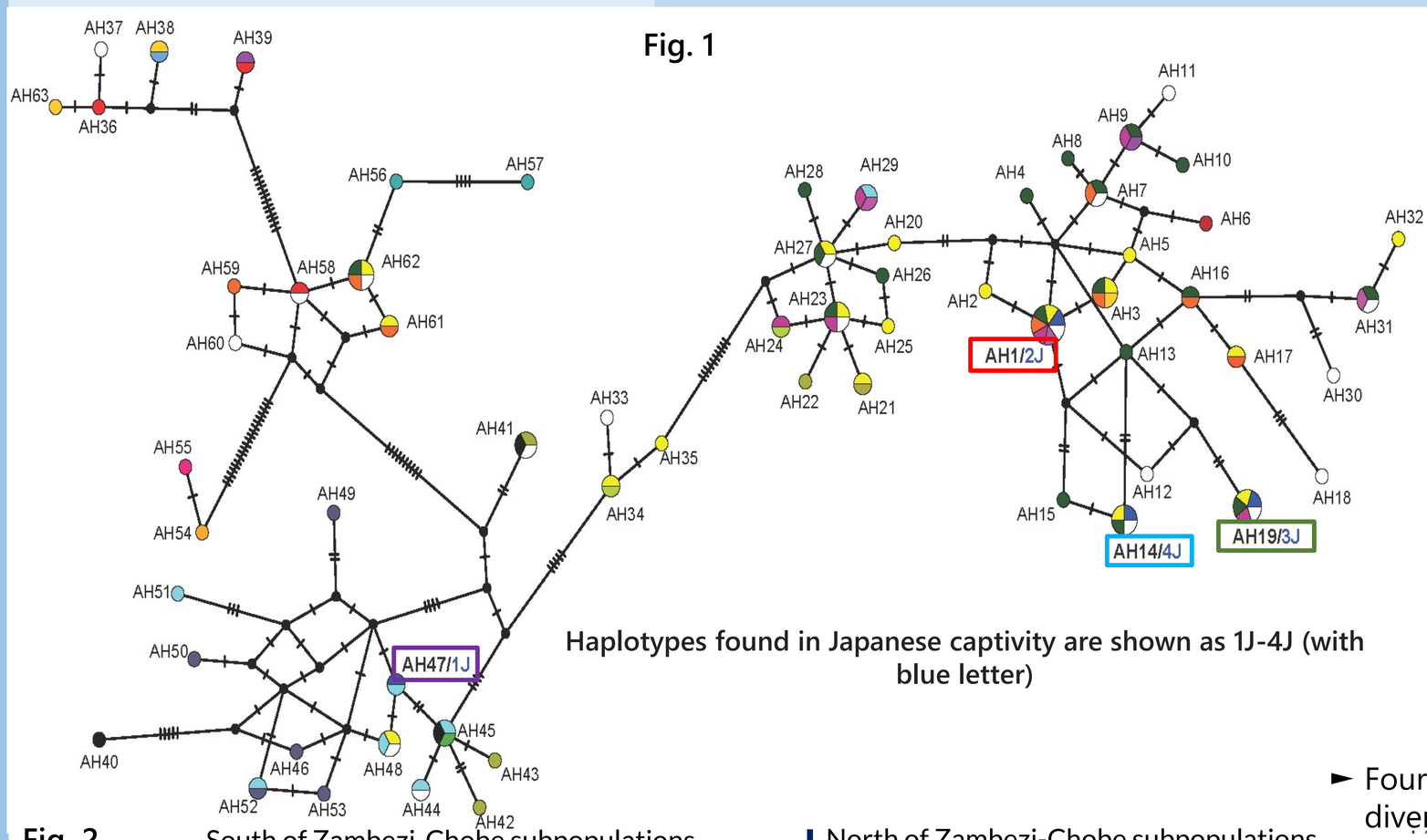
- **Data analysis and Phylogenetic analysis**

→ MEGA 11 (Tamura et al., 2021).
→ DnaSP v6 (Rozas et al., 2017).
→ PopART v1.7 (Leigh & Bryant, 2015).
→ Structure v2.3.4 (Hubisz et al., 2009).
→ GenAlEx 6.5 (Peakall & Smouse, 2011).

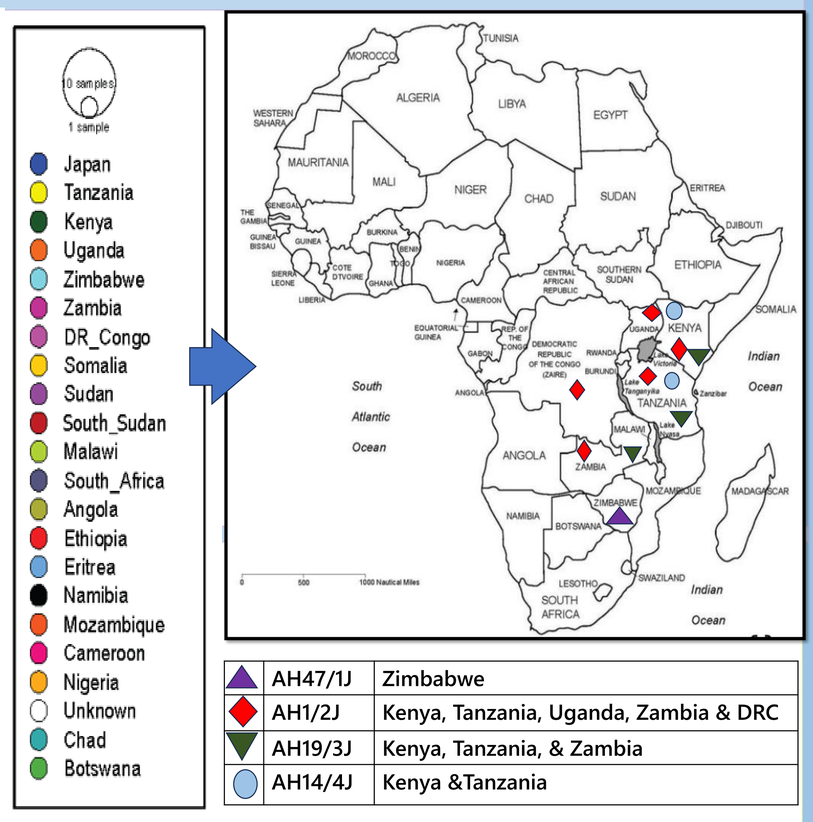


Results and Discussion

Fig. 1

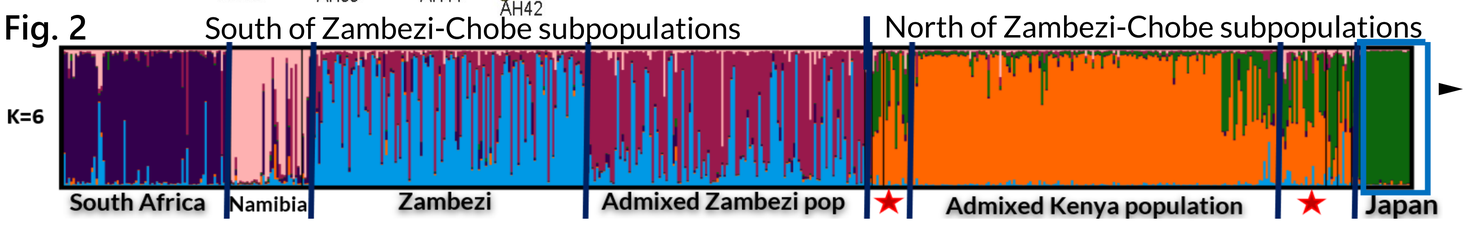


Haplotypes found in Japanese captivity are shown as 1J-4J (with blue letter)



▲	AH47/1J	Zimbabwe
◆	AH1/2J	Kenya, Tanzania, Uganda, Zambia & DRC
▼	AH19/3J	Kenya, Tanzania, & Zambia
●	AH14/4J	Kenya &Tanzania

Fig. 2



- Four haplotypes of maternal lineage with haplotype diversity of ***h* = 0.65** and STR loci with nuclear diversity of ***Ho* = 0.75** were identified.
- Improved nDNA diversity was observed with STR data and a slight loss of mtDNA diversity when compared with reported wild haplotype (***h* = 0.94**) and nuclear (***Ho* = 0.66**) variations.

→ Both mtDNA & nDNA estimated the geographical origin of captive black rhinoceros in Japan to **Kenya, Tanzania & DR of Congo (Fig 1 & 2)**
→ Reduction in mtDNA diversity can be attributed to decreased effective population size in the wild due to past genetic bottlenecks.
→ Slightly high nDNA diversity in this gene pool suggests that captive breeding practices have restored previously lost wild gene variants.
→ Selective breeding through pedigree-assisted pairing of individuals for mating, contributed to restoring genetic diversity and reducing inbreeding.
→ Captive black rhinoceros in Japan have East African origin of the *D.b michaeli* subspecies as they share a genetic ancestor.

Conclusion

1. The gene pool in Japan has the potential for genetic diversity's self-enhancement without external supplementation.
2. The current level of diversity should be maintained and further improved by allowing additional variation by re-introducing genetically diverse individuals.
3. These captive individuals provide ideal translocation or reintroduction candidates to other breeding programs with critically low genetic diversity

Acknowledgments

Our sincere appreciation to the Government of Japan (MEXT) for funding overall studies and Leading Program in Wildlife and Primatology (PWS) for sponsoring academic activities of the first author. We are very thankful to Japanese zoos for provision of specimens and Ms. Hiromi Kobayashi for unconditional help during the course of this work.