

**Genetic Diversities among Founder Populations of the Endangered  
Avian Species, the Japanese Crested Ibis and the Oriental Stork  
in Japan**

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## SUMMARY

The Japanese crested ibis *Nipponia nippon* and the Oriental stork *Ciconia boyciana* are endangered avian species and their conservation projects are intensively promoted in Japan. It is important for these conservation projects to obtain information on genetic relatedness by using molecular tools such as single nucleotide polymorphism (SNP) and short tandem repeat (STR) markers and maintain the genetic diversity among populations.

The current Japanese population of *N. nippon* originates from only 5 founders donated by the Chinese government. To discover genome-wide SNPs and STRs while obtaining genotype data of these polymorphic markers in each founder, reduced representation libraries were independently prepared from each of the founder genomes and sequenced on an Illumina HiSeq2000. This yielded 316 million 101-bp reads. Consensus sequences were created by clustering sequence reads, and then sequence reads from each founder were mapped to the consensus sequences, resulting in the detection of 52,512 putative SNPs and 162 putative STRs. Moreover, genotype data of 32,157 putative SNPs were estimated. The haplotypes numbers and STR alleles and the investigation of genetic similarities suggested that the total genetic diversity between the founders was low, although we could not identify a pair with closely related genome sequences.

In addition to SNP and STR makers, polymorphisms of functional genes could provide valuable information for the conservation of endangered species. The major histocompatibility complex (MHC) is a highly polymorphic genomic region that plays a central role in the immune system. Because of its high polymorphism and importance for disease resistance and other functions, the MHC has been an important focus in the

conservation of endangered species.

We investigated the structure and polymorphism of the *N. nippon* MHC class II region and the *C. boyciana* MHC region. In *N. nippon*, 3 contigs representing different haplotypes of MHC class II regions were assembled. A pair of MHC-IIA and -IIB genes was arranged head-to-head between the *COL11A2* and *BRD2* genes. The 3 haplotypes contained 1–3 copies of MHC-IIA/IIB gene pairs. Genotyping of the MHC class II region detected only 3 haplotypes among the 5 founders, suggesting that the genetic diversity of the current *N. nippon* population is extremely low. In *C. boyciana*, one contig (approximately 160 kb) was assembled. Partial sequences of subclones suggested that this contig contained a partial copy of *COL11A2*, 2 copies of MHC IIA/IIB pairs (*DAA1/DAB1* and *DAA2/DAB2*), *BRD2*, *DMA*, *DMB1*, *DMB2*, MHC I (*UAA*), *TAP1*, *TAP2*, 2 copies of MHC I (*UBA1* and *UBA2*), and a partial copy of *TNXB*. These results revealed that the MHC genomic structure of *N. nippon* and *C. boyciana* were conserved, but they were largely different from that of Galliformes. Nine MHC class II haplotypes were detected in 6 founders of *C. boyciana*, suggesting that the genetic diversity of the MHC region in the *N. nippon* population is lower than that in the *C. boyciana* population in Japan.

The present study provided important insight into protocols for genetic management of the captive breeding population of *N. nippon* and *C. boyciana* in Japan, toward the national project for the reintroduction of captive-bred individuals into the wild and for future studies on the evolution of the avian MHC. In addition, we proposed a simple, efficient, and cost-effective approach for the simultaneous detection of genome-wide polymorphic markers and their genotypes for species currently lacking a reference genome sequence.