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Author(s): Kensuke Urano, Kanako Tsubono, Yukio Taniguchi, Hirokazu Matsuda, Takahisa Yamada, Toshie Sugiyama, Kosuke Homma, Yoshinori Kaneko, Satoshi Yamagishi and Hiroaki Iwaisaki

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Kensuke Urano[1], Kanako Tsubono[1], Yukio Taniguchi[1], Hirokazu Matsuda[1], Takahisa Yamada[2], Toshie Sugiyama[2], Kosuke Homma[3], Yoshinori Kaneko[4], Satoshi Yamagishi[5], and Hiroaki Iwaisaki[1]*

[1]*Laboratory of Animal Breeding and Genetics, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*
[2]*Department of Agrobiology, Faculty of Agriculture, Niigata University, Nishi-ku, Niigata 950-2181, Japan*
[3]*Field Center for Sustainable Agriculture and Forestry, Niigata University, Nishi-ku, Niigata 950-2181, Japan*
[4]*Sado Japanese Crested Ibis Conservation Center, Sado, Niigata 952-0101, Japan*
[5]*Yamashina Institute for Ornithology, Abiko, Chiba 270-1145, Japan*

The Japanese crested ibis *Nipponia nippon* is a critically threatened bird. We assessed genetic diversity and structure in the Sado captive population of the Japanese crested ibis based on 24 and 50 microsatellite markers developed respectively for the same and related species. Of a total of 74 loci, 19 showed polymorphisms in the five founder birds of the population, and therefore were useful for the analysis of genetic diversity and structure. Genetic diversity measures, A, n_e, H_e, H_o and PIC, obtained by genotyping of the 138 descendants were similar to those of other species with population bottlenecks, and thus considerably low. The low level of genetic diversity resulting from such bottlenecks was consistent with the results of lower genetic diversity measures for the Sado captive relative to the Chinese population that is the source population for the Sado group as determined using previously reported data and heterozygosity excess by Hardy-Weinberg equilibrium tests. Further, individual clustering based on the allele-sharing distance and Bayesian model-based clustering revealed that the founder genomes were equally at population in total, and with various admixture patterns at individual levels inherited by the descendants. The clustering results, together with the result of inheritance of all alleles of the microsatellites from the founders to descendants, suggest that planned mating in captive-breeding programs for the population has succeeded in maintaining genetic diversity and minimizing kinship. In addition, the Bayesian model-based clustering assumed two different components of genomes in the Sado captive Japanese crested ibis, supporting a considerably low level of genetic diversity.

Key words: genetic diversity, genetic structure, Japanese crested ibis, microsatellite, Sado Island

INTRODUCTION

The Japanese crested ibis *Nipponia nippon* is a globally threatened bird (Collar et al., 1994) that became extinct in the wild in Japan. After the extinction in Japan, five individuals of the Japanese crested ibis (two individuals in 1999, one individual in 2000 and two individuals in 2007) were gifted by the Chinese government. The population size has now increased to about 150 captive individuals, composing the current Sado Japanese crested ibis population, as a result of conservation efforts involving captive-breeding programs implemented at the Sado Japanese Crested Ibis Conservation Center, Niigata, Japan.

* Corresponding author. Tel. : +81-75-753-6322;
Fax : +81-75-753-6340;
E-mail: iwaisaki@kais.kyoto-u.ac.jp
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A major concern in the conservation of small or captive populations is loss of genetic diversity through genetic drift and inbreeding (Lande, 1988). Further, the idea of maintaining captive-bred animals for eventual release into the wild is a major aim of modern zoological collections (Durrell and Mallinson, 1987), and the Ministry of the Environment of Japan launched the project for a tentative release of the Japanese crested ibis in Sado Island in 2008. Additionally, the project for merging of novel Japanese crested ibis from the Chinese population into the Sado captive population will be carried out in near future with the effort of the Ministry of the Environment of Japan. Knowledge of genetic diversity and structure can be vital to the administration of captive populations, and is fundamental to the success of metapopulation management strategies, including the reintroduction of captive-bred individuals into the wild, and the success of merging management strategy of novel individuals from the Chinese population into the Sado captive population. How-

ever, it is difficult to obtain precise knowledge of the genetic diversity and structure of the Sado captive population of Japanese crested ibis, as pedigree information for relatedness and kinship of the founders is deficient. Therefore, molecular genetics studies on genetic diversity and structure are clearly needed to enable further effective management of the conservation of this species as part of the national project for the reintroduction of captive-bred individuals into the wild and the merging of Chinese novel individuals into the Sado captive population.

Microsatellite DNA is the most suitable DNA marker currently available for the purpose of the study on genetic diversity and structure (Zhang and Hewitt, 2003). Therefore, in this study, we reported the assessment of genetic diversity and structure in the Sado captive population of the Japanese crested ibis based on 24 and 50 microsatellite markers developed for the same species and related species, respectively.

MATERIALS AND METHODS

Sample

We used the five founder birds for the Sado captive Japanese crested ibis population, and 138 descendant birds that had hatched from 2001 to 2009. Peripheral blood samples were collected from the founders at the Sado Japanese Crested Ibis Conservation Center (Niigata, Japan) and used for genomic DNA preparation. Post-hatch eggs stored at the Sado Japanese Crested Ibis Conservation Center (Niigata, Japan) were used for the descendants. The vascularized chorioallantois membrane samples were excised from the eggs with forceps and a scalpel and used for genomic DNA preparation, as described by Urano et al. (2011). DNA was extracted from these samples using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany).

Genotyping

We used a total of 74 microsatellite loci in this study. Of the 74 loci, 24 corresponded to the microsatellite markers developed for the Japanese crested ibis (Ji et al., 2004; He et al., 2006). Additionally, we used 50 microsatellite markers developed for species related to the Japanese crested ibis (Tomasulo-Seccomandi et al., 2003 for the wood stork *Mycteria americana*; Santos et al., 2006 for the scarlet ibis *Eudocimus ruber*; Sawyer and Benjamin, 2006 for the roseate spoonbill *Ajaia ajaja*; Yeung et al., 2009 for the black-faced spoonbill *Platalea minor*) which are likely to be useful to identify microsatellite loci of the Japanese crested ibis, as PCR primers derived from one species are often found to amplify microsatellite loci in related species (Engel et al., 1996; Primmer et al., 1996; Slate et al., 1998). With these 74 loci, one primer of each pair was end-labeled with a fluorescent dye, 6-FAM. PCR amplifications were carried out in a 10-μl volume using GeneAmp PCR System 9700 (Applied Biosystems, Foster city, CA), as described by He et al. (2006), Ji et al. (2004), Tomasulo-Seccomandi et al. (2003), Santos et al. (2006), Sawyer and Benjamin (2006), or Yeung et al. (2009). For allele scoring, a portion of PCR products (0.2 μl) from each locus was combined with 10 μl of formamide loading dye that contained the fluorescent-labeled GeneScan-500LIZ size standard (Applied Biosystems), and assayed using a 3730xl DNA analyzer (Applied Biosystems). We used GeneMapper software version 4.0 (Applied Biosystems) to create electropherograms and to score PCR products. To examine amplification and polymorphism of the 74 microsatellite loci in the Sado captive population of Japanese crested ibis, DNA panel of the five founders were first tested. Subsequently, the polymorphic loci were genotyped on the 138 descendants.

Data analysis

Standard genetic diversity was assessed by calculating allelic diversity (A), effective number of alleles (n_e), observed (direct count) heterozygosity (H_o), unbiased expected heterozygosity (H_e; Nei, 1987), and polymorphic information content (PIC; Botstein et al., 1980) of the 138 descendants in the Sado captive Japanese crested ibis population, using EXCEL MICROSATELLITE TOOLKIT version 3.1.1 (Park, 2001). Fisher's exact test was used to determine departure from Hardy-Weinberg equilibrium (HWE) using the GENEPOP version 1.2 (Raymond and Rousset, 1995). Unbiased estimates of exact P-values were obtained by the Markov chain Monte Carlo (MCMC) algorithm of Guo and Thompson (1992). The GENEPOP program was also used to test linkage disequilibrium for pairs of loci. Significance values for HWE and pair-wise comparisons of linkage disequilibrium were also Bonferroni-corrected to account for multiple tests.

To assess genetic relationships among individuals of the five founders and the 138 descendants in the Sado captive Japanese crested ibis population, the natural logarithm of the proportion of shared alleles (D_{ps}; Bowcock et al., 1994), calculated by MICROSATELLITE ANALYZER (Dieringer and Schlotterer, 2003), was used as a distance for individual clustering. Based on the D_{ps}, the neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) was applied to constructing a tree, with the NEIGHBOR in the PHYLIP version 3.69 (Felsenstein, 2005). The tree was edited using TREEEXPLORER in MEGA4 version 4.0 (Kumar et al., 2004).

To compare the composition of genomes among individuals of the five founders and the 138 descendants in the Sado captive Japanese crested ibis population, a model-based Bayesian clustering was conducted using the STRUCTURE version 2.3 (Pritchard et al., 2000). We carried out 10 independent runs for each K value ranging from 1 to 8. For all runs, we used the default settings, and an admixture model with correlated allele frequencies and the parameter of individual admixture alpha were set to be the same for all clusters. Further, we performed each run with 100,000 Markov chain Monte Carlo sampling after a burn-in period of 100,000 iterations. To determine the optimal number of genetic clusters (K), both the natural logarithm of the probability of the data [ln Pr (X/K)] (average values of 10 independent runs for each K) (Pritchard et al., 2000) and ΔK statistic (Evanno et al., 2005) were calculated. Using the program STRUCTURE, the most likely K is that where the ln Pr (X/K) and/or the ΔK statistic are maximized. Additionally, in the STRUCTURE analysis, we calculated F values that reflect the amount of drift for each cluster from a common ancestral population (Falush et al., 2003; Shoda-Kagaya et al., 2010).

We compared genetic diversity measures (A, n_e, H_e and H_o) between the Sado captive and the Chinese population that is the source or ancestral population for the Sado, based on the microsatellite markers developed from the Japanese crested ibis (Ji et al., 2004; He et al., 2006). We used data (captive population data were used for literature of He et al., 2006) obtained from the literature (Ji et al., 2004; He et al., 2006) for the Chinese population, and the present data for the Sado captive population.

RESULTS

Polymorphism of microsatellite markers

Of the 24 loci developed for the Japanese crested ibis (Ji et al., 2004; He et al., 2006), 22 were successfully amplified, and nine displayed polymorphisms in the five founders (Table 1), indicating that the nine markers provide the basis for the analysis of genetic diversity and structure in this study. Of the amplified 22 loci, five loci (NnEA9, NnAD10, NnEH10, NnGF4 and NnLF11; Ji et al., 2004) were monomorphic in both the founders in this study and the Chinese Japanese crested ibis population (Ji et al., 2004). Thus, 17 microsatellite markers developed from the Japanese crested ibis, except for the two not amplified and the monomorphic

five loci, were found to be useful for comparison of genetic diversity in the Sado captive and the Chinese populations of Japanese crested ibis (see below).

For 50 microsatellite markers developed for the related species (Tomasulo-Seccomandi et al., 2003; Santos et al., 2006; Sawyer and Benjamin, 2006; Yeung et al., 2009), 44 (eight from the wood stork, 10 from the scarlet ibis, six from the roseate spoonbill, and 20 from the black-faced spoonbill) succeeded in amplifying and 10 (one from the wood stork, one from the roseate spoonbill, and eight from the black-faced spoonbill) showed polymorphisms in the five founders (Table 1). Thus, the 10 polymorphic markers, together with the nine Japanese crested ibis markers, were chosen for large-scale genotyping of the 138 descendants for the analysis of genetic diversity and structure in this study.

Table 1. Nineteen microsatellite loci polymorphic in this study.

Locus name*	Source species	Allele size (bp)	Primer sequences obtained from
Nn01	Japanese crested ibis	179, 181, 187	He et al. (2006)
Nn04	Japanese crested ibis	192, 194	He et al. (2006)
Nn12	Japanese crested ibis	219, 221, 223	He et al. (2006)
Nn17	Japanese crested ibis	265, 269	He et al. (2006)
Nn18	Japanese crested ibis	159, 173	He et al. (2006)
Nn21	Japanese crested ibis	157, 167	He et al. (2006)
Nn25	Japanese crested ibis	155, 159	He et al. (2006)
Nn26	Japanese crested ibis	140, 149	He et al. (2006)
NnNF5	Japanese crested ibis	112, 115	Ji et al. (2004)
PM1-17	Black-faced spoonbill	400, 430	Yeung et al. (2009)
PM2-16	Black-faced spoonbill	298, 302	Yeung et al. (2009)
PM2-20	Black-faced spoonbill	363, 367	Yeung et al. (2009)
PM2-21	Black-faced spoonbill	246, 262, 284	Yeung et al. (2009)
PM2-28	Black-faced spoonbill	184, 188	Yeung et al. (2009)
PM2-29	Black-faced spoonbill	224, 228	Yeung et al. (2009)
PM2-37	Black-faced spoonbill	430, 466	Yeung et al. (2009)
PM3-13	Black-faced spoonbill	179, 181	Yeung et al. (2009)
Aaju02	Roseate spoonbill	296, 302	Sawyer and Benjamin (2006)
Wsu13	Wood stork	200, 203	Tomasulo-Seccomandi et al. (2003)

* Additional 47 loci amplified as a monomorphic microsatellite product in this study: Nn03 (He et al., 2006), NnAF4, NnBF7, NnCE11, NnCG3, NnDD9, NnEB12, NnHB12, NnEA9, NnAD10, NnEH10, NnGF4, and NnLF11 (Ji et al., 2004) from the Japanese crested ibis, WSu03, WSu09, WSu14, WSu17, WSu19, WSu23, and WSu24 (Tomasulo-Seccomandi et al., 2003) from the wood stork, Eru02, Eru03, Eru04, Eru05, Eru06, Eru07, Eru08, Eru09, Eru10, and Eru11 (Santos et al., 2006) from the scarlet ibis, Aaju01, Aaju03, Aaju04, Aaju05, and Aaju06 (Sawyer and Benjamin, 2006) from the roseate spoonbill, and PM1-4, PM1-13, PM2-68, PM3-15, PM3-16, PM3-17, PM3-20, PM3-22, PM3-25, PM3-28, PM3-29, and PM3-31 (Yeung et al., 2009) from the black-faced spoonbill.

Analysis of genetic diversity

The genetic diversity measures of the five founders and the 138 descendants are shown in Table 2. The result of the same *A* values in the founders and the descendants indicated that the descendants inherited all alleles of the microsatellites from the founders. Higher values of n_e, H_e, H_o and PIC in the descendants were observed at Nn01, Nn12 and PM2-21 loci having three alleles. Nn17 and PM2-37 loci showed lower values of n_e, H_e, H_o and PIC in the descendants, due to a rare allele derived only from a heterozygote of a founder gifted recently (in 2007) from the Chinese government. The result of genetic diversity measures indicated that genetic diversity in the Sado captive population was similar to that of other species with population bottlenecks (Bouzat et al., 1998; Tarr et al., 1998) and considerably low.

In HWE tests except for PM2-37 locus with only one copy of a rare allele in the descendants, significant deviations from the HWE were observed in six cases (Nn25, Nn26, NnNF5, PM2-20, PM2-21 and overall) (Table 2). In particular, three of six cases (NnNF5, PM2-20 and overall) significantly deviated from HWE expectations, even after correction using Bonferroni procedure (Table 2). The PM2-20 locus may be Z-linked since all homozygotes were female, as described in the black-faced spoonbill by Yeung et al. (2009), with the locus having a lower H_o than expected (Table 2). The NnNF5 and overall cases showed a higher H_o than expected, indicative of heterozygosity excess (Table 2). This excess may have resulted from the known severe bottleneck (Cornuet and Luikart, 1996). In addition, none of the loci, except for PM2-20, appeared to be in linkage disequilibrium with one another when alpha value was adjusted to 0.000327 (Bonferroni correction for 153 pairwise comparisons), supporting the idea that the 19 markers used in this

Table 2. Allelic diversity (*A*) in the founders, and *A*, effective number of alleles (n_e), unbiased expected heterozygosity (H_e), observed (direct count) heterozygosity (H_o), polymorphic information content (PIC) and deviation from HWE in the descendants of 19 microsatellite markers in the Sado captive Japanese crested ibis population.

Locus name	Founders	Descendants					
	A	*A*	n_e	H_e	H_o	PIC	HWE
Nn01	3	3	1.995	0.501	0.526	0.447	–
Nn04	2	2	1.923	0.482	0.504	0.365	–
Nn12	3	3	2.991	0.668	0.697	0.592	–
Nn17	2	2	1.015	0.014	0.014	0.014	–
Nn18	2	2	1.423	0.298	0.319	0.253	–
Nn21	2	2	1.994	0.500	0.558	0.374	–
Nn25	2	2	1.938	0.486	0.388	0.367	+
Nn26	2	2	1.614	0.382	0.452	0.308	+
NnNF5	2	2	1.567	0.363	0.474	0.296	+, (+)
PM1-17	2	2	1.907	0.477	0.500	0.362	–
PM2-16	2	2	1.321	0.244	0.283	0.214	–
PM2-20	2	2	1.299	0.231	0.156	0.204	+, (+)
PM2-21	3	3	2.336	0.574	0.543	0.494	+
PM2-28	2	2	1.676	0.405	0.448	0.322	–
PM2-29	2	2	1.836	0.458	0.416	0.352	–
PM2-37	2	2	1.007	0.007	0.007	0.007	na*
PM3-13	2	2	1.249	0.200	0.225	0.180	–
Aaju02	2	2	1.805	0.448	0.388	0.347	–
Wsu13	2	2	1.302	0.233	0.268	0.205	–
Overall	2.158	2.158	1.695	0.367	0.377	0.300	+, (+)

+, Significant ($P < 0.05$).
(+), Significant ($P < 0.05$) after correction using Bonferroni procedure.
* The test for departure from Hardy-Weinberg equilibrium is not applicable because of only one copy of a rare allele derived only from heterozygote of the founder gifted recently (in 2007) from the Chinese government.

study are useful for the analysis of genetic diversity and structure in the Sado captive population. However, we should note that the PM2-20 locus was excluded from the clustering analyses.

Clustering analysis

We next assessed genetic relationships among individuals of the founders and the descendants. Neighbor-joining trees depicting the relationships were constructed according to genetic distances based on the proportion of shared alleles between pairs of individuals (Fig. 1). The clustering pattern showed that the descendants were distributed almost radially and equally without bias against each of the five founders (Fig. 1), suggesting that the five founder genomes are equally at population in toto and with various mingling patterns at the individual level inherited by the descendants.

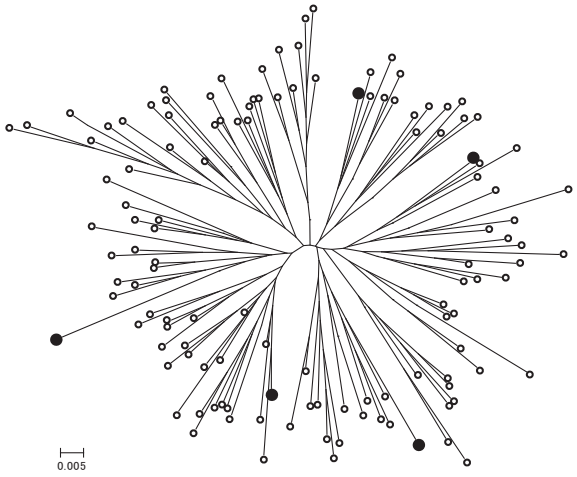

Fig. 1. Neighbor-joining trees depicting the relationships among the five founders and the 138 descendants of the Sado captive Japanese crested ibis population. Distance was measured using the natural logarithm of the proportion of shared alleles (D_{ps}; Bowcock et al., 1994) calculated from 19 microsatellite loci except for the PM2-20 locus. Closed and open circles indicate the founders and the descendants, respectively.

We further compared the composition of genomes among individuals of the founders and the descendants. Graphical displays of the results from the structure analysis are presented in Fig. 2. The maximum value of ln Pr (X/K) was obtained at K = 2. Further, the ΔK statistic was also obviously maximum at K = 2 [ΔK values were 73.37 (K = 2), 1.54 (K = 3), 4.44 (K = 4), 2.40 (K = 5), 3.85 (K = 6) and 1.78 (K = 7)], which provided a relatively meaningful explanation of the genetic structure for the Sado captive population. We therefore assumed that K = 2 was the number of clusters that captured the major structure of the data. At K = 2, one of the founders that had a specific component (colored with green) accounting for over 90% was distinct from the others of the founders that had a significantly low fraction of the green component (Fig. 2), suggesting that their genomes had different compositions. The two components were distributed with equal proportion across the descendants, and there was considerable inter-individual variation in the fraction of the two components (Fig. 2). These results indicate that the founder genomes are equally at population in total and with various admixture patterns at individual level inherited by the descendants. Importantly, the result of only the two different components assumed of genomes, the Sado captive Japanese crested ibis population, despite having been founded from only five birds, supported considerably low levels of genetic diversity. Further, higher F value was exhibited in the green cluster (0.180) than in the red cluster (0.013).

Comparison of genetic diversity in the Sado captive and the Chinese populations

In the present study, we found that the 17 Japanese crested ibis microsatellite markers (Nn01, Nn03, Nn04, Nn12, Nn17, Nn18, Nn21, Nn25, Nn26, NnAF4, NnBF7, NnCE11, NnCG3, NnDD9, NnEB12, NnHB12 and NnNF5) could be used for the comparison of the present data for the Sado population and the literature data for the Chinese population (see above). As described in the literature, all 17 markers were reported to be polymorphic, and a total of 42 alleles detected in the Chinese population (Ji et al., 2004; He et al., 2006). In contrast, there was less diversity in the Sado population, as eight loci were monomorphic and a total of 28 alleles were detected (Table 3). A of the Sado population was equal to or less than that of the Chinese population in each of the 17 markers, and average A was lower for the Sado population than the Chinese population (Table 3), although it

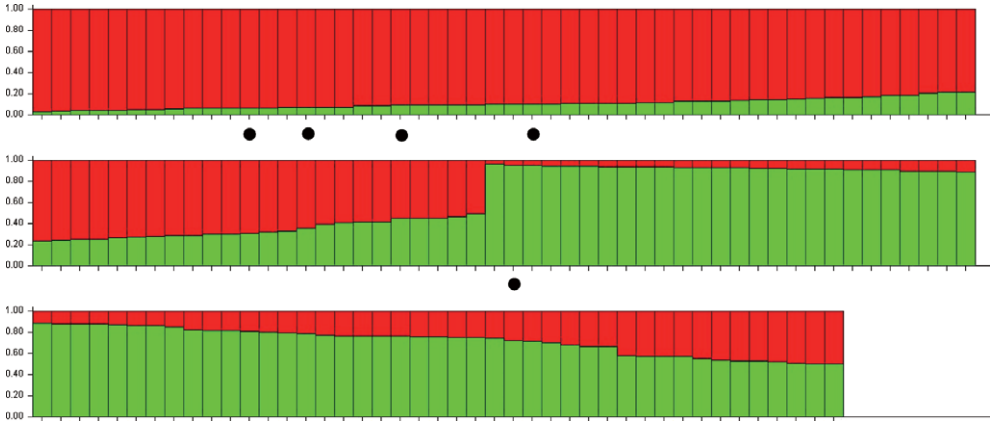

Fig. 2. Comparison of genomes among the five founders and 138 descendants of the Sado captive Japanese crested ibis population. Each individual animal is represented by a single vertical line divided into K = 2 colors, where K is the number of clusters assumed and the length of the colored segment represents the individual's estimated proportion of membership to a particular cluster. Closed circles indicate the five founders. Among the 10 runs carried out for K = 2, only graphical representation of the highest ln Pr (X/K) estimated value is shown.

Table 3. Comparison of genetic diversity measures (allelic diversity (*A*), effective number of alleles (n_e), unbiased expected heterozygosity (H_e) and observed (direct count) heterozygosity (H_o)) between the Sado captive and Chinese populations by using the data obtained from the literature.

Locus name	Sado captive population*				Chinese population**			
	A	n_e	H_e	H_o	*A*	n_e	H_e	H_o
Nn01	3	1.995	0.501	0.526	5	3.356	0.702	0.704
Nn03	1	1.000	0.000	0.000	2	1.443	0.307	0.222
Nn04	2	1.923	0.482	0.504	2	1.692	0.409	0.333
Nn12	3	2.991	0.668	0.697	3	3.012	0.668	0.370
Nn17	2	1.015	0.014	0.014	2	1.905	0.475	0.444
Nn18	2	1.423	0.298	0.319	2	2.028	0.507	0.778
Nn21	2	1.994	0.500	0.558	2	1.938	0.484	0.630
Nn25	2	1.938	0.486	0.388	4	3.333	0.700	0.926
Nn26	2	1.614	0.382	0.452	2	1.299	0.230	0.259
NnAF4	1	1.000	0.000	0.000	2	1.010	0.010	0.010
NnBF7	1	1.000	0.000	0.000	3	1.019	0.019	0.019
NnCE11	1	1.000	0.000	0.000	2	2.008	0.502	0.990
NnCG3	1	1.000	0.000	0.000	2	1.019	0.019	0.000
NnDD9	1	1.000	0.000	0.000	2	1.019	0.019	0.000
NnEB12	1	1.000	0.000	0.000	2	1.010	0.010	0.010
NnHB12	1	1.000	0.000	0.000	2	1.019	0.019	0.000
NnNF5	2	1.567	0.363	0.474	3	1.590	0.371	0.463
Overall	1.647	1.439	0.217	0.231	2.471	1.747	0.321	0.362

* The data from the descendants in this study were used for the Sado captive population.

** The data for Nn01, Nn03, Nn04, Nn12, Nn17, Nn18, Nn21, Nn25 and Nn26 in the Chinese population were obtained from captive population of He et al. (2006). The data for NnAF4, NnBF7, NnCE11, NnCG3, NnDD9, NnEB12, NnHB12 and NnNF5 in the Chinese population were obtained from Ji et al. (2004).

was unclear whether the 28 alleles were shared with the alleles reported in the Chinese population, representing a subset of the total alleles found. Average n_e, H_e and H_o were also lower for the Sado population relative to the Chinese population (Table 3). Most single-locus comparisons were consistent with the overall trends, but in three cases (~18% of the total) n_e and H_e were higher in the Sado population relative to the Chinese population (Table 3). These results suggested that the low levels of genetic diversity in the Sado captive population are the result of a bottleneck.

DISCUSSION

Genetic diversity and structure in the Sado captive Japanese crested ibis

The present study demonstrates that nine Japanese crested ibis microsatellite markers (Nn01, Nn04, Nn12, Nn17, Nn18, Nn21, Nn25, Nn26 and NnNF5) and 10 related species microsatellite markers (PM1-17, PM2-16, PM2-20, PM2-21, PM2-28, PM2-29, PM2-37, PM3-31, Aaju02 and Wsu13) show polymorphisms in the five founders, and were therefore useful for the analysis of genetic diversity and structure in the Sado captive Japanese crested ibis population. The usefulness of the 19 markers was further corroborated by the data showing no linkage disequilibrium among the markers. From the result of genetic diversity measures obtained by using the 19 markers, we showed that genetic diversity in the Sado population was similar to those of other species with population bottlenecks (Bouzat et al., 1998; Tarr et al., 1998) and thus considerably low. Further, the Bayesian model-based clustering with STRUCTURE (Pritchard et al., 2000) assumed the two different components of genomes in the Sado captive Japanese crested ibis. The result of only the two components, together with the maximum value of single-locus *A* = only 3, in spite of the founding on the five birds supported considerably low levels of genetic diversity in the Sado captive Japanese crested ibis. Additionally, we should note that one unique allele (187-bp allele of Nn01) was only detected from one founder having a high fraction of the component colored with green and distinct from the other founders (data not shown), which is likely consistent with a remarkably low level of genetic diversity in the Sado captive population. The low level of genetic diversity is thought to be caused by population size reduction, or bottleneck (in this case, the founding event by five individuals), as reported in a large number of studies (Vrijenhoek et al., 1985; Billington, 1991; Leberg, 1992; Stangel et al., 1992; Bouzat et al., 1998; Tarr et al., 1998).

Thus, to evaluate the effects of bottleneck on genetic diversity in the Sado captive Japanese crested ibis population, we performed a comparison of genetic diversity measures between the Sado captive and the Chinese population that is the source or ancestral population for the Sado. The result of comparison of genetic diversity measures obtained by using the 17 Japanese crested ibis markers was consistent with the idea that low level of genetic diversity in the Sado captive Japanese crested ibis resulted from bottleneck.

Populations that have experienced more severe size reduction should suffer greater decreases in the number of alleles and polymorphic loci (Nei et al., 1975; Maruyama and Fuerst, 1985), and the results for the Sado population generally matched this prediction. However, the decrease in allelic diversity that we observed was not as great as might be presupposed solely on the basis of the founding event on the five individuals. The decrease in allelic diversity following a size reduction does depend on the starting number (Maruyama and Fuerst, 1985), and the recent bottleneck on the Chinese population may have resulted in the loss of many rare alleles. When rare alleles have been lost, even small samples are likely to contain most of the allelic variation in a population, and subsequent size reduction may not result in substantial decreases in allelic diversity.

It is more difficult to detect founder effects through heterozygosity, due to its relative insensitivity to bottleneck (Nei et al., 1975). Moreover, single-locus heterozygosity has a large stochastic variance (Nei and Roychoudhury, 1974), and even when severe reductions in size have occurred, dramatic fluctuations in allele frequencies can result in unpredictable changes in single-locus heterozygosity (Leberg, 1992). Our results are consistent with the predictions, as erratic changes in single-locus heterozygosity following founder event were observed. Shifts in heterozygosity detected in this study were often in opposite directions. Thus, it is important to assay many loci, and in fact changes in multilocus heterozygosity more closely matched predictions, as average H_e and H_o were lower in the Sado population relative to the Chinese population.

As a result of the HWE tests, we found that the NnNF5

and overall cases showed heterozygosity excess, and suggested that this excess might result from bottleneck (Cornuet and Luikart, 1996). However, heterozygosity excess also can result from planned mating (Wright, 1969) to maintain genetic diversity and minimize kinship in captive-breeding programs implemented at the Sado Japanese Crested Ibis Conservation Center, Niigata, Japan. Further, we cannot exclude the possibility that heterozygosity excess results from the differential survival of genotypes. In this case, the excess may be attributable to heterosis (Falconer, 1989). Selection against one homozygous genotype that is completely recessive with respect to fitness also can result in heterozygosity excess (Falconer, 1989).

Conservation program of the Japanese crested ibis

Based on the result of *A*, we demonstrated that the descendants in the Sado captive population inherited all alleles of the microsatellites from the five founders. Additionally, individual clustering based on the allele-sharing distance showed that the descendants were distributed nearly radially and equally without bias against each of the five founders. Further, the clustering algorithm implemented in STRUCTURE (Pritchard et al., 2000) indicated equal distribution of two clusters across the descendants and considerable inter-individual variation in the fractions of the two components. The two clustering analyses revealed that the founder genomes were equally at population in total and with various admixture patterns at individual level inherited by the descendants. Taken together, we suggest that planned mating in captive-breeding programs for the Sado population has succeeded in maintaining genetic diversity and minimizing kinship.

Our STRUCTURE analysis produced an *F* value that indicated a high value for the green cluster. This result suggests that the green cluster may be a lineage that was influenced by strong genetic drift after divergence from the common ancestral population. Thus, the red cluster, which exhibited a lower *F* value, may be an important lineage for conservation of genetic diversity of the Sado captive population of the Japanese crested ibis.

Whereas pedigree information of the descendants of the Sado captive population is recorded, the information for relatedness and kinship of the five founders is deficient, which hampers the acquisition of exact knowledge of genetic diversity and structure. The present study provides useful information on genetic diversity and structure in the Sado captive population of the Japanese crested ibis, which can be vital to the administration of the captive population. A national project for the reintroduction of captive-bred individuals into the wild was launched recently by the Ministry of the Environment of Japan. An additional project for merging the Chinese novel individuals into the Sado captive population will also be carried out in near future. In these conservation programs, it is certainly important to increase genetic diversity in the population reintroduced into the wild and in the Sado captive population through release of captive-bred birds and merge of Chinese novel birds, respectively (Frankham et al., 2002). Selection of donor animals genetically distinct from individuals within the recipient populations will be critical. In this regard, the set of 66 microsatellite markers (22 Japanese crested ibis markers and 44 related species markers) amplified in this study would be useful for the future molecular genetics analysis to select the best donor individuals. Therefore, this study will provide an important insight on establishment of protocols for management of conservation of the Japanese crested ibis toward the national project for the reintroduction and the merging strategies.

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