

# Genetic diversity studies of endangered Grevy's zebra (*Equus grevyi*) in the captivity

Hideyuki Ito

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### Chapter 1. General introduction

The Grevy's zebra (*Equus grevyi*) inhabits arid to semi-arid regions in northern Kenya and Ethiopia. During the past few decades, the species has suffered a severe decline due to destruction of a habitat, overhunting and competition with livestock. The wild population is estimated to number about 2,800 and 508 are kept in captivity. This equid is one of the most endangered wild equids in the world, and is subject to an important captive breeding programme. However until now, there has been little information about the genetic diversity of the Grevy's zebra. Furthermore, hybridization between the Grevy's zebra and the plains zebra (*Equus quagga*) has been documented, leading to a requirement for conservation genetic management within and between these related species. Also, animals in the captivity are kept and cared by human, so understanding personality of each individual can enable better routine management of them. So, understanding of personality is important to manage not only individuals but also population. In this study, I aimed to develop microsatellite markers that can be useful for the characterization of genetic diversity of endangered Grevy's zebra to manage captive populations and to conserve wild populations. As far as I know, this study is the first attempt to develop molecular markers based on wild equid species. This study is organized into two parts. In chapter 2 of this thesis, I developed novel microsatellite markers in the Grevy's zebra using next-generation sequencing, and considered usefulness of developed markers (identification individuals, species and subspecies, and detection hybrid individuals), and determined the genetic diversity of Grevy's zebra populations using mitochondrial DNA and developed microsatellite markers. In chapter 3, I investigated the polymorphisms within androgen receptor (*AR*) gene, to clarify genetic information about *AR* gene in three zebra. This information will provide a foundation for developing genetic conservation programs of Grevy's zebra in captivity, and application tool to wild population.

### Chapter 2. Evaluation of genetic diversity with DNA markers in Grevy's zebra

#### 2.1 Using next generation sequencing to develop microsatellite markers for the endangered Grevy's zebra

Today the highly endangered Grevy's zebra inhabits only Kenya and Ethiopia. It is necessary to plan a conservation program that includes genetic management. However, there is little

information on Grevy's zebra genetics until now. I developed 21 novel microsatellite markers for the Grevy's zebra using next-generation sequencing. Forty-one Grevy's zebra individuals in captivity were analyzed. The number of alleles, observed and expected heterozygosity ranged from 2 to 9 (mean 4.6), 0.075-0.854 (mean 0.523) and 0.072-0.784 (mean 0.529), respectively. Cumulative probability of identity among siblings for all loci was  $2.78 \times 10^{-6}$ . I developed the first microsatellite marker derived from the Grevy's zebra genome. These novel microsatellite loci will be useful to study in population genetics, individual identification and parentage studies for conservation.

## 2.2 Population genetic diversity and hybrid detection in captive zebras

I developed 28 microsatellite markers in Grevy's zebra and assessed cross-amplification in the two other zebra species. The average expected heterozygosity in Grevy's zebra, plains zebra and mountain zebra (*E. zebra*) was 0.479, 0.595 and 0.531, respectively. Cumulative probability of identity over all loci in Grevy's zebra, plains zebra and mountain zebra was  $1.06 \times 10^{-6}$ ,  $5.67 \times 10^{-9}$  and  $9.77 \times 10^{-6}$ , respectively, indicating that these markers should be capable of identifying closely related individuals. These markers are useful for clarifying genetic structure among the three zebra species, identify species and subspecies, and discriminate endangered Grevy's zebra from stimulated hybridized individuals that may be mixed with Grevy's zebra in captivity. I believe that the microsatellite markers in this study will be useful for conservation of endangered zebra species in the wild and captivity.

## 2.3 Mitochondrial DNA sequence diversity of Grevy's zebra for management in captivity

In this Chapter, I examined the genetic diversity of the captive population of Grevy's zebra by analyzing the *cytochrome b* (*cytb*) and control region sequences of mitochondrial DNA (mtDNA). In addition, for comparative purposes, I investigated the same genetic markers in the congeneric species of plains zebra and mountain zebra. Even though there were more samples from the Grevy's zebra, the Grevy's zebra was found to have the lowest index of molecular genetic diversity compared to the plains zebra and mountain zebra at *cytb* and control region sequences. However, these results were inconsistent with pedigree data. The low genetic diversity of Grevy's zebra in captivity is likely to be derived from low genetic diversity of the founding population. Furthermore, these results may indicate that a reduction in genetic diversity in the wild populations occurred before the drastic population declines. Also, the mtDNA regions analyzed had extremely low genetic diversity, limiting their usefulness as tools for genetic management of captive Grevy's zebra. In this study, I showed that the genetic diversity of mtDNA in Grevy's zebra was low compared to congeneric zebras in captivity. Importantly, the comparative molecular data for Grevy's and mountain zebra contradict the expected relative genetic diversity in these species estimated from pedigree analysis of studbook records.

## 3. Androgen receptor gene polymorphism in zebra species

Androgen receptor (*AR*) genes have been found to have associations with reproductive development, behavioural traits, and disorders in humans. However, the influence of similar genetic effects on the behaviour of other animals is scarce. I examined the loci *AR* glutamine repeat (*ARQ*) in three zebra species and compared them with those of domesticated horses. I observed polymorphism among zebra species and between zebra and horse. I found that locus *ARQ* in three zebra species had longer alleles than that in horses. As zebras have not been domesticated it might be expected that they are more aggressive than horses. As androgens such as testosterone influence aggressiveness, *AR* polymorphism among equid species may be associated with differences in levels of aggression and tameness. My findings indicate that it would be useful to conduct further studies focusing on the potential association between *AR* and personality traits, and to understand domestication of equid species.

#### **4. GENERAL DISCUSSION AND FINAL REMARKS**

In this study, partial genome sequence data of Grevy's zebra that were obtained using NGS were screened for microsatellite repeats, and I developed 28 novel microsatellite markers. These de novo microsatellite markers will be useful in the conservation for the Grevy's zebra in the wild and the captivity. Moreover their discrimination ability will be used to investigate social system and ecology. The inconsistency between genetic diversity of pedigree data and genetic diversity that showed in this study indicate the importance of integrating two methods. This finding is first example showing the inconsistency, and will lead to develop more moderate breeding plan for the Grevy's zebra. The polymorphism that I showed in this study is the first finding of the polymorphism of *AR* in zebra species, and in non-domesticated equid as far as I know. These finding may be first step to discover relation between *AR* and phenotype, and may lead to develop keeping methods that moderate for each individual, and population management plans (such as developing bachelor group).