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Genetic Diversity and Differentiation of MHC Class I Genes in Red-Crowned Crane Populations

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The red-crowned crane (*Grus japonensis*) has been demoted to "vulnerable species" because its populations have apparently stabilized in Japan and Korea. Low variation and genetic drift may cause damage to the nascent recovery of the *G. japonensis* population. The major histocompatibility complex (MHC) is one of the most polymorphic gene families in the vertebrate genome and can reflect information on the adaptive evolution of endangered species. In this study, variations in MHC I exon 3 of captive *G. japonensis* in China were assessed and compared with those in cranes from Japan. Forty MHC alleles of 274 base pairs were isolated from 32 individuals from two captive populations in China. There was high variability in the nucleotide and amino acid composition, showing the proportion of polymorphic sites of 18.98 and 32.97%, respectively. Comparative analyses of the Chinese and Japanese populations based on 222 base pair sequences revealed more alleles and higher variation in the Chinese population. The lack of significant geographical differentiation of *G. japonensis* was supported by the genetic differentiation coefficient (0.04506) between the Chinese and Japanese populations. Positive selection of antigen-binding sites was observed, which contributed to maintaining the diversity of MHC class I genes. Phylogenetic analysis suggested the persistence of *trans*-species polymorphisms among MHC class I genes in Gruidae species. Our results may contribute to optimizing the management of *G. japonensis* populations and population recovery of this threatened species.

Keywords: *Grus japonensis*, major histocompatibility complex, genetic structure, balancing selection, *trans*-species polymorphism

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INTRODUCTION

The red-crowned crane (*Grus japonensis*) is a large wading bird distributed in East Asia and far east Russia (BirdLife International, 2021). This bird has been a long-term and auspicious symbol of East Asian culture (Lee et al., 2020). *G. japonensis* can be divided into two main populations based on differences in their geographical distribution and life history, namely the migratory population on the Asian continent and the non-migratory population in Japan. Continental populations can be further divided into a subpopulation that winters in coastal intertidal zones and lakes along the

middle and lower reaches of the Yangtze River in China and a subpopulation that winters in the demilitarized zone of the central Korean Peninsula (BirdLife International, 2021). With the rapid decline of the *G. japonensis* population because of the loss and degradation of suitable wetlands through conversion of land for agriculture and industrial development purposes, this crane was listed as an endangered species by the International Union for Conservation of Nature from 2000 to 2020 (Ye et al., 2021; Zhou et al., 2021). To protect birds from anthropogenic threats, governmental and non-governmental actions have been taken, such as enacting protection laws and setting up nature reserves and supplementary food in winter (Gong et al., 2020). Population trends in Japan and subpopulation wintering in Korea have rapidly increased, whereas subpopulation wintering in China continues to decline. With the apparent stabilization of populations in Japan and Korea, *G. japonensis* was downgraded to the vulnerable level by the International Union for Conservation of Nature in 2021 (BirdLife International, 2021).

Monitoring changes in the genetic diversity of endangered species over time contributes to understanding potential risks associated with genetic drift and reduced genetic variation (Zhang et al., 2015; Williams et al., 2020). Compared with neutral markers, the genetic variation of major histocompatibility complexes (MHC) under selection pressure can provide more accurate information on the adaptive evolution of endangered species (Sutton et al., 2015; He et al., 2017; Manlik et al., 2019; Minias et al., 2021). MHC form one of the most polymorphic gene families in the vertebrate genome, and their protein are involved in triggering immune response. MHC molecules can be divided into class I (MHC I) and class II (MHC II) molecules according to the antigen they can recognize. Exons 2–3 of MHC IA genes and exon 2 of MHC IIB genes encode the peptide-binding regions of MHC protein molecules, respectively (Minias et al., 2019; Williams et al., 2020). The level of variation of these MHC exons is often used in conservation genetic studies of endangered species, such as population differentiation, mate choice, and disease association (Luo et al., 2012; Sutton et al., 2015, 2016; Zhang B. Y. et al., 2020; Zhu et al., 2020).

The genetic diversity of *G. japonensis* has been widely examined, which is beneficial for population management and conservation (Miura et al., 2013a,b; Sugimoto et al., 2015; Akiyama et al., 2017a,b; Sun et al., 2021). However, most studies have been performed on island populations from Japan, whereas the genetic information between continent and island populations has not been extensively compared. Moreover, the ethology (vocalization patterns) and morphology (coloration and egg size) differ between the continental and island populations (BirdLife International, 2021). Studies focusing on the genetic diversity of the continental population are needed because of the rapid decline of the wintering subpopulation in China. In this study, we assessed the genetic diversity of captive *G. japonensis* in China by cloning and sequencing of MHC I exon 3. We then compared the genetic information between the Chinese (CHN population) and Japanese (JPN population) populations. This study was conducted to understand the genetic diversity and differentiation of MHC class I genes in *G. japonensis* populations and contribute to the conservation efforts and recovery of this threatened bird.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

The Jiangsu-Yancheng Coastal Wetlands Rare Birds National Nature Reserve (JYNNR) is one of the world's largest winter habitats for red-crowned cranes. In addition to the wild population of red-crowned cranes, there are large captive populations in Jiangsu province, which are important complementary populations of west-flying red-crowned cranes. The captive populations of JYNNR in Yancheng and Nanjing Hong Shan Forest Zoo (NHSFZ) in Nanjing are the two largest populations in Jiangsu Province. A certain number of chicks hatch and are raised in these two locations each year. In this study, 16 pairs of captive *G. japonensis* (11 pairs from JYNNR and five pairs from NHSFZ) involved in reproduction were sampled. These cranes contributed to the gene pool of captive and even wild populations of the west-flying red-crowned crane. Blood samples were collected during periodic physical examination, labeled, and stored at −80°C. Total genomic DNA was extracted using DNAiso reagent (Takara, Beijing, China) and used for subsequent PCR amplification.

PCR Amplification and Cloning Sequencing

A pair of universal primers (MHC I-E3-F: 5′-TCA GCC CCR TCT CCC TGG TC-3′, MHC I-E3-R: 5′-GTA GAA GCC GTA AGC GCG GCA-3′) to amplify the complete exon 3 of Gruidae MHC class I genes was designed according to five MHC sequences from *Grus grus* (Sequence ID: Grgr-UA*01, Grgr-UA*02; GenBank accession: MK034099.1, MK034100.1) and *Bugeranus carunculatus* (Sequence ID: Buca-UA*01, Buca-UA*02, Buca-UA*03; GenBank accession: MK034101.1, MK034102.1, MK034103.1). PCR amplification was performed in a 50 μL volume containing 25 μL Premix Taq^{TM} (Takara, Beijing, China), 5 μL DNA template (∼100 ng), 1.5 μL MHC I-E3-F (1 μmol/L), 1.5 μL MHC I-E3-R (1 μmol/L), and 17 μL sterile water. The PCR program consisted of initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. After 0.5% agarose gel electrophoresis, the target products were excised from the agarose gel and purified using a MiniBEST agarose gel DNA extraction kit (Takara, Beijing, China). The purified PCR products were inserted into the pMD 18-T vector (Takara, Beijing, China) and introduced into *Escherichia coli* DH 5α competent cells. Positive clones were identified by PCR using the original and universal M13 primers. Ten positive clones per individual were sequenced unidirectionally by Tsingke Biotechnology (Nanjing, China) using the M13 primer.

Statistical Analysis

The MHC sequences of *G. japonensis* were identified using BLAST for the MHC sequences of *G. japonensis* and other

cranes in GenBank.[1] The generated MHC sequences were longer than those (partial exon 3) isolated in previous studies of the genetic diversity of *G. japonensis* in Japan (GenBank accessions: LC132723.1–LC132738.1) (Akiyama et al., 2017a). Comparative analyses were performed based on 274-bp sequences (complete exon 3) for two populations in China and analyses based on 222-bp sequences for six populations between China and Japan. LaserGene software (version 7.0; DNASTAR, Inc., Madison, WI, United States) was used to align and edit the obtained MHC sequences (Burland, 2000). The variable sites in the nucleotide and amino acid sequences were counted using MEGA v10.0 software (Kumar et al., 2018). The nucleotide diversity (Pi) and overall mean distance of nucleotide sequences were calculated using DnaSP 6.0, and Mega v10.0, respectively (Rozas et al., 2017; Kumar et al., 2018). Antigen-binding sites (ABSs) were predicted from MHC class I molecules from *Nipponia nippon* (Chen et al., 2015). Non-synonymous substitution (d_N) and synonymous substitution (d_S) were calculated using MEGA v10.0 (Kumar et al., 2018). Evidence for positive, purifying or neutral selection was assessed using two methods. First, d_N/d_S with z-test in Mega v10.0 was employed to assess selection of the ABSs, non-ABSs, and entire codons, respectively (Kumar et al., 2018). Second, the selection coefficient of each codon was estimated using the mixed effects model of evolution (MEME), fixed effects likelihood, and fast unconstrained Bayesian approximation on Datamonkey online[2] (Weaver et al., 2018). The population differentiation of *G. japonensis* was evaluated based on the genetic differentiation coefficient (Fst) using Arlequin v3.5 (Excoffier and Lischer, 2010). A phylogenetic tree of the MHC alleles of *G. japonensis* was reconstructed based on Bayesian inference using the PhyloSuite software package (Zhang D. et al., 2020). The allele sequences from *B. carunculatus*, *G. grus*, and *Grus canadensis* (Sequence ID: Grca-UA*01; GenBank accession: AF033106.1) as outgroups were also used for phylogenetic analysis. The phylogenetic tree was visualized and edited using iTOL v5.0 (Letunic and Bork, 2021).

RESULTS

Allelic Diversity of MHC Class I Genes in Red-Crowned Crane

MHC class I fragments of 450 bp in length were obtained through cloning and sequencing of the PCR products. After comparing the nucleotide sequences of Grgr-UA and Buca-UA, we removed the redundancies and confirmed 40 complete exon 3 sequences of MHC class I that were 274 bp in length. There were 2–7 unique sequences per individual, with an average of four sequences per individual (**Supplementary Table 1**). This result indicates the existence of four MHC class I genes in red-crowned cranes. As 16 partial exon 3 sequences of Grja-UA from four sampling locations of the JPN population were previously isolated, these sequences were labeled as Grja-UA*17–56 (GenBank accessions: OM772895–OM772934). Fourteen alleles were shared between two sampling locations of the CHN population, whereas 7 and 19 alleles were private to Nanjing and Yancheng, respectively. The exon 3 sequences exhibited high variability in nucleotide composition, as 52 of 274 bases were variable among the 40 alleles from the CHN population (**Table 1**). No pseudogene sequences were detected. All Grja-UA alleles could be translated into amino acid sequences. The amino acid sequences also showed high variability, with 30 variable amino acids among the 91 residues (**Figure 1**). To synthetically analyze the allelic diversity of MHC class I genes in red-crowned cranes, nucleotide and amino acid sequences from the CHN population were shortened to sequence length from the JPN population. As a result, some sequences were regarded as identical alleles, and 43 alleles were used for analysis (**Supplementary Table 2**). Nucleotide and amino acid diversity in the CHN population was higher than that in the JPN population (**Table 2**). Overall, the allelic diversity of MHC class I genes was high in red-crowned cranes, with an overall proportion of polymorphic nucleotide and amino acid of 18.55 and 31.51%, respectively (**Table 2**).

Genetic Structure of Red-Crowned Crane Populations

Ten alleles were common to the CHN and JPN populations (**Supplementary Table 2**). Furthermore, Grja-UA*47, Grja-UA*49, Grja-UA*50, and Grja-UA*51 were shared by all six *G. japonensis* populations (**Figure 2**). However, the frequencies of the common alleles greatly differed between the CHN and JPN populations. The frequency of Grja-UA*47 ranged from 20% (in the Hongshan population) to 100% (in the Nemuro and Abashiri populations), whereas the frequencies of Grja-UA*49, Grja-UA*50, and Grja-UA*51 ranged from 10% (in the Hongshan population) to 100% (in the Abashiri population) (**Figure 2B**). Fst analysis revealed slight differentiation between the CHN and JPN populations in MHC class I genes (Fst = 0.04506, P = 0.00000). The minimum and maximum pairwise Fst values were 0.02490 (between the Yancheng and Abashiri populations) and 0.07170 (between the Nanjing and Tokachi populations), respectively. In contrast, the pairwise Fst values were all less than zero within the four sampling locations of the JPN population and 0.01038 between the two sampling locations of the CHN population (**Table 3**). These results suggest that gene flow occurred internally in the JPN and CHN populations.

Selection on MHC Class I Exon 3

Seventeen codon positions were inferred as ABSs compared to the *N. nippon* MHC sequences (**Figure 1**). The d_N/d_S values of the putative ABSs were 1.280, 1.176, and 1.271 for the MHC in the CHN, JPN, and entire populations, respectively, suggesting that these sites are under positive selection (**Table 2**). Positive selection also occurred at two non-ABS sites (3rd and 46th within the 73 codons), supporting that positive selection has affected Grja-UA. The d_N/d_S values of non-ABS and all sites were less than 1.0 supporting purifying selection (**Table 2**). In addition, MEME analysis indicated that three codons (3rd, 27th,

[1] https://www.ncbi.nlm.nih.gov/

[2] http://www.datamonkey.org/

TABLE 1 | MHC class I genetic diversity of two populations of *Grus japonensis* in China.

Population	*N*	Variable sites	Total alleles	Private alleles	Pi
Nanjing	10	43	21	7	0.044 ± 0.003
Yancheng	22	48	33	19	0.044 ± 0.002
All	32	52	40	–	0.044 ± 0.002

All the analyses were based on 274-bp sequences.N presents Number of individuals.Pi presents nucleotide diversity.

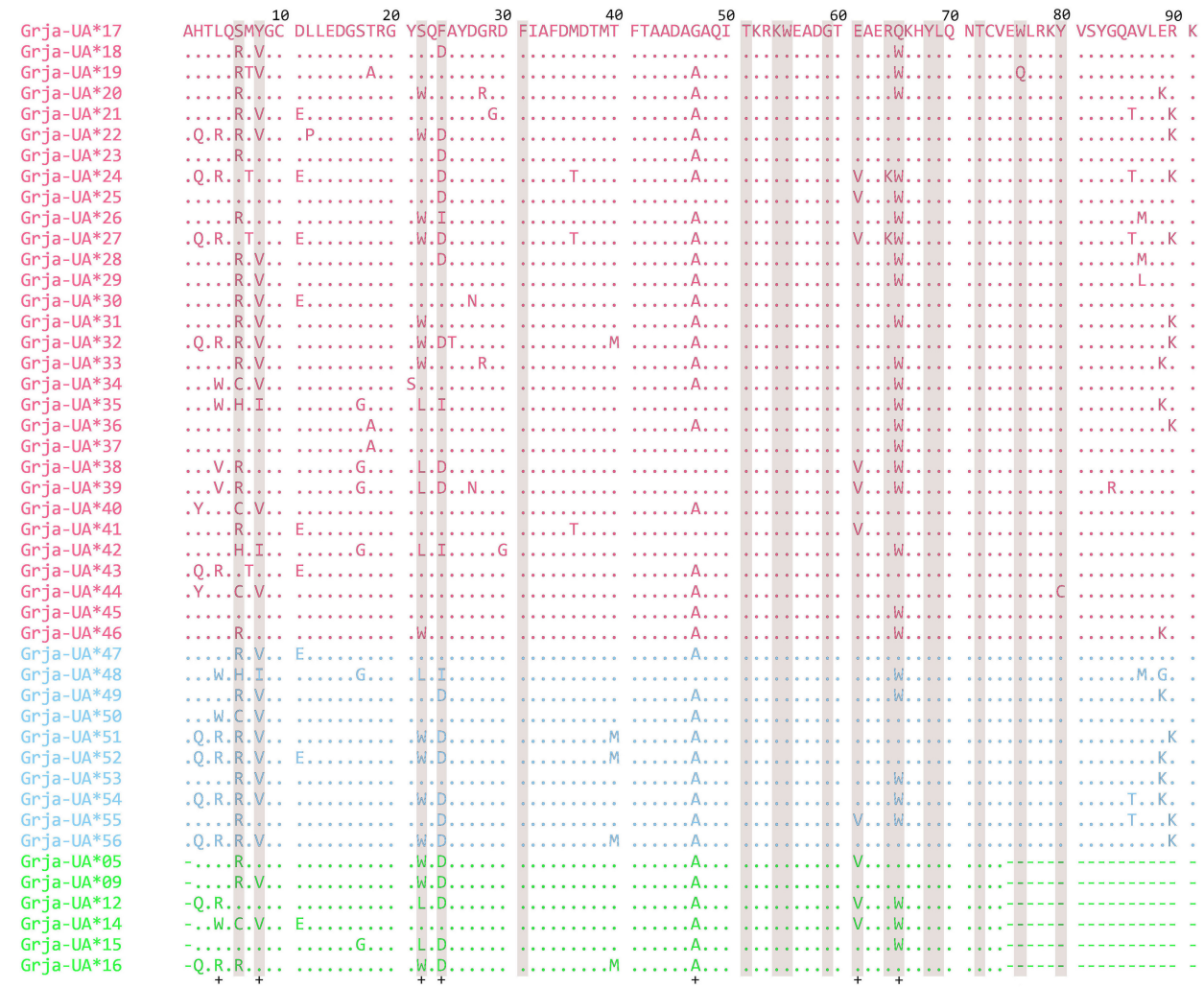

FIGURE 1 | Amino acid sequences of MHC class I exon 3 molecules from *Grus japonensis*. Dots indicate identity to reference sequence. Dashes indicate missing amino acid residues. Shaded areas present antigen-binding sites (ABSs) predicted from MHC class I molecules of *N. nippon* (Chen et al., 2015). Crosses present sites deduced to be under positive selection estimated by mixed effects model of evolution (MEME) analysis. Private alleles of the Chinese (CHN) population, private alleles of Japanese (JPN) population, and common alleles are marked as red, green, and blue, respectively.

and 56th among the 91 codons) were under strong purifying selection (**Figure 3**).

Phylogenetic Relationships Among MHC Class I Alleles

A Bayesian phylogenetic tree was constructed to illustrate the phylogenetic relationships among MHC class I alleles within Gruidae species (**Figure 4**). Grja-UA*41 and Grja-UA*50 first clustered with Grca-UA*01 and Grgr-UA*01, respectively, indicating that the alleles did not cluster by species. MHC alleles from some species were closer to alleles from other species than to alleles from the same species, indicating an evolutionary pattern of *trans*-species polymorphism within Gruidae. Additionally, the relationships among the Grja-UA alleles were not congruent with the sampling locations of the CHN and JPN populations. Private alleles

TABLE 2 | Polymorphism and d_N/d_S values of MHC class I exon 3 in CHN and JPN populations.

	CHN population	JPN population	CHN and JPN population
No of nucleotide polymorphic sites	41 (18.55%)	25 (11.31%)	41 (18.55%)
No of amino acid polymorphic sites	23 (31.51%)	12 (16.44%)	23 (31.51%)
Overall mean distance of nucleotide sequences	0.052 ± 0.009	0.048 ± 0.010	0.052 ± 0.010
PSS-MEME	3rd*, 7th, 23th, 46th*, 60th, 64th	3rd, 7th, 23th, 60th, 64th	3rd*, 7th, 21th*, 23th 46th*, 60th, 64th
PSS-FEL	3rd*, 5th, 21th, 46th*	–	3rd*, 5th, 21th*, 46th*
PSS-Baysin	3rd*, 5th, 10th, 21th, 46th*, 64th	3rd, 5th, 21th, 64th	3rd*, 5th, 10th, 21th*, 46th*, 64th
d_N/d_S value	0.766/1.280/0.490	0.662/1.176/0.362	0.742/1.271/0.460

All the analyses were based on 222-bp sequences. Data on JPN population (including: Abashiri, Kushiro, Nemuro, Tokachi) was cited from Akiyama et al. (2017a). Positively selected sites (PSS) shared by three methods are marked with an asterisk. The three values for d_N/d_S are for the entire region, ABS region, and non-ABS region, respectively.

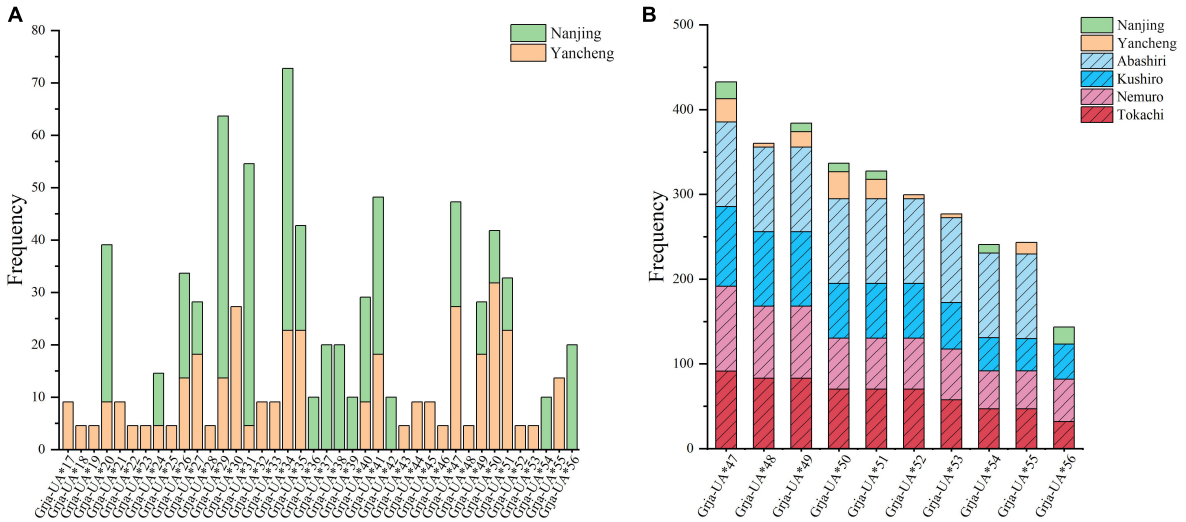

FIGURE 2 | Distributions and frequencies of MHC class I alleles in *Grus japonensis* populations. **(A)** Distributions and frequencies of alleles in the Chinese (CHN) population. **(B)** Distributions and frequencies of common alleles in the CHN and Japanese (JPN) populations. Data on the JPN population (including: Abashiri, Kushiro, Nemuro, Tokachi) was cited from Akiyama et al. (2017a).

did not group into a clade but were mixed with common alleles. Grja-UA*16 clustered with Grja-UA*51, and Grja-UA*35 clustered with Grja-UA*42. However, no clear and overall phylogenetic relationships were identified because of the many parallel clades and low posterior probabilities in the Bayesian tree.

DISCUSSION

Genetic Diversity and Differentiation of Red-Crowned Crane

In this study, we examined the MHC class I allelic variation in the threatened red-crowned crane *G. japonensis*. We identified 40 alleles (complete exon 3), including 52 nucleotide variable sites, from 32 individuals in two CHN subpopulations (**Table 1**). These results highlight the high diversity of MHC class I genes in the CHN population of red-crowned cranes. However, previous data indicated low genetic variability in MHC class I genes in the JPN population, as only 16 alleles were isolated from 152 individuals (Akiyama et al., 2017a). The difference in genetic variability between the CHN and JPN populations were congruent with previous results based on neutral markers (Hasegawa et al., 1999; Miura et al., 2013b). In addition, shorter amplified fragments in the JPN populations may also prevent the identification of certain alleles. The effects of bottlenecks on genetic variation differ widely among different species. Low genetic variation was generally observed in most bottlenecked species (Babik et al., 2012; Kubota and Watanabe, 2013; Sutton et al., 2013, 2015; Li et al., 2017; Saka et al., 2018; Lan et al., 2019; Arauco-Shapiro et al., 2020), whereas high variation was also detected (Galaverni et al., 2013; Newhouse and Balakrishnan, 2015; Fulton et al., 2017; MacDougall-Shackleton, 2020; Cruz-Lopez et al., 2021). Multiple MHC copies may be a major cause of this high variation (Wan et al., 2011; Stervander et al., 2020; He et al., 2021). However, the copy number of MHC class I genes in *G. japonensis* has not been precisely defined because the number of exon 3 sequences varies greatly among individuals. It remains unclear whether multiple

TABLE 3 | Population pairwise Fst values (below the diagonal) and *P*-values (above the diagonal) between six populations of *Grus japonensis*.

Population	Abashiri	Kushiro	Nemuro	Tokachi	Nanjing	Yancheng
Abashiri		0.95495	0.93694	0.97297	0.00000	0.03604
Kushiro	−0.01091		0.99099	0.99099	0.00000	0.00000
Nemuro	−0.01138	−0.00317		0.96396	0.00000	0.00000
Tokachi	−0.01316	−0.00171	−0.00312		0.00000	0.00000
Nanjing	0.06228	0.06813	0.06171	0.07170		0.09910
Yancheng	0.02490	0.03518	0.03216	0.03606	0.01038	

All the analyses were based on 222-bp sequences. Data on JPN population (including: Abashiri, Kushiro, Nemuro, Tokachi) was cited from Akiyama et al. (2017a).

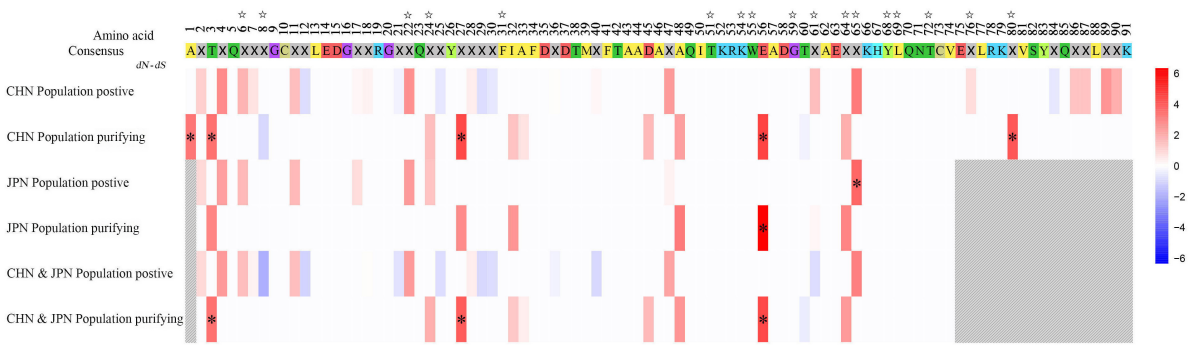

FIGURE 3 | Selection on amino acid residues of MHC class I exon 3 in different populations of *Grus japonensis*. Stars above the consensus amino acid sequence indicate antigen-binding sites. Crosses in the consensus amino acid sequence indicate variable sites. Asterisks indicate strong selection ($|dN–dS| > 10$). Gray slashes indicate missing data.

gene copies or multiple sequences at a certain locus lead to high diversity.

Evolutionary significant units and management units have been developed to better protect endangered species populations on separate evolutionary trajectories (Moritz, 1994; Zhu et al., 2013; Carlson et al., 2016). The definition of evolutionarily significant units highlights the high distinctiveness of separate populations, specifically the monophyly for mitochondrial alleles as well as the significant divergence of frequency for nuclear alleles. In practical situations, management units have often been defined and used and only revealed the significant divergence of allele frequencies at mitochondrial or nuclear loci but neglected the phylogenetic distinctiveness of the alleles (Hoglund et al., 2011). Although there were differences in the ethology and morphology of red-crowned cranes, MHC allele frequencies (Fst = 0.04506, P = 0.00000) and evolutionary relationship of haplotypes did not support the separate evolution of the continental and island populations (Hasegawa et al., 1999; Miura et al., 2013b). Nevertheless, a larger gene pool in the continental population can be inferred because of the higher diversity of MHC and other neutral markers (Hasegawa et al., 1999; Miura et al., 2013b; Akiyama et al., 2017a). The low genetic variation in the JPN population can be increased *via* translocations by introducing continental individuals. According to recent reports, continental individuals flew to Japan and may have enriched the low gene pool of the JPN population (Akiyama et al., 2017a; Kawasaki et al., 2022). Recovering *G. japonensis* may be more difficult, as the CHN population with high genetic variation is still decreasing.

Historical Balancing Selection and *Trans*-Species Polymorphism

Nucleotide sites under positive selection can accumulate more non-synonymous sites, which can alter the encoded amino acid sequence, structure, and function of the protein. The ABS region, subjected to positive selection, is a well-known feature of MHC alleles (Luo et al., 2012; Zeng et al., 2016). We found significant evidence for positive selection of the ABS region of MHC class I alleles in *G. japonensis*, as five of seven positively selected sites were ABSs and the d_N/d_S value for the ABS region was more than 1 (**Figure 1** and **Table 2**). This indicates that balancing selection for immunological responses to pathogens is involved in generating and maintaining MHC diversity in *G. japonensis* undergoing a bottleneck. However, multiple MHC gene copies were included in the analysis because of the difficulty of locus assignment, which may artificially inflate MHC variation and bias our conclusion (Knafler et al., 2014).

Trans-species polymorphisms are another well-known feature of MHC alleles (Kohyama et al., 2015; Qin et al., 2021). The phylogenetic tree showed that the allelic lineage of cranes did not form monophyletic groups among species, suggesting that Grja-UA alleles are involved in several instances of

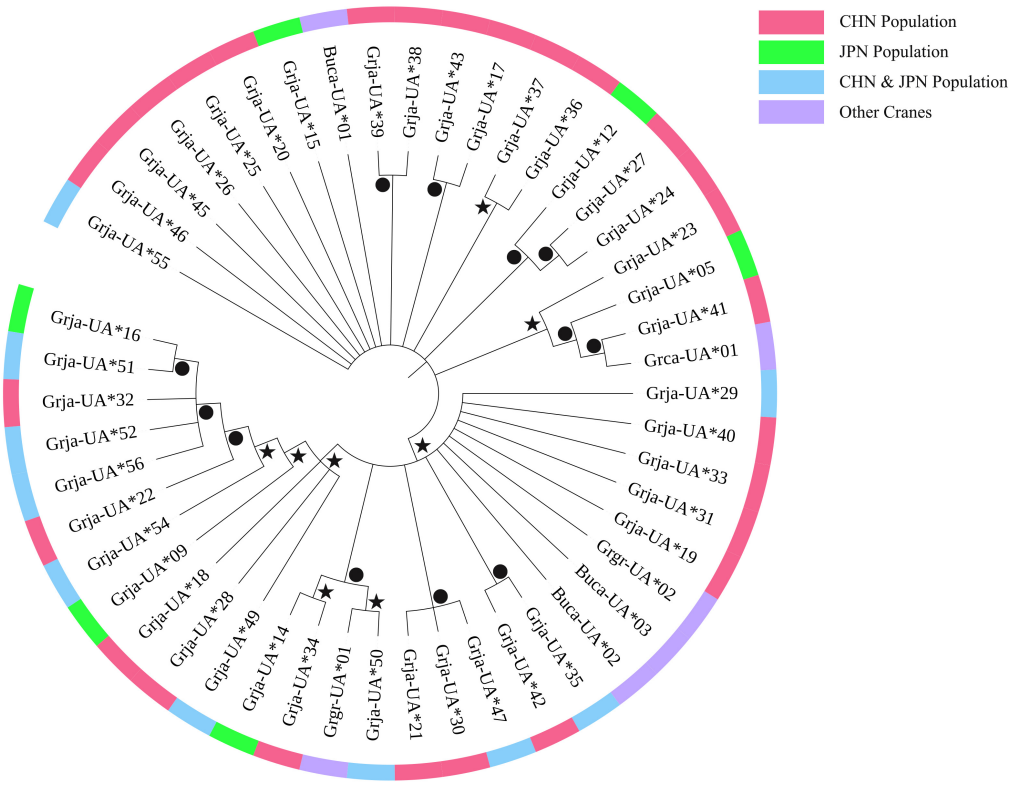

FIGURE 4 | Bayesian phylogenetic tree for MHC class I alleles (222-bp sequences) from *Grus japonensis* and other three species in *Gruidae*. Dots indicate posterior probabilities >0.8. A star indicates 0.5 < posterior probabilities <0.8.

trans-species polymorphism (**Figure 4**). This may be because allelic lineages present in a common ancestor were retained by balancing selection, even after species-level diversification (Akiyama et al., 2017a; Nishita et al., 2018). But sharing of polymorphism between unrelated or distantly related species may be owing to convergent evolution rather than maintenance of ancestral sequence (Yeager and Hughes, 1999). The analyzed *G. japonensis* samples were restricted to populations in Japan and captive populations in China. However, wild populations of this species are distributed over a much wider area from Far East Russia to Korea and northeast and east China. Only the three species were used in phylogenetic analysis. In further studies, novel MHC class I sequences could be detected in other *G. japonensis* populations and in Gruidae species, which will more precisely reveal the relationships between species divergence and evolution of MHC class I genes among Gruidae species.

CONCLUSION

In this study, 40 MHC class I exon 3 alleles with 274 bp were isolated from 32 individuals from two captive populations in China. High polymorphism was found at both the nucleotide and amino acid levels. Comparative analyses between the CHN and JPN populations based on 222 bp sequences showed more alleles and higher variation in the former. The low Fst value (Fst = 0.04506) suggests no significant geographical differentiation of *G. japonensis* between the CHN and JPN populations. Low genetic variation in the JPN population may be increased *via* translocations by introducing individuals from the CHN population. Well-known features of MHC class I genes are found in *G. japonensis*, such as positive selection on ABSs and *trans*-species polymorphism within Gruidae species. Our study suggests that genuine rejuvenescence of *G. japonensis* requires conservation of the CHN population.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found here: https://www.ncbi.nlm.nih.gov/genbank/, OM772895–OM772934.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics Committee of Nanjing Forestry University.

AUTHOR CONTRIBUTIONS

HyL conceived the study. HyL and NX acquired the funds. NX and CS conducted the experiments. WY and KH carried out the bioinformatics analysis. NX drafted the manuscript. YZ, HL, and CL reviewed and revised the manuscript. All authors approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.898581/full#supplementary-material

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