Genetic Analyses in the Japanese Captive Population of Japanese Crested Ibis (\textit{Nipponia nippon}) using Pedigree Information

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The Japanese captive population of Japanese crested ibis (\textit{Nipponia nippon}) was established using 5 founders derived from the Chinese captive population. Its size has increased rapidly, and the maintenance phase is about to start. Thus, this study was designed to perform genetic analyses in this population with pedigree information, considering the adoption of mean kinship strategy as the breeding strategy suited to the maintenance phase. Because the relationships among the 5 founders were unknown, different assumptions were set up ranging from 0 to 0.25 of kinship coefficients between the 5 founders. Assuming that the 5 founders were non-inbred in all the assumptions, the results showed that the gene diversity and the mean inbreeding coefficient would fluctuate largely from \( \sim 65\% \) to \( \sim 82\% \) and from \( \sim 0.07 \) to \( \sim 0.29 \), respectively. Moreover, the genetic importance of individuals based on mean kinship shifted largely. This study suggested that the Japanese captive population had low gene diversity and high mean inbreeding coefficient even under the assumption that the 5 founders were unrelated and non-inbred. In addition, the study also suggested that it became more effective to analyze the genetic status and to introduce mean kinship strategy into this population with more credible molecular evaluation of the relationships among founders.

Key words: founder, genetic diversity, Japanese crested ibis (\textit{Nipponia nippon}), mean kinship, pedigree information

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Introduction

Although Japanese crested ibis (\textit{Nipponia nippon}) ranged over much of Japan in the Edo period (Yasuda, 1988), after the Meiji period, excessive hunting and environmental deterioration decimated this species (Yasuda, 1984). In 1981, it completely disappeared from the wild (Japanese Ministry of the Environment, 2002). Artificial breeding of the individuals brought into captivity was unsuccessful (Niigata Prefectural Government, 2000). Thus, the last individual indigenous to Japan died in captivity in 2003.

The habitats of this species were also widespread throughout China, Russia, the Korean Peninsula and Taiwan in the beginning of the 20th century (BirdLife International, 2001), however, these populations also began to decline after the middle of the 20\textsuperscript{th} century. Thus, it was thought to have become extinct in these countries, until 7 individuals composed of 2 pairs and 3 nestlings which were the offspring of 1 pair of them were rediscovered in China in 1981 (Yasuda, 1985). In China, the various projects for the conservation in the wild and captivity have been launched since 1981 (Ding, 2007), resulting in the wild population of about 1000 individuals and the captive population of about 600 individuals as of 2012.

In 1999, the Chinese government first gifted 1 pair to Japan. After that, 1 individual and 2 individuals were provided from the Chinese government in 2000 and 2007, respectively. As a national project, Japan has constructed the captive population in which these 5 individuals were used as founders. As of June 2013, there were 212 individuals in this population, and then, a total of 125 individuals were reintroduced into the wild since 2008.

The size of the Japanese captive population has increased rapidly (Wajiki \textit{et al.}, 2014), reaching the number of individuals which was thought to be sustainable for this population. Therefore, this population is about to make the transition from the growth phase to the maintenance phase.

Although recovery of lost alleles depends only on introductions of additional founders or mutation, the disparities in founder allele frequencies due either to random drift or to selection are partly reversed depending on preferential
breeding of individuals having unique or rare alleles during the maintenance phase (Lacy, 1994). The individual mean kinship (MK) enables us to find individuals that are most likely to have such alleles and to construct the breeding strategy, named as MK strategy, to select them (namely, individuals with low MK) as mating pairs (Ballou and Lacy, 1995). The efficacy of MK strategy was confirmed by a simulation study (Ballou and Lacy, 1995) and an experimental study using Drosophila melanogaster (Montgomery et al., 1997). Therefore, MK strategy is commonly used in the present captive breeding programs.

In this study, we assessed the genetic status of this population, considering the adoption of MK strategy. For the purpose, molecular markers were thought to be very useful, and the amplifiable and polymorphic microsatellite markers of this species had been developed (Urano et al., 2013). However, the number of them was still small. Thus, in this study, we utilized only pedigree information, investigating how well the genetic status could be assessed and MK strategy could be executed by only using the pedigree information known currently.

Materials and Methods

Current Breeding Strategy of the Japanese Captive Population

One pair, namely, YOUYOU (YO: male) and YANYAN (YA: female) was brought into Japan in 1999 and named A pair (Fig. 1). In the same year, only 1 offspring (male 19) of A pair was born through artificial breeding. MEIMEI (ME: female) was introduced into Japan in 2000 and mated with male 19 (named B pair) (Fig. 1). A and B pairs showed high reproductive performances. Then, avoiding full-sib mating in order to prevent inbreeding, by 2010, we formed the 20 mating pairs, named C, D, E, F, G, H, I, J, K, L, M, N, O, P, S, T, U, V, W and X pairs, between those of the offspring of A pair (named A lineage) and those of the offspring of B pair (named B lineage) whose kinship coefficients were as low as possible (<0.25) under the assumption that the 3 founders were unrelated and non-inbred (Fig. 1). When HOAYAN (HO: male) and IISHUI (II: female) were introduced into Japan in 2007, we selected the mating combinations, named Q and R pairs, of HO and one of A lineage (female 54), and II and one of B lineage (male 82), respectively, whose kinship coefficients were both 0 under the assumption that the 5 founders were unrelated and non-inbred (Fig. 1). Subsequently, by 2010, we formed the 2 mating pairs (named Y and Z pairs) between those of B lineage and those of Q lineage (the offspring of Q pair) whose kinship coefficients were as low as possible (<0.25) under the assumption that the 5 founders were unrelated and non-inbred (Fig. 1).

Genetic Analyses under the Various Assumptions about Levels of Kinships among the Founders

Because the 5 founders without pedigree information were derived from the Chinese captive population, they had the high possibility of being related. Therefore, we first assumed 4 levels (assumptions 1-4) of kinships among the 5 founders as described below. Under all the assumptions, we assumed that the 5 founders were non-inbred and all the kinship values of each of the founders itself were 0.5.

We assumed that the 5 founders were unrelated, namely, all the kinship values among them were assumed to be 0 as assumption 1 (Table 1).
conventional approach, as is assumed in current breeding strategy of the Japanese captive population.

For more realistic kinships among the founders, we created the hypothetical pedigree. Shirai previously designed some hypothetical pedigrees in which YO, YA and ME traced back to their grandparents or great-grandparents utilizing the information on the birthplaces and the living condition (namely, the wild or captivity) of these 3 founders, their parents and their grandparents (unpublished data, 2005). Among these hypothetical pedigrees, we adopted the pedigree in which both each of kinships among the 3 founders and the average mean kinship of the 3 founder population showed the maximum values as assumption 2 (Fig. 2). For HO and II, we utilized the information in which there were no common ancestries over 2 generations and no direct relationships between these 2 founders and the other 3 founders. Based on the former information, we created the hypothetical pedigree in which the 2 founders traced back to their great-grandparents. At that time, assuming that the 2 founders had the common great-grandparents, the kinship between the 2 founders resulted in the maximum value of 0.016 (Fig. 3). Using the latter information, we created the hypothetical pedigrees which demonstrated the relationships between these 2 founders and the other 3 founders. However, there existed too many pedigree patterns. Therefore, in assumption 2 as the extreme assumption, HO or II and YO, YA or ME were assumed to be first-degree relatives, with the kinship values of 0.25 between them (Table 1).

In assumption 2, because of little information on the relationships between HO or II and the other 3 founders, they

| Table 1. The kinship matrixes under assumptions 1-4 |
|-----------------------------------------------|
| Assumption 1                                  |
| YOUYOU | YANYAN | MEIMEI | HOAYAN | IISHUI|
| YOUYOU | 0.500 | 0.000 | 0.000 | 0.000 | 0.000 |
| YANYAN | 0.000 | 0.500 | 0.000 | 0.000 | 0.000 |
| MEIMEI | 0.000 | 0.000 | 0.500 | 0.000 | 0.000 |
| HOAYAN | 0.000 | 0.000 | 0.000 | 0.500 | 0.000 |
| IISHUI | 0.000 | 0.000 | 0.000 | 0.000 | 0.500 |

The average mean kinship of the founder population is 0.100

| Assumption 2                                  |
| YOUYOU | YANYAN | MEIMEI | HOAYAN | IISHUI|
| YOUYOU | 0.500 | 0.063 | 0.031 | 0.250 | 0.250 |
| YANYAN | 0.063 | 0.500 | 0.031 | 0.250 | 0.250 |
| MEIMEI | 0.031 | 0.031 | 0.500 | 0.250 | 0.250 |
| HOAYAN | 0.250 | 0.250 | 0.250 | 0.500 | 0.016 |
| IISHUI | 0.250 | 0.250 | 0.250 | 0.016 | 0.500 |

The average mean kinship of the founder population is 0.231

| Assumption 3                                  |
| YOUYOU | YANYAN | MEIMEI | HOAYAN | IISHUI|
| YOUYOU | 0.500 | 0.063 | 0.031 | 0.010 | 0.250 |
| YANYAN | 0.063 | 0.500 | 0.031 | 0.010 | 0.250 |
| MEIMEI | 0.031 | 0.031 | 0.500 | 0.010 | 0.250 |
| HOAYAN | 0.010 | 0.010 | 0.010 | 0.500 | 0.016 |
| IISHUI | 0.250 | 0.250 | 0.250 | 0.016 | 0.500 |

The average mean kinship of the founder population is 0.174

| Assumption 4                                  |
| YOUYOU | YANYAN | MEIMEI | HOAYAN | IISHUI|
| YOUYOU | 0.500 | 0.250 | 0.250 | 0.250 | 0.250 |
| YANYAN | 0.250 | 0.500 | 0.250 | 0.250 | 0.250 |
| MEIMEI | 0.250 | 0.250 | 0.500 | 0.250 | 0.250 |
| HOAYAN | 0.250 | 0.250 | 0.250 | 0.500 | 0.250 |
| IISHUI | 0.250 | 0.250 | 0.250 | 0.250 | 0.500 |

The average mean kinship of the founder population is 0.300
had the possibility that the kinships of HO and YO, YA or ME and the kinships of II and each of them were quite different. We estimated that this fact had a large influence on results of the analyses. Thus, based on assumption 2, in order to make a difference between the kinships of HO and YO, YA or ME and the kinships of II and each of them, we assumed that the former values were 0.01, the latter values were 0.25 in assumption 3 (Table 1).

As assumption 4, we assumed that all the 5 founders corresponded to first-degree relatives. Namely, all the kinship values among the 5 founders were assumed to be 0.25 in assumption 3 (Table 1).

Subsequently, we calculated the gene diversity (GD) which was expressed as the proportion of the expected heterozygosity of the Japanese captive population to that of the Chinese captive population, the mean inbreeding coefficient (MeanF) and MK. These parameters were computed from 158 Japanese captive individuals which were alive as of December 2010 using pedigree information obtained in the previous study (Wajiki et al., 2014). They were calculated utilizing the population management software (PM2000) v1.214 (Pollak et al., 2005). In the calculation for GD and MeanF, the founders themselves were excluded. Furthermore, in the calculation of GD, 1000 iterations of gene dropping simulation (MacCluer et al., 1986) were performed.

### Results

The GD and the MeanF under assumptions 1–4 were given in Table 2. Under assumption 1, namely, conventional approach, the average mean kinship of the founder population was 0.100, and the GD and the MeanF were 82.2% and 0.072, respectively. Under assumption 2 which had the value of 0.231 as the average mean kinship of the founder population, the GD and the MeanF were 77.1% and 0.130, respectively. Under assumption 3, the average mean kinship of the founder population, the GD and the MeanF were 0.174, 78.7% and 0.113, respectively. The GD and the MeanF under assumption 4 which had the value of 0.300 as the average mean kinship of the founder population were much lower and much higher values of 65.0% and 0.286, respectively, than them under assumption 1.

We showed the distribution of the frequency of MK under assumptions 1–4 in Fig. 4. This distribution clearly shifted to higher MK value under the assumption that the average mean kinship of the founder population was higher. We listed 10 individuals which had the lowest or highest MK under assumptions 1–4 in Table 3. According to the levels of kinships among the 5 founders, the individuals and lineages which had low or high MK shifted largely. For example, although II and HO had the extreme low values of 0.008 and 0.017, respectively, under assumption 1, II had the highest MK of females in the population under assumptions 2 and 3, and HO had the second highest MK of the population under assumption 2. As only exception, male 19 paired with ME kept the highest MK of the population through assumptions 1–4.

### Discussion

The results indicated that assumption 1 showed the highest GD and the lowest MeanF of all the assumptions, and that when the higher average mean kinship of the founder population was assumed, the lower GD and the higher MeanF were shown. Therefore, if all the kinship values among the 5 founders were equal to or lower than that of first-degree relatives, under the assumption that the 5 founders were non-inbred, the GD would fluctuate largely from ~65% to ~82% and the MeanF from ~0.07 to ~0.29.
Most management programs use the expected heterozygosity as the index of genetic variability, and the widely accepted goal for genetic managements of captive populations is to retain 90% of the GD found in the wild or source population over 100 years (Frankham et al., 2007). Even based on most optimistic estimates, namely, assumption 1, the GD of the Japanese captive population was the low value of 82.2%, compared with the GD (94.6% in 1994, 95.2% in 1999) of the domestic captive population of oriental white stork (Ciconia boyciana) which was established for re-introduction (Hosoda, 2000). Because inbreeding generally leads to a cumulative loss of reproductive fitness including the quantity and the quality of sperm, the mating ability, the number of offspring produced, the maternal ability and the survival proportion of chicks, it increases the risk of extinction (Frankham et al., 2007). In the Japanese captive population, even under assumption 1, the MeanF was elevated to the extent of the inbreeding coefficient (0.0625) expected in first-cousin mating.

Table 3 showed that the individuals with low MK changed largely depending on the levels of kinships among the 5 founders. Thus, if MK strategy would be introduced into this population, we required greater consideration of selection of individuals as mating pairs. Moreover, although we are performing the current breeding programs towards the increase of the descendants of HO and II in order to equalize founder contributions under the assumption that the founders are unrelated, our present study inspires us the importance of the relationships among the founders.

Jones et al. (2002) suggested that conventional breeding programs carried out under the assumption that founders were unrelated would be to preserve the alleles at only frequencies present in the founder population, however, for populations that had experienced a bottleneck, post-bottleneck allele frequencies might be drastically different than pre-bottleneck frequencies. They concluded that the preservation of the rare alleles was accomplished by integrating the information of founder relationships (Jones et al., 2002). Some papers reported the estimation of the relationships among founders by utilizing molecular markers and the integration of the molecular data into conventional breeding programs under the assumption that founders were unrelated (Jones et al., 2002; Wisely et al., 2003; Russello and Amato, 2004).

On the other hand, Rudnick and Lacy (2008) examined the impact of assumptions about founder relationships on GD and MeanF in MK strategy through computer simulation, suggesting that the overall long-term benefit for knowing founder relationships was only modest. In addition, the studies on the parma wallaby (Macropus parma) (Ivy et al., 2009) and the lowland tapir (Tapirus terrestris) (Gonçalves da Silva et al., 2010) populations corroborated this suggestion. However, it is unclear whether this suggestion is directly applicable to the Japanese captive population in

**Fig. 4.** The distribution of the frequency of the individual mean kinship (MK) in the Japanese captive population of Japanese crested ibis as of December 2010 under assumptions 1–4.
which the adoption of MK strategy is considered. Rudnick and Lacy (2008) and Ivy et al. (2009) performed the simulations under the assumption that the accurate pedigrees were recorded and that the individuals without pedigree information were not introduced in the process of MK strategy. In the Japanese captive population, however, continuous introductions of new founders from the Chinese population will be exclusively required in order to increase and maintain the genetic diversity (Wajiki et al., 2014), and then we have the high possibility that the relationships among the existing founders and newly introduced founders are unknown. In addition, Rudnick and Lacy (2008) concluded that the effectiveness of MK strategy depends on the accuracy of pedigree information on recent, rather than historical, ancestors.

In conclusion, we indicated that the Japanese captive

| Rank | Assumption 1 | Assumption 2 | Assumption 3 | Assumption 4 |
|------|--------------|--------------|--------------|--------------|
|      | Male | Female | Male | Female | Male | Female | Male | Female |
| 1    | HOAYAN | IISHUI | YOUYOU | MEIMEI | HOAYAN | 253Q | HOAYAN | IISHUI |
|      | (0.017) | (0.008) | (0.218) | (0.158) | (0.027) | (0.125) | (0.259) | (0.254) |
| 2    | 265R | 193R | 89A | YANYAN | 239Q | 269Q | 239Q | 253Q |
|      | (0.099) | (0.099) | (0.220) | (0.218) | (0.125) | (0.125) | (0.305) | (0.305) |
| 3    | 239Q | 226R | 105A | YANYAN | 21A | 245Q | MEIMEI | 245Q |
|      | (0.103) | (0.103) | (0.220) | (0.220) | (0.125) | (0.150) | (0.305) | (0.305) |
| 4    | 245Q | 258R | 144A | 32A | 182Q | 286Z | 182Q | 193R |
|      | (0.103) | (0.103) | (0.220) | (0.220) | (0.127) | (0.173) | (0.307) | (0.309) |
| 5    | 182Q | 261R | 212A | 26A | 164Q | 288Z | 164Q | 226R |
|      | (0.106) | (0.099) | (0.220) | (0.221) | (0.130) | (0.173) | (0.309) | (0.309) |
| 6    | 164Q | 253Q | 39A | 104A | 283Z | 241Y | 265R | 258R |
|      | (0.108) | (0.103) | (0.221) | (0.221) | (0.173) | (0.175) | (0.309) | (0.309) |
| 7    | 283Z | 269Q | 86A | 190B | 238Y | 290Y | 238Y | 286Z |
|      | (0.147) | (0.103) | (0.221) | (0.221) | (0.175) | (0.175) | (0.335) | (0.312) |
| 8    | 238Y | MEIMEI | 178B | 195B | 289Y | 293Y | 289Y | 286Z |
|      | (0.150) | (0.123) | (0.221) | (0.221) | (0.175) | (0.175) | (0.337) | (0.335) |
| 9    | 289Y | 262Z | 219B | 247B | 291Y | YANYAN | 289Y | 286Z |
|      | (0.150) | (0.147) | (0.221) | (0.221) | (0.175) | (0.210) | (0.337) | (0.335) |
| 10   | 291Y | 288Z | 259B | 297B | YOUYOU | 21A | 291Y | 288Z |
|      | (0.150) | (0.147) | (0.221) | (0.221) | (0.210) | (0.212) | (0.337) | (0.335) |

YOUYOU, YANYAN, MEIMEI, HOAYAN and IISHUI are founders. The other individuals are presented by numbers and alphabets in which the former and the latter show the pedigree registry numbers and their lineages, respectively. When multiple individuals have the same MK values, they are ranked in ascending order of the pedigree registry numbers. The numbers shown in parentheses are MK values which the individuals have.
population had the inadequate GD and MeanF even under the assumption that the founders were unrelated and non-inbred. In addition, the study also suggested that it became more effective to assess the genetic status and to introduce MK strategy into this population with more credible molecular evaluation of the relationships among founders.

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