

Genetic research into the Japanese golden eagle (*Aquila chrysaetos japonica*) for conservation management

Yu SATO

CONTENTS

Chapter 1. General introduction.....	1
Chapter 2. Genetic diversity of Japanese golden eagles.....	2
Chapter 3. Sustainability of Japanese golden eagles.....	2
Chapter 4. Population genomics of three golden eagle subspecies.....	2
Chapter 5. General discussion and final remarks.....	3

Chapter 1. General introduction

The golden eagle (*Aquila chrysaetos*) has six sub-species found across a circumpolar distribution in the temperate northern hemisphere. The Japanese golden eagle (*A. c. japonica*) is one of sub-species, distributed only in Japan and possibly a part of the Korean Peninsula. There are now estimated to be only around 500 individuals in Japan. The Japanese Ministry of the Environment has been categorized this as endangered. Ecologies of this bird has been studied for more than 40 years in the wild, and decreasing of the number of individuals is concerned. Captive breeding program has also been acted from 1997 with a total of 16 founder birds, and forty eight Japanese golden eagle individuals are alive in nine zoos at 2018 as *ex situ* conservation. To conserve the animals which has genetic risks to the extinction such as loss of genetic diversity, or inbreeding depression, reintroduction from the other population is one of the solution for genetic rescue. In case of Japanese golden eagle, captive population must be a source population. However, loss of genetic diversity and inbreeding has been concerned in both wild and captive, and there is no empirical evidence and no studies to examine individual relatedness. In this study, I tried to reveal the genetic diversity of Japanese golden eagle and their sustainability. In Chapter 2 of this thesis, genetic diversity of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) were analyzed to assess the genetic status. Additionally, the diversity was compared with other non-endangered subspecies, American and Scottish golden eagles (*A. c. crysaetos* and *A. c. canadensis*), to consider the urgency of conservation. In Chapter 3, the sustainability of captive population was simulated under population viability analysis (PVA) to forecast their demographic and genetic trajectories. In Chapter 4, population genomic analysis was conducted to reveal the fluctuation of effective population size in the past for evaluating the current and future population decreasing.

Chapter 2. Genetic diversity of Japanese golden eagles

For analyzing genetic diversity, this study used haplotypes of mtDNA Control Region and pseudo Control Region or sixteen microsatellite markers of nDNA. This study analyzed fifty-one samples obtained from the wild population (Iwate prefecture $n = 46$, Tochigi prefecture $n = 3$, Aomori prefecture $n = 2$) and twenty samples obtained from the captive population including nine wild origin founder birds and eleven captive bred birds. As a result, it was revealed that Japanese golden eagle of both wild and captive have maintained relatively high genetic diversity even compared with other subspecies. On the other hand, wild population showed higher inbreeding coefficient ($F = 0.08$) than that of captive (-0.07) in Japan, and that was almost similar value as that of Scottish (0.09) which experienced bottleneck. These results suggested that inbreeding might be promoted rapidly after generation changes by limited numbers of offsprings in future. It is important to maintain current diversity, and effective conservation managements should be started as soon as possible.

Chapter 3. Sustainability of Japanese golden eagles

Chapter 2 revealed that captive (zoo) Japanese golden eagle population maintains roughly equivalent levels of diversity with wild population. For improving the situation of the population declining in the wild, one candidate approach is reintroduction from captivity. It was required to reveal the sustainability under current breeding program of Japanese golden eagle, and I tried to reveal this by simulation under PVA with actual captive situation. In the result, it was suggested that current captive population will be extinct within 156 years. For long term sustainability (> 200 years), combination of two strategies were essential; increasing the number of mating pairs for expanding the population size, and supplementation of some birds from wild for maintaining genetic diversity.

Chapter 4. Population genomics of three golden eagle subspecies

Effective population size (N_e) is one of the number of individuals which was calculated from genetic diversity, and it is one of the most important factors for understanding genetic diversity because N_e refers the historical genetic variation of breeding individuals who contribute to maintain or change of genetic diversity. Recently, it becomes possible to estimate the history of change in N_e over time from genomic information of a diploid individual by pairwise sequential Markovian coalescent (PSMC) model. I tried to reveal the fluctuation of N_e in the past of three golden eagle subspecies (Japanese, American, and Scottish). The PSMC results revealed that the effective population size of three

golden eagle subspecies have been decreased from about 0.1 Mya. The N_e of Japanese reached to 5,000 individuals, that of American was 6,000, and Scottish was 2,000 in 10 kya. Additionally, it was also suggested that N_e of American golden eagle has been decreased from about 6,000 in 10 kya to 400 at present. Because the genetic diversity of Japanese golden eagle is lower than that of American, this endangered Japanese subspecies is probably facing much more critical situation to extinction. In addition, genomic difference rate among three subspecies were also calculated, and that of Japanese–American was 0.14 % and Japanese–Scottish was 0.17 %. This indicating that American golden eagle is more related to Japanese than Scottish. Although careful and adequate consideration must be required, relocation from other subspecies is probably one of the approach for conservation of Japanese golden eagles.

Chapter 5. General discussion and final remarks

This study revealed that the endangered Japanese golden eagle has maintained relatively high genetic diversity within mtDNA and nDNA even compared with other subspecies. However it is also suggested that the future possibility of inbreeding in wild. For conservation of the animals which has genetic risks to the extinction, reintroduction from the other population is an approach as genetic rescue, but source population is required to be genetically sustainable. In case of Japanese golden eagle, captive population carries the role of *ex situ* conservation, but it was also revealed that captivity is not sustainable. Reintroduction from captive should be conducted for conserving wild Japanese golden eagles, but supplementation from wild to captive is also important for sustainability of captivity. This study suggests that the importance of circle-like relationship between wild (*in situ*) and captive (*ex situ*) through reintroduction and supplementation for conservation of endangered Japanese golden eagle. Supplementation of new individuals from wild to captive seems to be a topic sometimes among zookeepers, but no research has investigated the effect to sustainability of captivity. This study is probably a first report about importance of a close relationship between introduction and supplementation for conservation of endangered wildlife. However, in case of Japanese golden eagle, current endangered situation has been caused by not only genetics but also by some environmental factors such as degradation of habitual environment. Improving these problems is also important and essential for conservation. It is required to strengthen collaborations among administrations, zoos, field conservationists, and conservation geneticists for conservation of Japanese golden eagle.