

**Studies on Genetic Diversity and Its Maintenance in the
Japanese Population of Japanese Crested Ibis
(*Nipponia nippon*)**

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General Introduction

Japanese crested ibises (*Nipponia nippon*) were once widespread from Hokkaido to Kyushu in Japan (Yasuda, 1988). However, due to continuing habitat degradation and overhunting, the population began a drastic decline after the Edo period (Yasuda, 1984). In 1981, the Japanese government made a decision to bring the last five wild individuals into captivity, in order to prevent extinction of the species (Niigata Prefectural Government, 2000). Artificial breeding of the individuals brought into captivity was unsuccessful (Niigata Prefectural Government, 2000). Thus, the last individual indigenous to Japan died in 2003.

Although the Japanese crested ibis was also widely distributed in China, Russia, the Korean Peninsula, and Taiwan at the outset of the 20th century (BirdLife International, 2001), these populations also reduced after the middle of the 20th century. The species was therefore thought to be extinct until the discovery of seven individuals in China in 1981. The seven individuals included two pairs and three chicks that were the offspring of one of the adult pairs (Yasuda, 1985). The seven individuals were conserved both in the wild and captivity (Ding, 2004). Owing to the conservation efforts made in China, the wild and captive Chinese populations had grown to 1,040 and 660, respectively, by 2012 (Japanese Society for Preservation of Birds, 2013).

In 1999, one pair derived from the Chinese captive population was gifted to Japan from the Chinese government. Subsequently, one and two individuals, respectively, were provided in 2000 and 2007. The Japanese government launched a conservation project and created a captive population founded by the five individuals originating from the Chinese captive population. With the efforts of this conservation project to solve technical problems regarding captive breeding, the size of the Japanese captive population has increased rapidly since. Currently, the population has been divided among breeding

facilities, including the Sado Japanese Crested Ibis Conservation Center, which has managed the breeding program including selection of mating pairs and translocations to other facilities. As of June 2013, the Japanese captive population included 212 individuals, which were thought to be sustainable for this population. Therefore, this population is about to make the transition from the growth phase to the maintenance phase.

In September 2008, the first reintroduction took place on Sado Island. With the Japanese conservation project's efforts to solve technical problems concerning training methods for reintroduction and reintroduction methods, eight reintroductions were carried out on Sado Island and a total of 125 individuals were released from September 2008 to June 2013. The breeding success of the reintroduced population has been confirmed since 2012. According to the Japanese Ministry of the Environment, 73 individuals were thought to be alive in the wild as of June 2013. The conservation project for the Japanese crested ibis has been regarded as a model for efforts towards conservation of various endangered animal species in Japan.

The International Union for Conservation of Nature and Natural Resources (IUCN) proposed the need to conserve genetic diversity as one of three global conservation priorities (McNeely et al., 1990). Genetic diversity is an expression of differences in the DNA base sequence that may produce differences in the amino acid sequence. This, in turn, may result in differences either in which protein is produced or in the amount of protein a specific genetic locus encodes. These differences may result in physiological, morphological or functional differences, which, in turn, could lead to variation in individual fecundity, viability and behavior (Frankham et al., 2002). Genetic diversity is needed in order for populations to adapt to environmental changes, for example, climatic change associated with global warming, intrusion of new competitors,

fluctuations in pathogens and so on (Frankham et al., 2002). Populations need to adjust to these environmental changes, otherwise, they would go extinct. Genetic diversity of populations contributes to their evolvability (Frankham et al., 2002). Furthermore, the loss of genetic diversity could result in reduced fecundity in both sexes (Roelke et al., 1993; Eldridge et al., 1999), increased expression of deleterious genetic traits (Wang et al., 1999), increased disease susceptibility (O'Brien and Evermann, 1988), and increased extinction risk (Frankham, 1995a).

The population of the Japanese crested ibis in China has increased substantially from four individuals, and the Japanese captive population was established by only five individuals transferred from the Chinese captive population. Hence, the Japanese captive population has gone through two genetic bottlenecks. A genetic bottleneck, which means a rapid reduction in population size, may cause severe loss of genetic diversity (Frankham et al., 2002). Because the reintroduced population originates from the Japanese captive population, the reintroduced population, as well as the captive population, requires maintenance of genetic diversity. This is needed in order to accomplish the final goal in this project, which is to establish a viable population in the wild where environmental changes are more significant than in captivity.

In Chapter 1, demographic analyses of the Japanese captive population were conducted. The purpose of the demographic analyses was to calculate basic life tables, population dynamics, as well as predict the future genetic diversity. Furthermore, the aim was to determine the number of newly introduced founders and the carrying capacity compatible with genetic and demographic limitations. In Chapter 2, based on the relationships among the five founding individuals, we performed genetic analyses on the Japanese captive population using pedigree information. In this chapter, we considered

the adoption of mean kinship strategy (MK strategy) as the breeding strategy suitable for the maintenance phase. In Chapter 3, we determined the number of individuals to be released in the future, in order to preserve genetic diversity of the reintroduced population. This was performed by estimating the probability that the reintroduced population would retain a rare allele existing in the captive population after 50 years. The estimation was based on demographic parameters obtained from either Japanese captive or reintroduced populations, or Chinese wild population.

Chapter 1

Prediction of genetic diversity based on demographic analyses in the Japanese captive population

Introduction

The purpose of this chapter was to estimate the demographic parameters and to discuss future prospects for retaining the genetic diversity including the introductions of new founders and the carrying capacity. The studbook for the Japanese captive population has already been constructed using the Single Population Analysis and Records-Keeping System software (SPARKS) v1.54 (Scobie and Laurie, 2004) which was provided from the International Species Information System. We utilized these official studbook data for the purpose.

Materials and Methods

Studbook data used

In this chapter, the studbook data as of December 2010 were used for the analyses. Any unique specimen identifiers (ID numbers, tags and so on), sexes, sires, dams, birth and death dates, full transaction histories and any data on reproductive potential of living animals are generally registered for each specimen in the studbook (Wilcken and Lees, 2012). Studbooks are proposed to provide accurate, up-to-date information in a standard format that can easily be used for demographic and genetic analyses of a single population (Thompson and Earnhardt, 1996; Wilcken and Lees, 2012). For the Japanese captive population, these studbook data have been recorded using the software SPARKS which is used by most studbook keepers in the world (Thompson and Earnhardt, 1996; Wilcken and Lees, 2012).

As of December 2010, there were 297 individuals registered in the SPARKS including 12 individuals protected from the wild before the extinction, three individuals transiently introduced from China for breeding, five individuals introduced from China

and 277 progeny of these five individuals (Table 1-1). The 277 individuals included 153 alive and 54 dead individuals in captivity, 42 individuals reintroduced into the wild and 28 individuals transferred to China for the reason stated below (Table 1-1). All of the 12 protected and the three transiently introduced individuals were not alive in the Japanese captive population as of 2010, and they had no descendants. All of the five introduced individuals were alive and participated in breeding until 2010. Birth years of eight from the 12 protected individuals and all the three transiently introduced individuals were treated as unknown. Sex of one dead individual in captivity was also unknown. With regard to 42 reintroduced individuals, only the records before reintroduction were analyzed.

Table 1-1. The classification of individuals analyzed in this chapter

1, Individuals protected from the wild before the extinction ¹⁾	12	
2, Individuals transiently introduced from China for breeding ¹⁾	3	
3, Individuals introduced from China ²⁾	5	
	4, Alive individuals in captivity	153
4-7, Progeny of	5, Dead individuals in captivity	54
individuals introduced	6, Individuals reintroduced into the	42
from China	wild	
	7, Individuals transferred to China	28
Total		297

1) All of them were not alive in the Japanese captive population as of 2010, and they had no descendants.

2) All of them were alive and participated in breeding until 2010.

The five individuals introduced from China, namely, YOUYOU (denoted as YO: male), YANYAN (YA: female), MEIMEI (ME: female), HOAYAN (HO: male) and IISHUI (II: female) were regarded as founders of the Japanese captive population, since their pedigree information was unknown. YO and YA was brought into Japan in 1999 and named A pair (Figure 1-1). In the same year, only one offspring (male 19) of A pair was born through artificial breeding. ME was introduced into Japan in 2000 and mated with male 19 (named B pair) (Figure 1-1). 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 three founders were unrelated and non-inbred (Figure 1-1). Their offspring were named C-P and S-X lineages. When HO and II 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 zero under the assumption that the five founders were unrelated and non-inbred (Figure 1-1). Subsequently, by 2010, we formed the two 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 five founders were unrelated and non-inbred (Figure 1-1). Their offspring were named Y and Z lineages. Moreover, two unplanned offspring whose sire and dam were one of I lineage and one of D lineage, respectively, were produced.

According to the agreements for cooperation in the protection for this species

between the Japanese and Chinese governments, it has been determined that the half of B and Q lineages and the offspring of R pair (R lineage), and all the offspring of the pair of HO and II belong, and are transferred, to China at the request of the Chinese government. HO and II have not been paired up to the present. Thirty-three individuals as of December 2010 were applicable to this case. Among them, 28 individuals had been already transferred to China, and the remaining five individuals were scheduled to be transferred after 2010.

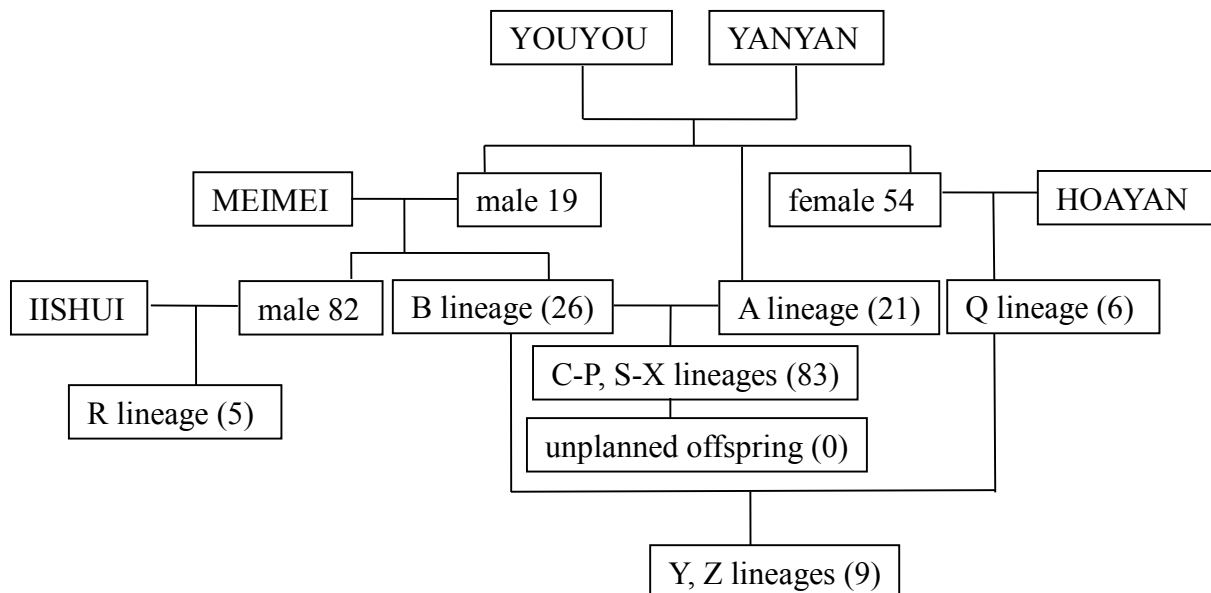


Figure 1-1. The simplified pedigree. YOUYOU, YANYAN, MEIMEI, HOAYAN and IISHUI are founders. These founders were assumed to be unrelated and non-inbred. The numbers following male or female show the pedigree registry numbers. The A-Z lineages represent the offspring of A-Z pairs. The numbers in parentheses are the numbers of individuals of each lineage in the Japanese captive population as of December 2010. Two unplanned offspring whose sire and dam were one of I lineage and one of D lineage, respectively, were produced.

Demographic analyses

Based on the studbook data explained above, the demographic parameters were calculated including the number of individuals and the sex ratio in each age class.

Three demographic parameters (Foose and Ballou, 1988; Ballou and Foose, 1996; Caughley and Gunn, 1996; Pianka, 1999; Wilcken and Lees, 2012) computed were the mortality rate (q_x), the survivorship (l_x) and the fecundity rate (m_x). q_x is defined as the probability dying during age class x and provides the prediction of the life-span and high risk term to death. It is calculated from the number of individuals which die during age class x divided by the number of individuals that are alive at the beginning of the age class. l_x is the proportion of individuals surviving to the beginning of age class x . It is the cumulative measure, which is one at age class zero and the product of the survival rates ($p_x = 1 - q_x$) from age class zero to $x-1$. In the Japanese captive population, there were no males and females older than 14 years of age previously, except for one female protected from the wild that survived to 36 years of age and died in 2003. Thus, the q_x of males at age class of 15 years and females at age class of 36 years were calculated as one, and the l_x of males at age class of 16 years and females at age class of 37 years were calculated as zero. m_x means the average number of same-sex live births produced at the season of births by an individual aged x and is calculated by dividing the number of same-sex live births produced by individuals at age class x by the number of individuals alive at the beginning of the age class. However, because we used relatively small sample sizes, males and females were each credited with one-half of one reproduction for every live birth in this chapter (Pianka, 1999). m_x enables us to predict the starting, peak and stopping ages for physiological reproductive ability.

Moreover, as to the population growth, the intrinsic rate of natural increase (r)

and the finite rate of increase (λ) were calculated. r is the exponential yearly growth rate of the population and is determined by iteration using Euler's implicit equation (Foose and Ballou, 1988; Ballou and Foose, 1996; Caughley and Gunn, 1996; Pianka, 1999; Wilcken and Lees, 2012):

$$\sum e^{-rx} l_x m_x = 1, \quad (1)$$

where the summation is across all ages x . λ represents the estimate of the expected yearly growth rate of the population and is defined as (Foose and Ballou, 1988; Ballou and Foose, 1996; Caughley and Gunn, 1996; Pianka, 1999; Wilcken and Lees, 2012):

$$\lambda = e^r, \quad (2)$$

When a population is growing or declining, r is greater or less than zero, respectively. Therefore, unlike λ , r is converted to equal scales for growing or declining populations (Caughley and Gunn, 1996; Wilcken and Lees, 2012).

In addition, the generation length (T) was evaluated. This parameter has many definitions and they provide quite different estimates (Caughley and Gunn, 1996). The one used herein was the average age of males (females) producing an offspring during a season of births, which is expressed as (Ballou and Foose, 1996; Caughley and Gunn, 1996; Wilcken and Lees, 2012):

$$T = \sum x e^{-rx} l_x m_x, \quad (3)$$

where the summation is across all ages x . It should be noted that this T is not the age of first reproduction.

All of these parameters (q_x , l_x , m_x , r , λ , T) were calculated separately for each sex.

In this chapter, the effective population size (N_e) was also assessed, by calculating the variance effective population size rather than the inbreeding effective population size. The former is defined as the size of an idealized population (a randomly mating population of constant size with equal sex ratio and a Poisson distribution of family sizes) which gives rise to the rate of change in variance of gene frequencies observed in the population under consideration, and the latter is defined as that relevant to the rate of inbreeding (Wright, 1931). The form most commonly used in the design of captive breeding program is the variance effective population size (Lacy, 1995). Although many demographic and genetic methods were developed for calculating the variance effective population size (Frankham et al., 2002), the estimate based on demographic approaches is not often practical because too many demographic data are required (Frankham et al., 2002). As the variance effective population size, therefore, we calculated N_e estimated from the rate of decay of heterozygosity according to generation. Concretely, we first calculated the average number of generations (t) in which the founder generation was defined as zero generation and the living founders were excluded from the calculation (Wilcken and Lees, 2012), and the gene diversity (GD), namely, the proportion of heterozygosity expected in the Japanese captive population to the Chinese captive population as a source population. In the calculation of GD, we assumed that each founder had two unique alleles and it was estimated by 1,000 iterations of gene dropping simulation (MacCluer et al., 1986). There would be a high possibility that the five founders in the Japanese captive population are related to each other, because they were derived from the Chinese captive population. Due to the missing pedigree data for the five founders, however, we calculated the GD based on the conventional approach under the assumption that the five founders were unrelated and non-inbred. The N_e was

subsequently calculated from the following equation (Wilcken and Lees, 2012):

$$GD = \left(1 - \frac{1}{2N_{f0}}\right) \left(1 - \frac{1}{2N_e}\right)^t, \quad (4)$$

where N_{f0} is the number of founders, and potential founders defined as founders that has not yet produced any living descendants in the population are not included in this study. Further, we calculated N_e/N where N is the actual number of individuals in the population. For N , we did not use the total number, but used the median of the number of all the individuals (not only the alive ones) registered in the studbook in each generation (zero, 1-1.99, 2-2.99 and 3-3.99 generations) (Wilcken and Lees, 2012).

In order to investigate the transition of r , λ , T , N_e and N_e/N , the values in December 2001 and December 2008 were also calculated. The former and the latter years corresponded to the first year when ME participated in breeding in addition to YO and YA and when HO and II participated in breeding in addition to YO, YA and ME, respectively.

In the calculation of these parameters except for the age structure and N_e/N , we used the population management software (PM2000) v1.214 (Pollak et al., 2005).

Simulation study to estimate the number of newly introduced founders and the carrying capacity to retain the gene diversity after 50 and 100 years

The widely accepted goal for genetic management of captive populations is to retain 90% of the genetic diversity found in the wild or source population over 100 years (Frankham et al., 2002; Association of Zoos and Aquariums, 2012), and by compromise, 90% over 50 years or 80% over 100 years (Frankham et al., 2002). Most management programs use GD as the index of genetic diversity (Frankham et al., 2002; Association of Zoos and Aquariums, 2012). GD is lost at the rate of $1/(2N_e)$ per generation (Ballou and

Foose, 1996; Frankham et al., 2002). N_e is calculated from N_e/N multiplied by the number of individuals. The number of individuals for a given year is estimated from the one in the previous year multiplied by λ , but it cannot exceed a carrying capacity. Additionally, one year is equivalent to $1/T$ generation. Hence, using λ , T and N_e/N , the GD for a given year can be estimated. Moreover, using founder genome equivalent (FGE), which is defined as the number of equally contributing founders with no random loss of founder alleles in descendants that would be expected to produce the same genetic diversity as in the population under study (Lacy, 1989; Lacy, 1995), the value of the GD can be also approximated by $1-1/(2FGE)$ (Lacy, 1989; Lacy, 1995). So, when new founders are added to a population, the new GD is calculated by the following equation:

$$\text{newGD} = 1 - \frac{1}{2\{FGE_{\text{current}} + (FGE_{\text{perfounder}} \times N_{\text{additional}})\}}, \quad (5)$$

where FGE_{current} and $FGE_{\text{perfounder}}$ are the current FGE estimated from GD before new founders are added, and the one expected to be recruited per new founder, respectively, and $N_{\text{additional}}$ shows the number of new founders added.

Based on the method shown above, we estimated the GD after 50 and 100 years of 2010 with PM2000 using the obtained values of λ , T and N_e/N in 2010, in order to estimate the number of newly introduced founders and the carrying capacity required to retain 90% after 50 or 100 years or 80% over 100 years of genetic diversity found in the Chinese captive population. It is important in this simulation that λ , T and N_e/N are assumed to be constant over 50 and 100 years. The average λ and T values for both sexes were used. Although the N_e/N utilized by PM2000 corresponded to the demographic estimate, N_e/N regarded as the genetic estimate was used in the present study. The supplements from China were assumed to be introduced every five years for 50 and 100 years and their $FGE_{\text{perfounder}}$ were to be 0.4. The value of 0.4 is generally considered a

reasonable estimate of the average FGE that would be recruited per supplemented founder (Traylor-Holzer, 2011). Moreover, we assumed that the five founders and supplements were unrelated and non-inbred.

Results and Discussion

Age structure

The age structure is shown in Figure 1-2. The numbers of males and females were 81 and 77, respectively. This age structure was typical of a rapid growing population because the number of younger individuals was larger. However, the numbers of males and females in age classes of 2-4 years were both small. This is mainly due to increases of the rate of natural breeding, which causes lower hatchability as compared to artificial breeding, and of the donation to reintroduction in the individuals in these age classes. The number of individuals of founder lineages showed no remarked increase since 2008, although HO and II in addition to YA, YO and ME had participated in breeding since 2008. This extent of increase is likely to be attributable to an influence of natural breeding, transference of the half of B, Q and R lineages to China and deterioration of reproductive performance of A pair since 2005.

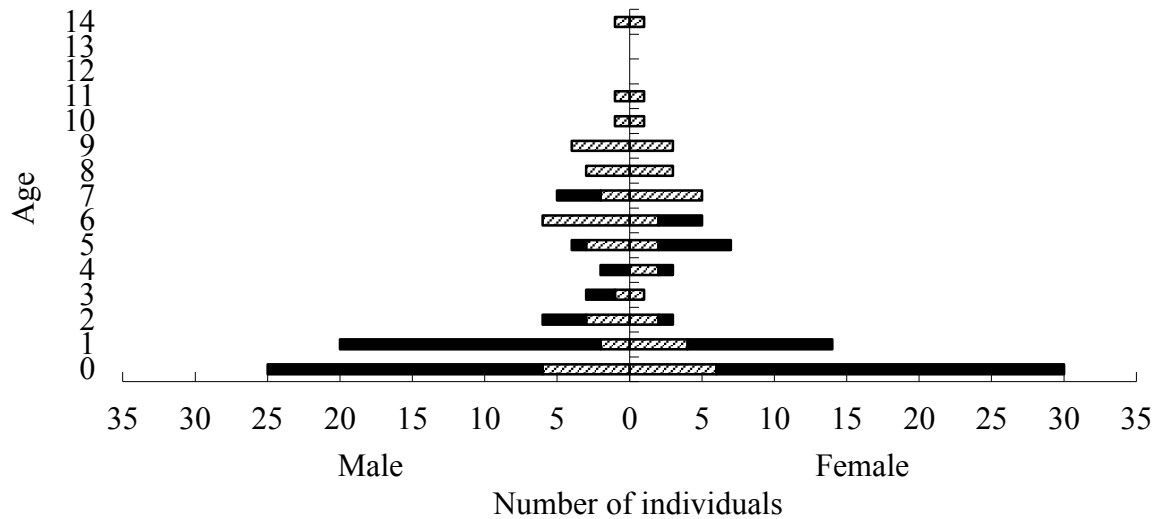


Figure 1-2. The age structure in the Japanese captive population as of December 2010. The slash parts denote the number of individuals of founder lineages (founders and A, B, Q and R lineages). Mating pairs were selected from these individuals. The black parts denote the number of individuals of non-founder lineages.

Life table

The values for q_x , l_x and m_x are presented in Table 1-2 and Figure 1-3. The q_x at age classes of zero and five years were high for both males and females. The high value of q_x at zero year of age is due to a high mortality rate within 30 days after birth, which is caused mainly by squeezing by parents, shortage of feed supply from parents and fall from nest in the case of natural breeding, and bacterial infection in artificial breeding. The high q_x at five years of age is likely to be caused by various factors, but remains unexplained. As to l_x , we found that 72.4% of males and 59.1% of females survived at

the time of six years and eight years of age, respectively, and that all the individuals equal to or older than these ages did not die except for one female that survived to 36 years of age. In every age class younger than 15 years, except for age class of zero year, the l_x for females was lower compared with that for males, which might be coincident with the fact that females tend to be more sensitive to the captive environment than males. The result of m_x showed that males and females could mate from one year of age. Empirically, however, this species can mate from the second breeding season except for the birth year (once a year for mating). It should be noted that there was the case in which the starting age for mating was regarded as one year, depending on birth date. The peak of sexual maturity was about 4-10 years of age for males and 6-11 years of age for females. The physiological reproductive ability was likely to terminate approximately at the time of 14 years of age. The m_x should be lower in younger age classes with higher rates of non-mating individuals, because non-mating individuals as well as mating individuals were used for the calculation. The age classes older than seven years were composed of only the individuals selected for mating and thus may provide more realistic data for physiological reproductive ability. However, it should be noticed that a sample size of 20 or more is needed for the calculation of the demographic parameters (Wilcken and Lees, 2012). Only the sample sizes of age classes younger than six years in both sexes were applicable to this suggestion. Therefore, future consecutive analyses for the demographic parameters will be required.

Table 1-3 shows the transition of the r , λ and T values for each sex. The r and λ resulted in 0.31 and 1.36 for males and 0.27 and 1.31 for females in 2010, respectively. It is important to note that the Japanese captive population has shown a continuous growth. It was reported that the λ was 1.04 for males and 1.02 for females in the Hokkaido

population of red-crowned crane (*Grus japonensis*) (Inoue, 1999), and 1.14 for males and 1.15 for females in the domestic captive population of oriental white stork (*Ciconia boyciana*) (Hosoda, 2000). It should be noted that the r and λ in the Japanese captive population of Japanese crested ibis would be reduced in the future because of the restriction of carrying capacity. The T for both sexes increased remarkably from 2001 to 2008 and slightly from 2008 to 2010, resulting in 4.87 for males and 5.12 for females in 2010. It was also reported that the T was 16.9 for males and 12.8 for females in the Hokkaido population of red-crowned crane (Inoue, 1999), and 10.6 for males and 10.9 for females in the domestic captive population of oriental white stork (Hosoda, 2000). Because random genetic drift occurs at the transition of generations, the increase of T reduces the loss of genetic diversity (Foose et al., 1986). Thus, it is desirable to increase T to prevent the loss of genetic diversity. While we showed that the r and λ of females were lower than those of males, the T of females was found to be longer than that of males, consistent with the lower l_x values in females as compared with males. We also showed that there was an extreme difference in r , λ and T between males and females in the population as of 2001, consistent with the lower m_x values as well as the lower l_x values in females than males.

Table 1-2. Life table calculations (q_x , l_x and m_x) for the Japanese captive population as of December 2010

Male				Female			
Age class ¹⁾	q_x	l_x	m_x	Age class ¹⁾	q_x	l_x	m_x
0	0.12	1.00	0.00	0	0.14	1.00	0.00
1	0.01	0.88	0.07	1	0.06	0.86	0.02
2	0.02	0.87	0.18	2	0.04	0.81	0.33
3	0.05	0.85	0.42	3	0.02	0.78	0.33
4	0.03	0.81	0.83	4	0.05	0.76	0.59
5	0.08	0.79	1.02	5	0.12	0.72	0.68
6	0.00	0.72	0.84	6	0.00	0.64	1.10
7	0.00	0.72	0.88	7	0.07	0.64	1.19
8	0.00	0.72	1.55	8	0.00	0.59	1.12
9	0.00	0.72	1.53	9	0.00	0.59	1.00
10	0.00	0.72	0.94	10	0.00	0.59	1.10
11	0.00	0.72	0.62	11	0.00	0.59	1.10
12	0.00	0.72	0.50	12	0.00	0.59	0.00
13	0.00	0.72	0.00	13	0.00	0.59	0.25
14	0.00	0.72	0.00	14	0.00	0.59	0.00
				15	0.00	0.59	0.00
				16	0.00	0.59	0.00

1) Data after 14 and 16 years of age are excluded for males and females, respectively, because the numbers of individuals are zero and one.



Figure 1-3. The fecundity rate (m_x) of the Japanese captive population as of December 2010. Data after 14 and 16 years of age are excluded for males and females, respectively, because the numbers of individuals are zero and one.

Table 1-3. The intrinsic rate of natural increase (r), the finite rate of increase (λ) and the generation length (T) for the Japanese captive population as of December in the three specified years

Year	r		λ		T	
	Male	Female	Male	Female	Male	Female
2010	0.31	0.27	1.36	1.31	4.87	5.12
2008	0.32	0.29	1.37	1.33	4.76	4.91
2001	0.54	0.21	1.72	1.23	2.25	3.57

Effective population size

Table 1-4 shows the transition of the N_e and N_e/N values. The N_e increased from 2001 to 2008, but decreased slightly from 2008 to 2010, resulting in the very low value of 10.7 in 2010. Franklin (1980) and Soulé (1980) suggested that N_e of 50 is necessary to avoid inbreeding depression. Additionally, it was indicated that N_e should be much higher than 50 to avoid inbreeding depression for longer generations (Latter et al., 1995; Bryant et al., 1999). Furthermore, it was also suggested that N_e of at least 500 should be required in order to maintain the evolutionary potential (Franklin, 1980; Lande and Barrowclough, 1987). Unequal sex-ratios, variance in family sizes and fluctuations in population size over generations make N_e smaller than an actual population size (Frankham, 1995b; Frankham et al., 2002). Frankham (1995b) suggested that fluctuation in population size is the most important factor. It is definitely important to know N_e/N for estimating N_e based on the census size, and N_e/N is decreased by same factors as N_e . The N_e/N in the Japanese captive population was also reduced through 2001 to 2010, showing the value of 0.17 in 2010, which was relatively low, taking into consideration the report that N_e/N seemed typically to fall in the range of 0.2-0.4 for the populations of captive endangered species (Mace, 1986). Therefore, we definitely need to make efforts to increase N_e and N_e/N in the Japanese captive population. For the stabilization in population size over generations and the equalization in family size, breeding strategies and reintroduction programs undertaken using pedigree information may be important for the Japanese captive population.

Table 1-4. The average generation number (t), the gene diversity (GD), the effective population size (N_e), the median of the number of individuals in each generation (MNG) and the N_e/N of the Japanese captive population as of December in the three specified years

Year	t	GD	N_e	MNG	N_e/N
2010	1.90	0.82	10.7	64	0.17
2008	1.79	0.81	11.2	50	0.22
2001	1.18	0.78	9.7	17	0.57

Simulation study to estimate the number of newly introduced founders and the carrying capacity to retain the gene diversity after 50 and 100 years

The GD as of December 2010 was estimated to be 82.2%. The results of simulation are given in Table 1-5. The carrying capacity of 200 individuals in Table 1-5 is approximately maximum allowable population size which was calculated based on captive spaces of breeding facilities in 2010. When we assumed that no supplements were introduced from China in the future, under the condition that the carrying capacity was 200 individuals, the GD was estimated to be 70.6% and 60.9% after 50 and 100 years, respectively. Furthermore, it was estimated that it would be required to continue introductions of five or four supplements every five years for 50 or 100 years, in order to retain 90% after 50 or 100 years, respectively, at the carrying capacity of 200 individuals. Similarly, for maintenance of 80% over 100 years, one supplement was estimated to be needed. If two supplements were introduced every five years for 50 or 100 years, we estimated the need to prepare the carrying capacity of about 560, 90 and 380 individuals, in order to retain 90% after 50 years and 80% and 90% after 100 years, respectively.

Additionally, assuming that no supplements were introduced in the future, we estimated that the goals of retention of 90% after 50 or 100 years could not be achieved even if the carrying capacity of 100 million individuals was set up. Similarly, even when we adopted the goal of retention of 80% over 100 years, we estimated that the carrying capacity of more than 4,000 individuals was needed.

Table 1-5. Predicted gene diversity after 50 (a) and 100 (b) years under various conditions of number of supplements and carrying capacity

(a)		Number of supplements introduced every five years for 50 years					
		0	1	2	3	4	5
Carrying capacity	100	60.7	75.9	81.3	84.1	85.9	87.2
	200	70.6	81.9	86.2	88.5	89.9	90.9
	300	74.1	84.2	88.1	90.2	91.5	92.4
	400	75.9	85.4	89.1	91.1	92.3	93.2
	500	76.9	86.1	89.7	91.7	92.9	93.7
	600	77.6	86.6	90.2	92.1	93.3	94.1

(b)		Number of supplements introduced every five years for 100 years					
		0	1	2	3	4	5
Carrying capacity	100	45.1	75.1	81.2	84.1	85.9	87.2
	200	60.9	81.9	86.6	88.8	90.1	91.1
	300	67.2	84.8	88.9	90.8	92.0	92.7
	400	70.5	86.5	90.2	92.0	93.0	93.7
	500	72.5	87.5	91.1	92.7	93.7	94.4
	600	73.9	88.3	91.7	93.3	94.2	94.8

Chapter 2

Genetic analyses on the Japanese captive population using pedigree information

Introduction

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 chapter, we assessed the genetic status of Japanese captive 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 chapter, 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

In Chapter 1, we could not help assuming that the five founders were non-inbred and unrelated, because they had no pedigree information. However, because the five founders were derived from the Chinese captive population, they had the high possibility of being related. Therefore, we first assumed four levels (assumptions 1-4) of kinships

among the five founders as described below. Under all the assumptions, we assumed that the five founders were non-inbred and all the kinship values of each of the founders itself were 0.5.

We assumed that the five founders were unrelated, namely, all the kinship values among them were assumed to be zero as assumption 1 (Table 2-1). This assumption is often utilized in conventional approach, as is assumed in current breeding strategy of the Japanese captive population (in Chapter 1).

For more realistic kinships among the founders, we created the hypothetical pedigree. Shirai (2005, unpublished data) 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 three founders, their parents and their grandparents. Among these hypothetical pedigrees, we adopted the pedigree in which both each of kinships among the three founders and the average MK of the three founder population showed the maximum values as assumption 2 (Figure 2-1). For HO and II, we utilized the information in which there were no common ancestries over two generations and no direct relationships between these two founders and the other three founders. Based on the former information, we created the hypothetical pedigree in which the two founders traced back to their great-grandparents. At that time, assuming that the two founders had the common great-grandparents, the kinship between the two founders resulted in the maximum value of 0.016 (Figure 2-2). Using the latter information, we created the hypothetical pedigrees which demonstrated the relationships between these two founders and the other three 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 2-1).

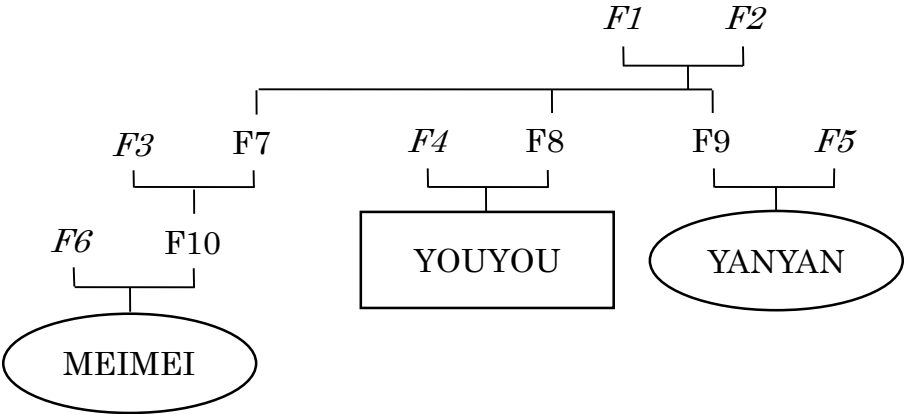


Figure 2-1. The excerpt of the hypothetical pedigrees which show the relationships among YOUYOU, YANYAN and MEIMEI (Shirai, 2005, unpublished data). The six individuals (F1-F6) indicated by italics are the hypothetical founders.

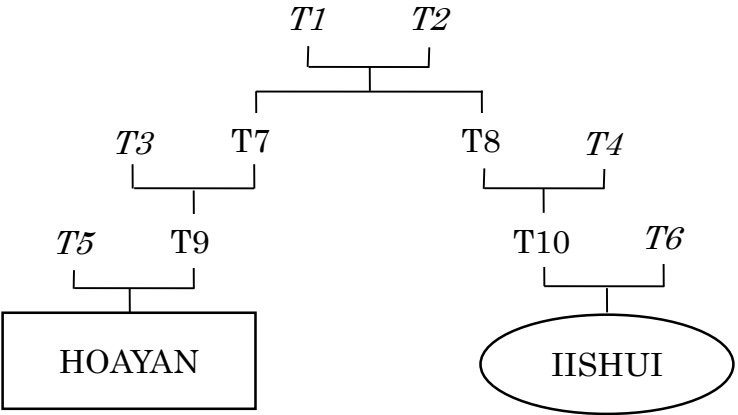


Figure 2-2. The hypothetical pedigree which shows the relationship between HOAYAN and IISHUI. The six individuals (T1-T6) indicated by italics are the hypothetical founders.

In assumption 2, because of little information on the relationships between HO or II and the other three founders, they 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 2-1).

As assumption 4, we assumed that all the five founders corresponded to first-degree relatives. Namely, all the kinship values among the five founders were assumed to be 0.25 (Table 2-1).

Table 2-1. The kinship matrixes under assumptions 1-4

Assumption 1					
	YOUYOU	YANYAN	MEIMEI	HOAYAN	IISHUI
YOUYOU	0.500	0	0	0	0
YANYAN	0	0.500	0	0	0
MEIMEI	0	0	0.500	0	0
HOAYAN	0	0	0	0.500	0
IISHUI	0	0	0	0	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.

Subsequently, we calculated 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 Chapter 1. They were calculated utilizing PM2000. In the calculation for GD and MeanF, the founders themselves were excluded. Furthermore, in the calculation of GD, 1,000 iterations of gene dropping simulation (MacCluer et al., 1986) were performed.

Results and Discussion

The GD and the MeanF under assumptions 1-4 were given in Table 2-2. Under assumption 1, namely, conventional approach, the average MK 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 MK of the founder population, the GD and the MeanF were 77.1% and 0.130, respectively. Under assumption 3, the average MK 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 MK of the founder population were much lower and much higher values of 65.0% and 0.286, respectively, than them under assumption 1. In this way, the results indicated that assumption 1 showed the highest GD and the lowest MeanF of all the assumptions, and that when the higher average MK of the founder population was assumed, the lower GD and the higher MeanF were shown. Therefore, if all the kinship values among the five founders were equal to or lower than that of first-degree relatives, under the assumption that the five founders were non-inbred, the GD would fluctuate

largely from 65.0% to 82.2% and the MeanF from 0.072 to 0.286.

Table 2-2. The gene diversity (GD) and the mean inbreeding coefficient (MeanF) of the Japanese captive population of Japanese crested ibis as of December 2010 under assumptions 1-4

Assumption	GD	MeanF
1	0.822	0.072
2	0.771	0.130
3	0.787	0.113
4	0.650	0.286

We showed the distribution of the frequency of MK under assumptions 1-4 in Figure 2-3. This distribution clearly shifted to higher MK value under the assumption that the average MK of the founder population was higher. We listed 10 individuals which had the lowest or highest MK under assumptions 1-4 in Table 2-3. According to the levels of kinships among the five 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.

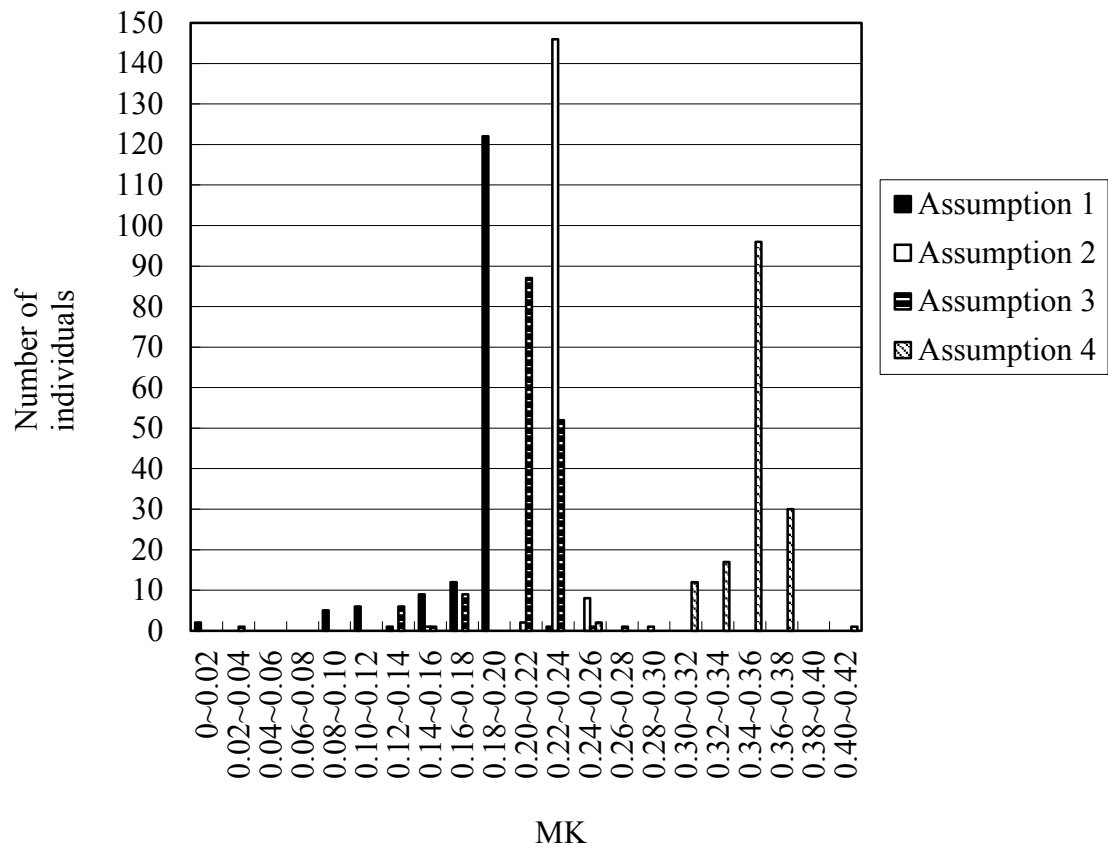


Figure 2-3. The distribution of the frequency of the individual mean kinship (MK) in the Japanese captive population of Japanese crested ibis as of December 2010 under assumptions 1-4.

Table 2-3. The genetic importance of individuals based on the individual mean kinship (MK) in the Japanese captive population of Japanese crested ibis as of December 2010 under assumptions 1-4¹⁾

Rank		Assumption 1		Assumption 2		Assumption 3		Assumption 4	
		Male	Female	Male	Female	Male	Female	Male	Female
↑ the lowest MK	1	HOAYAN (0.017)	IISHUI (0.008)	YOUYOU (0.218)	MEIMEI (0.158)	HOAYAN (0.027)	253Q (0.125)	HOAYAN (0.259)	IISHUI (0.254)
	2	265R (0.099)	193R (0.099)	89A (0.220)	YANYAN (0.218)	239Q (0.125)	269Q (0.125)	239Q (0.305)	253Q (0.305)
	3	239Q (0.103)	226R (0.099)	105A (0.220)	21A (0.220)	245Q (0.125)	MEIMEI (0.150)	245Q (0.305)	269Q (0.305)
	4	245Q (0.103)	258R (0.099)	144A (0.220)	32A (0.220)	182Q (0.127)	286Z (0.173)	182Q (0.307)	193R (0.309)
	5	182Q (0.106)	261R (0.099)	212A (0.220)	26A (0.221)	164Q (0.130)	288Z (0.173)	164Q (0.309)	226R (0.309)
	6	164Q (0.108)	253Q (0.103)	39A (0.221)	104A (0.221)	283Z (0.173)	241Y (0.175)	265R (0.309)	258R (0.309)
	7	283Z (0.147)	269Q (0.103)	86A (0.221)	190B (0.221)	238Y (0.175)	290Y (0.175)	283Z (0.335)	261R (0.309)
	8	238Y (0.150)	MEIMEI (0.123)	178B (0.221)	195B (0.221)	289Y (0.175)	293Y (0.175)	238Y (0.337)	MEIMEI (0.312)
	9	289Y (0.150)	286Z (0.147)	219B (0.221)	247B (0.221)	291Y (0.175)	YANYAN (0.210)	289Y (0.337)	286Z (0.335)
	10	291Y (0.150)	288Z (0.147)	259B (0.221)	297B (0.221)	YOUYOU (0.210)	21A (0.212)	291Y (0.337)	288Z (0.335)
	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
	10	204M (0.193)	272G (0.190)	265R (0.237)	193R (0.237)	204M (0.225)	181M (0.225)	204M (0.361)	181M (0.361)
	9	207M (0.193)	279G (0.190)	238Y (0.238)	226R (0.237)	207M (0.225)	199M (0.225)	207M (0.361)	199M (0.361)
	8	223M (0.193)	84C (0.192)	289Y (0.238)	258R (0.237)	223M (0.225)	224M (0.225)	223M (0.361)	224M (0.361)
	7	225M (0.193)	85C (0.192)	291Y (0.238)	261R (0.237)	225M (0.225)	231M (0.225)	225M (0.361)	231M (0.361)
	6	230M (0.193)	23B (0.193)	239Q (0.243)	241Y (0.238)	230M (0.225)	264M (0.225)	230M (0.361)	264M (0.361)
	5	250M (0.193)	181M (0.193)	245Q (0.243)	290Y (0.238)	250M (0.225)	193R (0.233)	250M (0.361)	163B (0.362)
	4	251M (0.193)	199M (0.193)	182Q (0.245)	293Y (0.238)	251M (0.225)	226R (0.233)	251M (0.361)	48B (0.363)
	3	257M (0.193)	224M (0.193)	164Q (0.248)	253Q (0.243)	257M (0.225)	258R (0.233)	257M (0.361)	58B (0.363)
	2	80B (0.194)	231M (0.193)	HOAYAN (0.255)	269Q (0.243)	265R (0.233)	261R (0.233)	80B (0.368)	40B (0.364)
	1	19A (0.239)	264M (0.193)	19A (0.281)	IISHUI (0.246)	19A (0.273)	IISHUI (0.246)	19A (0.401)	23B (0.367)
the highest MK ↓									

1) 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.

Chapter 3

**Numbers of individuals to be released in the future on Sado
Island from the perspective of allelic diversity**

Introduction

The reintroduction of this species into Sado Island aimed to establish a self-sustaining population consisting of 60 individuals, based on population viability analysis (Japanese Ministry of the Environment, 2003). As IUCN (1998) suggested, the build-up of reintroduced population should be modelled under a variety of conditions, in order to specify the most appropriate number and composition of individuals to be released per year and the numbers of years necessary to promote establishment of a viable population. Nagata and Yamagishi (2011) simulated the release numbers required to maintain the population of more than 60 individuals, varying parameters such as annual survival rate, fledging rate and predation pressure in the wild.

Some papers indicate that reintroduced populations which were founded by release of small numbers of individuals into an isolated area including an island, seemed not to be representative of the source population's gene pool and would generally retain lower genetic diversity (Stockwell et al., 1996; Tarr et al., 1998; Mock et al., 2004). So, the adequate number of individuals should be released to avoid loss of genetic diversity (Frankham et al., 2002; Allendorf and Luikart, 2007). However, no guidelines for the appropriate release numbers exist (Tracy et al., 2011). Thus, the purpose of this chapter was to determine number of individuals to be released in the future in order to preserve genetic diversity in the reintroduced population.

Materials and methods

Index of genetic diversity

Most captive management programs use GD as the index of genetic diversity (Frankham et al., 2002; Association of Zoos and Aquariums, 2012). Heterozygosity can

be used as a measure of a population's ability to respond to selection immediately after genetic bottleneck. In the reintroduced population, in order to target the survival of the population over longer-term, the other index was thought to be more effective. Allelic diversity determines a population's ability to respond to long-term selection over many generations, and ultimately the survival of the population (Allendorf, 1986). Also, Allendorf (1986) suggested that while even extreme genetic bottlenecks for a short time would have little effect on heterozygosity, they were expected to diminish quite a number of alleles. More alleles will give the population more options to respond to natural selection, and retention of rare alleles may be especially important for disease resistance and elsewhere (Slade and McCallum, 1992). In this chapter, we estimated the allelic diversity over the long-term, specifically, the extent to which a rare allele existed in the captive population would be retained in the reintroduced population in the future.

Simulation procedure

The rare alleles were held in the population due to survival of individuals passing them on via their reproduction, and were lost from the population by Mendelian sampling and as a result of death. Considering these factors, in this chapter, the stochastic simulation was carried out assuming parameters shown in Table 3-1.

Existing released individuals and individuals to be released additionally year by year of hypothetical number were randomly assigned genotypes which include 0-2 copies of a hypothetical neutral allele (rare allele) with a hypothetical frequency in the captive population. All these individuals were assumed to be sexually mature (two year at age or older) and have sex ratio of 1:1 based on facts. This species retains the same mating pairs in monogamous systems basically, and mates once a year. The mating pairs were assumed to produce offspring of hypothetical number having sex ratio of 1:1 every year until the

males were 12 years of age. Depending on these breeding systems, their offspring inherit the rare allele from their parents according to Mendelian rules. Alternatively, some of the released individuals were assumed to die immediately post-release based on average survival rate for three months after each of seven reintroductions (74%). Also, the annual survival rates of released individuals were assumed to be 49% at zero year of age, 66% at one year of age, 65% for adult females and 72% for adult males. These annual survival rates were determined based on the Japanese reintroduced population and the Chinese wild population. The annual survival rates for breeding adults were assumed to reduce after 13 years of age, reaching zero at 25 years of age based on information from the Chinese wild population. The annual survival rates for other individuals were assumed to reduce depending on density. If the reintroduced population reached the hypothetical carrying capacity, the population growth was stopped by removing randomly selected individuals except for breeding adults, and the annual survival rates at zero and one year of age were assumed to become 25% and 50%, respectively.

In this way, we determined whether the rare allele would be retained in the reintroduced population after 50 years. Fifty years corresponds to about 10 generations in the Japanese captive population (in Chapter 1), although the T has a high possibility of being longer in the future. The parameters shown in Table 3-1 including those described above were based on Japanese reintroduced or captive populations. Because the reintroduction of this species in Japan is recent, some parameters which could be obtained by accumulation of data over long-term in the wild were based on Chinese wild population and may vary in the future. However, except for average number of chicks produced per mating pair each year, these parameters were thought to approximate those of the Chinese wild population.

Important factors in the simulation

Because some parameters used in the simulation were estimated to have a significant impact on simulation results, they were given multiple values. These factors were the frequency of the rare allele in the captive population (Q), the number of individuals to be released per year (R), the average number of chicks produced per mating pair each year (C) and the carrying capacity on Sado Island (K) (Table 3-1). Q was given 0.05 which was suggested to be a frequency of the rare allele retained even in species that has experienced a prolonged genetic bottleneck (Grueber and Jamieson, 2008), and given 0.1 additionally. R was assumed as 5, 10, 15 or 30, mainly considering the release number in each reintroduction in the past, namely, less than 20. With regard to C, the average number of chicks produced per mating pair in 2013 on Sado Island was 0.58 (14 individuals/ 24 pairs, Japanese Ministry of the Environment, 2013a). While it increased slightly from that in the previous year in which the breeding success of the reintroduced population was confirmed initially, it was considerably lower than 2.26 in the Chinese wild population during the period of 1981 to 2003 (Ding, 2004). To be exact, 2.26 was the average number of chicks per nest (495 individuals/ 219 nests), and that per mating pair was expected to rise further since mating pairs occasionally build multiple nests as observed in the Japanese reintroduced population in which 24 mating pairs built 34 nests (Japanese Ministry of the Environment, 2013a). However, we used these values as C, namely, 0.58 and 2.26 as results of the Japanese reintroduced population and the Chinese wild population, respectively, because of the relative lack of information on Chinese wild population. As for K, no detailed research has been performed. Based on the fact that 85 individuals including 12 individuals born in the wild were alive, we assumed 100 as K along with 200 and 300. We varied any one of Q, R, C and K at a time keeping all other

parameters.

Then, we examined alternative scenarios in which many individuals were released over several recent years and the subsequent reintroductions were never carried out. Specifically, we assumed that 30 individuals were reintroduced each year for the first 5, 10 or 15 years and then the reintroductions were aborted at $Q = 0.05$ or 0.1 , $C = 2.26$ and $K = 300$.

Table 3-1. The parameters used in the simulation

Parameter	Assigned value	Source
Frequency of the rare allele in the captive population (Q)	0.05, 0.1	A
Size of the captive population	212 individuals	JC
Number of existing released individuals	99 ¹⁾	JR
Number of individuals to be released additionally every year (R)	5, 10, 15, 30	A
Average number of chicks produced per mating pair each year (C)	0.58, 2.26	JR, CW
Age at which males can breed	2-12 years	JC
Initial survival rate immediately post-release	0.74	JR
Annual survival rate at zero year of age	0.49	CW
Annual survival rate at one year of age	0.66	CW
Annual survival rate of adult females	0.65 ²⁾	JR
Annual survival rate of adult males	0.72 ²⁾	JR
Maximum allowable lifespan	25 years	CW
Age after which annual survival will be reduced by senescence	13 years ³⁾	A
Carrying capacity (K)	100, 200, 300 individuals	A
Annual survival rate at zero year of age when the population is at K	0.25 ⁴⁾	A
Annual survival rate at one year of age when the population is at K	0.50	A

Source; A: assumptive values, CW: result of Chinese wild population (Ding, 2004), JC: result of Japanese captive population (in Chapter 1), JR: result of Japanese reintroduced population (Japanese Ministry of the Environment, 2013a; Japanese Ministry of the Environment, 2013b)

- 1) We assumed that number of existing released individuals was decreased by initial survival rate immediately post-release ($= 0.74$), and resulted in 73 living individuals as of June 2013. Therefore, the back calculation was performed. All the 99 individuals were assumed to be released at once. Although 12 out of individuals born in the wild in 2012 and 2013 were alive as of June 2013, they were excluded.
- 2) Annual survival rates in 1-4 years after each of six reintroductions were first calculated. Next, the annual survival rates of each reintroduction were estimated by geometric mean. In this chapter, the averages of these annual survival rates were adopted.
- 3) No clear information existed. But, according to Ding (2004), in the Chinese wild population as of July 2003, the number of individuals after 13 years of age was extreme small.
- 4) The half of annual survival rate at zero year of age when the size of the population does not reach the carrying capacity was adopted as indicated by Weiser et al. (2013).

Implementation of simulation

The number of replicates was 1,000 and the proportion of replicates in which the rare allele was retained over 50 years was translated into the probability of retaining the rare allele in the reintroduced population. The implementation of simulation was conducted by using AlleleRetain (Weiser et al., 2012) in R program (R Development Core Team, 2012).

Results and Discussion

The probabilities of retaining the rare allele with $Q = 0.05$ in the reintroduced population after 50 years at $K = 100, 200$ and 300 are given in Figure 3-1a, 3-1b and 3-1c, respectively. Those under similar condition except for $Q = 0.1$ are shown in Figure 3-2a-c. For example, Figure 3-1a shows that if the average number of chicks produced per mating pair each year is maintained at result of the Japanese reintroduced population in 2013 ($C = 0.58$), under the condition that the carrying capacity on Sado Island is 100 individuals ($K = 100$), the reintroductions of five individuals every year ($R = 5$) resulted in the probability of allele retention of 66.7%. Under the same condition, the reintroductions of 15 and 30 individuals ($R = 15$ and 30) resulted in the probabilities of allele retention of more than 90% (Figure 3-1a). Thus, more individuals to be released resulted in increase of the probability of allele retention. But the increase of probability of allele retention became blunt in accordance with the increase of release number. The probabilities was almost similar between 15 and 30 individuals to be released under any condition. If the reintroductions are continued over 50 years, it seems that, in terms of allelic diversity, the reintroductions of more than 15 individuals would provide little benefit and the reintroductions twice or more a year would not be needed since 15

individuals were within the carrying capacity of training facility.

At $Q = 0.05$, $R = 5$, $K = 100$, while the probability of allele retention showed 66.7% assuming C as 0.58, when assuming C as 2.26, it showed much higher value of 80.3% (Figure 3-1a). The increase in C resulted in the increase of the probability of allele retention, especially at low R . The populations that grow slowly retain less allelic diversity than those that grow more quickly, and thus lower reproductive rates diminish the probability of allele retention (Allendorf and Luikart, 2007). In addition, the breeding success is unequal among individuals in many species (Emlen and Oring, 1977), and this variance can generally lead to a failure to equalize founder representation which produces decreased N_e and genetic diversity and increased inbreeding (Haig et al., 1990; Anthony and Blumstein, 2000; Meffert et al., 2005).

With regard to K , when we assumed $Q = 0.05$, $C = 0.58$ and $R = 5$, the probabilities of allele retention were 66.7%, 67.0% and 66.0% at $K = 100$, 200 and 300, respectively (Figure 1a-c). In this way, K had little effect on allele retention because the size of the reintroduced population reached the limit of carrying capacity only when $R = 15$ and 30, $C = 2.26$ and $K = 100$. If the reintroduced population reached the limit of carrying capacity, more individuals to be released would be required in order to increase the probability of allele retention (Mills and Allendorf, 1996; Grueber and Jamieson, 2008) because the probability of breeding success may decay as reported in the Chinese wild population (Ding, 2004). The parameters utilized when the reintroduced population reached the limit of carrying capacity were invalid at this time and thus should be fully examined in the future.

As expected, at $Q = 0.1$, the probabilities of allele retention were higher than at $Q = 0.05$ under each condition.

Figure 3-3 indicates that if the average number of chicks produced per mating pair each year is maintained in line with the Chinese wild population between 1981 and 2003 ($C = 2.26$), on the basis that the carrying capacity on Sado Island is 300 individuals ($K = 300$), the reintroductions of 30 individuals every year for the first 15 years and the absence of subsequent reintroductions resulted in the low values of 52.3% and 77.3% for the probabilities of retaining the rare alleles with frequencies in the captive population of 0.05 and 0.1 ($Q = 0.05$ and 0.1), respectively. The probabilities of allele retention reduced drastically immediately after discontinuation of reintroduction (data not shown). The probability of allele retention under this condition was lower than the probability under the condition that only five individuals were released each year over 50 years, in which the sum of number of individuals to be released for 50 years was less.

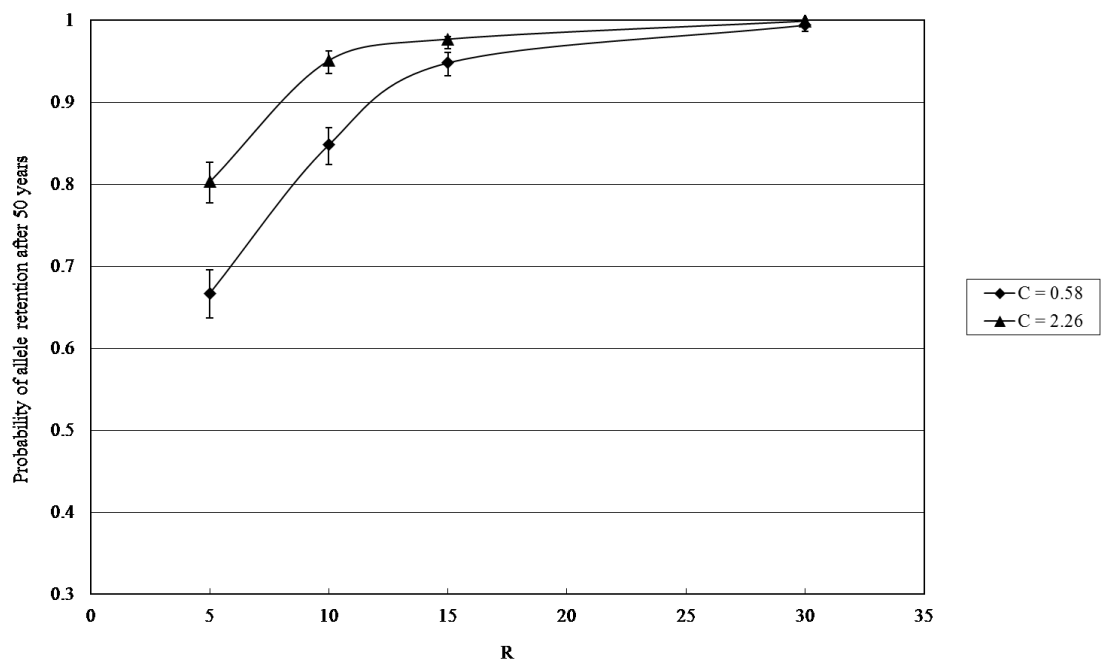


Figure 3-1a

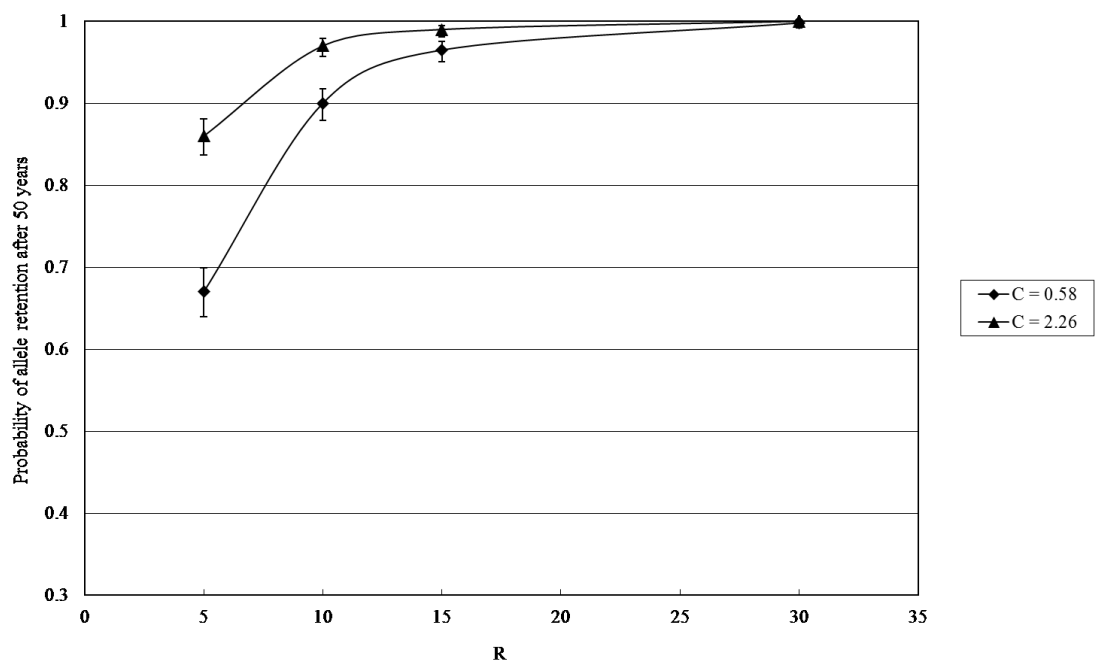


Figure 3-1b

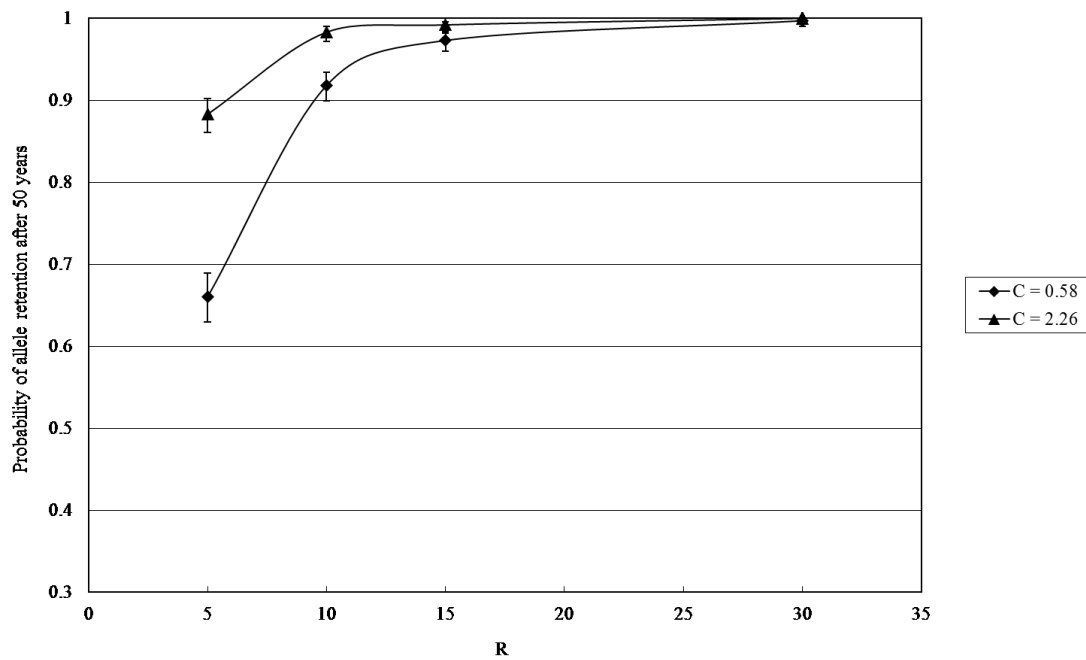


Figure 3-1c

Figure 3-1. The probabilities of retaining the rare allele with a frequency in the captive population of 0.05 in the reintroduced population after 50 years according to number of individuals to be released every year for 50 years (R) when the carrying capacity on Sado Island is 100 (a), 200 (b) and 300 (c). Assuming that the reintroduced population maintained the average number of chicks produced per mating pair each year (C) in the Japanese reintroduced population as of 2013 (0.58) or in the Chinese wild population between 1981 and 2003 (2.26) over 50 years, we compared them. Bars indicate 95% confidence limits.

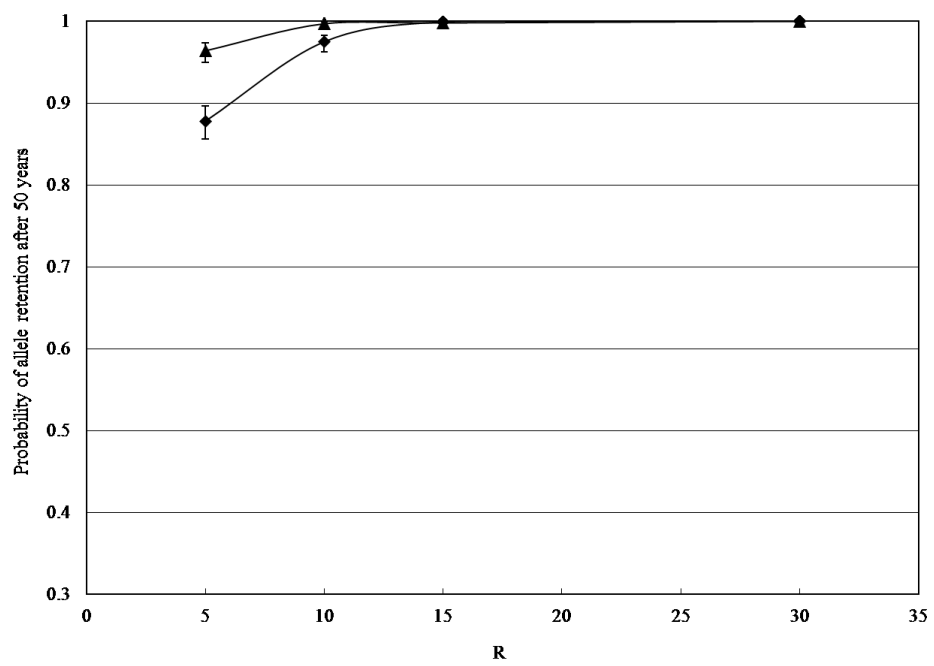


Figure 3-2a

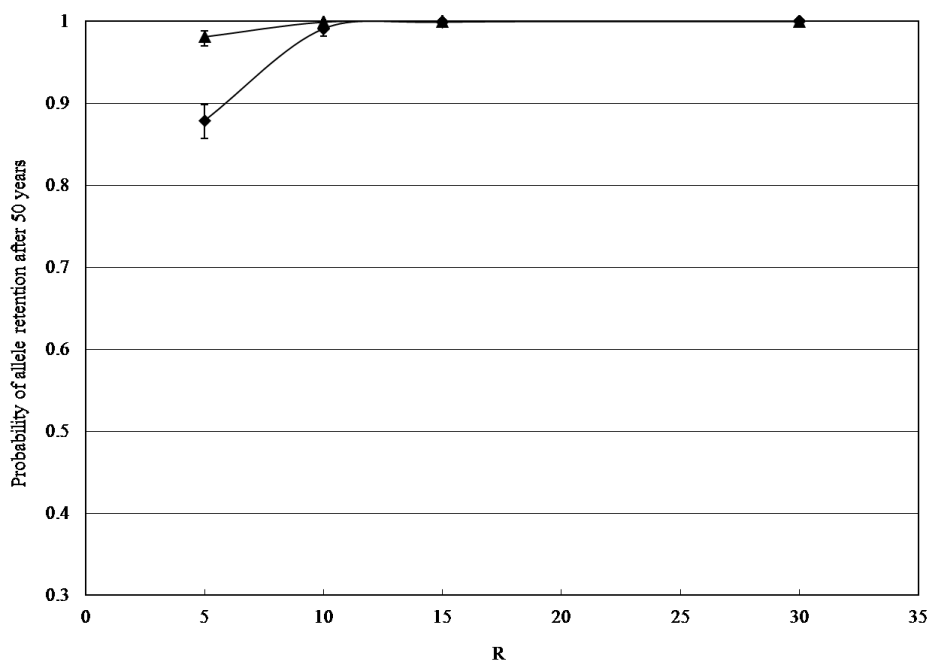


Figure 3-2b

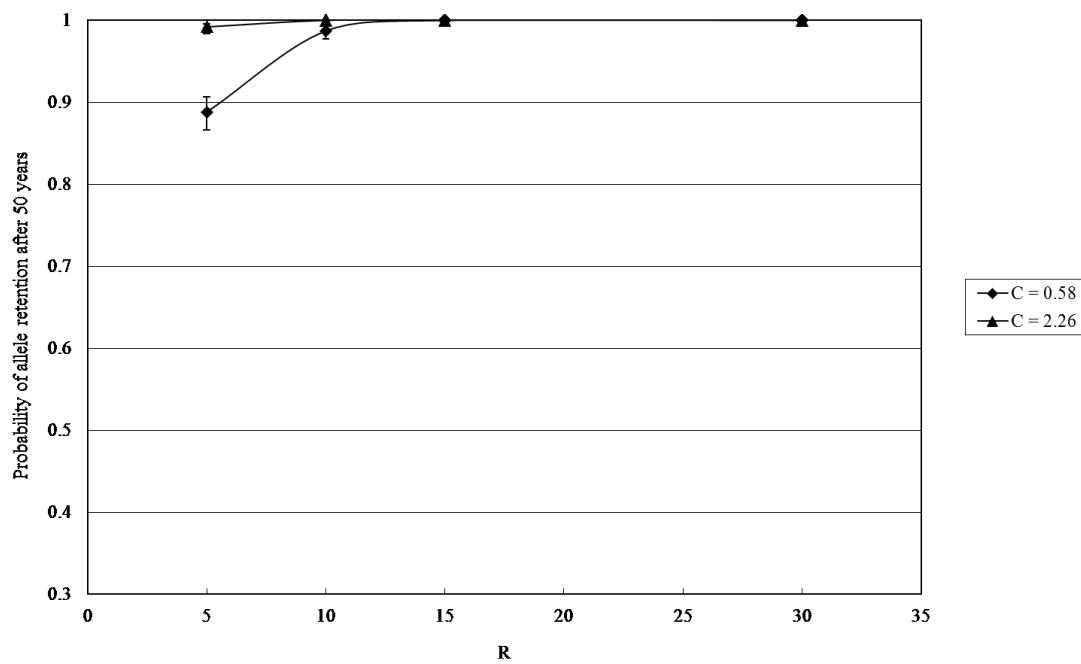


Figure 3-2c

Figure 3-2. The probabilities of retaining the rare allele with a frequency in the captive population of 0.1 in the reintroduced population after 50 years according to number of individuals to be released every year for 50 years (R) when the carrying capacity on Sado Island is 100 (a), 200 (b) and 300 (c). Assuming that the reintroduced population maintained the average number of chicks produced per mating pair each year (C) in the Japanese reintroduced population as of 2013 (0.58) or in the Chinese wild population between 1981 and 2003 (2.26) over 50 years, we compared them. Bars indicate 95% confidence limits.

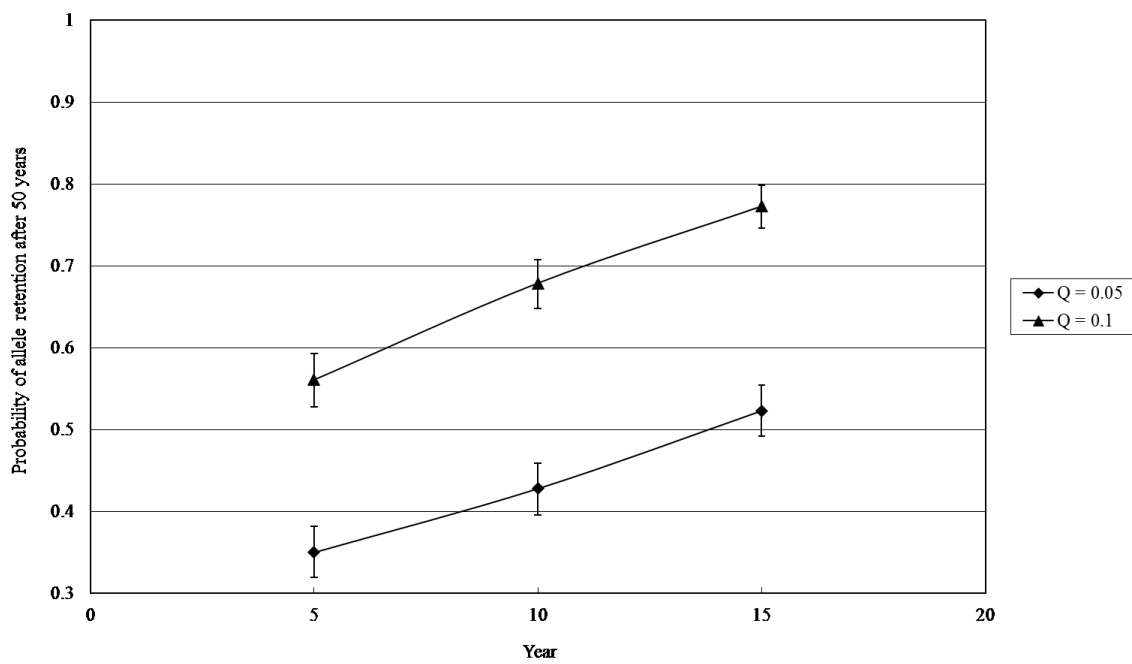


Figure 3-3

Figure 3-3. The probabilities after 50 years of allele retention when we assumed that 30 individuals were released each year for the first 5, 10 or 15 years and then the reintroductions were aborted. The frequencies of the rare allele in the captive population (Q) were assumed to be 0.05 and 0.1, the carrying capacity on the Sado Island was to be 300 and the average number of chicks produced per mating pair each year was to be 2.26. Bars indicate 95% confidence limits.

General Discussion

In Chapter 1, we estimated the demographic parameters of the Japanese crested ibis captive population, in order to predict the future genetic diversity considering the number of newly introduced founders and the carrying capacity. We found that, while a significant population growth was observed, T and N_e were still found to be short and small, respectively. Under the condition that the carrying capacity is 200 individuals, the GD after 100 years was estimated to be 60.9% in the absence of supplements from the Chinese population. Even when we adopted the compromise goal of retaining 80% of the genetic variation in the source population over 100 years, it was suggested that under the condition that the carrying capacity was 200 individuals, continuous introductions of at least one supplementing individual would be required. However, future demographic analyses should be performed to acquire more updated demographic parameter values. On that basis, this simulation should be performed continually to obtain exact estimates of the number of newly introduced founders and the carrying capacity required.

In Chapter 1, we assumed that the five founding individuals were non-inbred and unrelated, because we had no pedigree information for these individuals. This was done in spite of the fact that the Japanese crested ibis had experienced a severe genetic bottleneck in China. Therefore, it is likely that the genetic estimates, as shown in Chapter 1, are the most optimistic estimates. However, even based on these optimistic estimates, the Japanese captive population was estimated to be in an undesirable genetic situation. Therefore, conservation efforts to maintain and increase the genetic diversity, such as introductions of new founders and increases in carrying capacity, will definitely be required in the future.

Using the results found in Chapter 1, we investigated how low the current genetic diversity of this species was, when relationships among founders were considered. Under

the assumption that the five founders were non-inbred, in Chapter 2, we considered four scenarios in which all the kinship values among the five founders were equal to or lower than that of first-degree relatives. As a result, the GD would fluctuate largely from 65.0% to 82.2%, and the genetic importance of individuals based on MK also varied. If MK strategy would be introduced into this population, a greater consideration of selection of individuals as mating pairs would be required. 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 they were unrelated, our present study illustrates the importance of the relationships among the founders.

Rudnick and Lacy (2008) examined the impact of assumptions about founder relationships on genetic diversity in MK strategy using computer simulations. They found that the overall long-term benefit of knowing founder relationships was only modest. Population studies of the parma wallaby (*Macropus parma*) (Ivy et al., 2009) and the lowland tapir (*Tapirus terrestris*) (Gonçalves da Silva et al., 2010) corroborated this suggestion. However, it is unclear whether this also applies to the Japanese crested ibis captive population in which the adoption of MK strategy is considered. Rudnick and Lacy (2008) and Ivy et al. (2009) performed simulations under the assumption that 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 required in order to maintain and increase the genetic diversity. There is therefore a high possibility that the relationships among the existing founders and newly introduced founders are unknown. Future efforts are needed to develop more molecular markers to estimate the relationships among the founders. Further, as Jones et al. (2002) suggested, the result

should be incorporated into the breeding programs based on MK strategy.

Based on Chapters 1 and 2, the reintroduced population should also be analyzed in terms of genetic diversity in the wild population, which probably experiences more significant environmental changes than the captive population. For the survival of the reintroduced population, it is clear that more release number produces a better result (Beck et al., 1994; Fischer and Lindenmayer, 2000). In the reintroduction program for this species on Sado Island, however, the most release number per reintroduction is less than 20 individuals, due to the limited carrying capacity of the reintroduction training facility. Furthermore, the number of reintroductions per year is limited to one in autumn and sometimes two in spring or autumn depending on the required training period, maintenance of the training facility after training and the field environment at the time of release (Japanese Ministry of the Environment, 2013b). Moreover, because a reintroduction requires a financial infusion, excessive individuals should not be reintroduced. In addition, the number of individuals to be released affects the financial cost and labor needed in the captive-breeding programs, which supply the individuals to be released.

Weiser et al. (2013) aimed to retain an allele in the reintroduced population with a frequency in the source population of 0.05 with a probability of 90% for 10 generations. In Chapter 3, it was shown that, if this goal was applied to the Japanese crested ibis, it would be desirable to release 10-15 individuals annually over 50 years. The estimated number 10-15 individuals approximates the number of individuals that are currently released in the annual reintroduction, and therefore, a continuation of the current reintroduction scheme is necessary.

The goal of the national conservation project for the Japanese crested ibis is to

establish a viable population in the wild. To achieve this goal, maintaining the genetic diversity of the reintroduced population is one of the main factors. The genetic diversity of the captive population, which supplies the individuals to be released, is also essential. Through continued reintroductions, the introductions of new founders and breeding programs by integration of the molecular data into MK strategy are considered to play a key role for the success of this national project.

Summary

Japanese crested ibis (*Nipponia nippon*) completely disappeared from the wild in 1981, and the last individual indigenous to Japan died in captivity in 2003. Then, as a national project, the Japanese captive population has been established by using five individuals derived from the Chinese captive population as founders. Its size has increased rapidly, and the maintenance phase is about to start. In 2008, the reintroduction programs were initiated on Sado Island. In order for the Japanese reintroduced population to survive in the wild where environmental changes are frequent, the retention of genetic diversity in the population is needed. Because the reintroduced population is founded by the captive population, it is also very important to maintain the genetic diversity in the Japanese captive population. We first predict the number of founders to be introduced newly and the carrying capacity needed to maintain the genetic diversity in the captive population. It was suggested that under the condition that the carrying capacity was 200 individuals which was calculated as the current one, the gene diversity after 100 years was estimated to be about 60% with no supplements from China. Moreover, it was estimated that it would be required to continue introductions of four supplements every five years for 100 years, in order to retain 90% of the gene diversity after 100 years at the carrying capacity of 200 individuals. The target of retention of 90% after 100 years could never be achieved with no supplements. Because these results were based on the assumption that the five founders were unrelated and non-inbred, the genetic analyses when they were assumed to be related were performed using pedigree information. The adoption of mean kinship strategy as the breeding strategy suited to the maintenance phase was also investigated. When different assumptions were used ranging from zero to

0.25 of kinship coefficients between the five founders, the results showed that the gene diversity and the mean inbreeding coefficient would fluctuate largely from 65% to 82 % and from 0.07 to 0.29, respectively. We also found that the introduction of mean kinship strategy into the Japanese captive population should require adequate consideration because the genetic importance of individuals based on mean kinship shifted largely. It would become 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. One of the most important factors to ensure the genetic diversity in the reintroduced population is the number of individuals to be released. Our simulation indicated that 10-15 individuals, which were currently released in the annual reintroduction, would be needed to be released each year over 50 years in order that the reintroduced population after 50 years retains a rare allele with a frequency in the captive population of 0.05 with a probability of more than 90%. Therefore, the current reintroduction program appears to be reasonable and should be continued.

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