African Journal of Biotechnology Vol. 10(65), pp. 14684-14693, 24 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2228
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

cDNA cloning and polymorphic domains of the major histocompatibility complex (MHC) class Iα in two Chinese native chicken breeds

Yin Dai[1, 2], Xuelan Liu[1], Hong Ye[1], Fangfang Chen[1], Shengjie Liu[1] and Weiyi Yu[1]*

[1]Key Laboratory of Zoonoses of Anhui Province, Anhui Agricultural University, Hefei, China.
[2]Institute of Animal Husbandry and Veterinary Science, Anhui Academy of Agricultural Science, Hefei, China.

Accepted 23 September, 2011

Major histocompatibility complex (MHC) is a highly polymorphic gene and plays an important role in immune system for vertebrate. To understand the polymorphism character of domestic, we cloned 32 cDNAs of MHC class I *α* genes of two local chicken breeds in different areas of China. There were 112 variable amino acid residues in all five domains (leader peptide, α1, α2, α3 and TM/CY domains) of the putative α chain, and 76 of them were located in α1 and α2 domains. There were 23 to 25 polymorphic sites with high mutation frequency in α1 and α2 domains. Comparison of chicken with duck, human and mouse revealed that the two domains were highly similar among different species, and some highly polymorphic sites were located at the sites 9, 111(114), 113 (116) and 153 (156). Analysis of the phylogenetic tree indicated no relationship between the breeds and polymorphic alleles. All these results therefore indicate that MHC I class molecule of domestic chickens was more influenced by the pressure of common pathogens rather than geographic differences.

Key words: Chinese native chicken, MHC class Iα, α1 and α2 domains, polymorphism.

INTRODUCTION

Major histocompatibility complex (MHC) plays a crucial role in the susceptibility/resistance to pathogens for animals and is also a highly polymorphic gene in vertebrate genomes (Takeshima et al., 2009). This kind of polymorphism is the result of natural selection (Furlong and Yang, 2008). In mammals, MHC gene region spans approximately 4 Mb, while in avian it is greatly reduced in size and gene content (Kaufman et al., 1999). The turkey MHC gene is mapped to two distinct regions (B and Y) of a single chromosome (Chaves et al., 2009) and the exon 2 and exon 3 in chicken MHC class I gene encode the α1 and α2 domains (Hunt and Fulton, 1998). The peptide binding domains (PBD) of α1 and α2 domains in MHC gene appears the most polymorphic domain in vertebrate taxa (Silva and Edwards, 2009). A number of studies have focused on studying and analyzing MHC polymorphism and its evolution process in mammals (Furlong and Yang, 2008; O'Leary et al., 2009), whales (Xu et al., 2009) wild animals (Koutsogiannouli et al., 2009; Becker et al., 2009) and avian (Ewald and Livant, 2004; Westerdahl et al., 2000).

Restriction fragment length polymorphism (RFLP) has been used to identify MHC allele diversity (Westerdahl et al., 2000), and cDNA-PCR was more often applied to sequencing MHC gene (Silva and Edwards, 2009; O'Leary et al., 2009; Becker et al., 2009). Domestic animals have been artificially selected and bred for a long time. Hence, to know the polymorphic MHC character of domestic chicken in the past evolution, we cloned and analyzed the MHC class I α gene from two local breeds, Wenchang (WC) and Huaibei Partridge (HBP) chicken distributed in the two different areas in China and with diversity of produce and form characters.

*Corresponding author. E-mail: yuweiyi@ahau.edu.cn. Tel: 0086-551-5786891. Fax: 0086-551-5786013.

MATERIALS AND METHODS

Study materials

Individuals of HB and WC chicken used in the study were derived from Breeding Center of Feixi Farming Group in the Province of Anhui (China). In most cases, the family members and breeding records were obtained. Peripheral blood mononuclear cells (PBMCs) were separated from venous blood by density gradient centrifugation. From the GenBank database, we retrieved six amino acid sequences of MHC class Iα chains from Leghorn chicken, Silky chicken, *Numida meleagris*, duck, human and mouse with corresponding accession numbers; AY989897, AB178042, AB178051, AB115245, NM_002116 and NM_010380, respectively.

RNA extraction, cDNA synthesis and PCR amplification

Total RNA was isolated using TRIZOL Reagent (Invitrogen). Thereafter, first-strand cDNA was constructed in accordance with the cDNA synthesis kit instructions (TaKaRa Biotechnology, Dalian, China). The primers were designed using Oligo6.0 software, based on known complete cDNA sequence of chicken MHC class I glycoprotein (GenBank accession no. S78682); to amplify overlapping conserved domains from the 5′UTR to the 3′UTR. The sense primer sequence was 5′-GAGAGTGCAGCGGTGCGAG GCGAT-3′ and the antisense primer sequence was 5′-AATGCTGGTGTGGACTGTTGGCTC-3′. The PCR amplifications were carried out in a 60 µl reaction volume containing 50 ng cDNA, 20 pmol of each primer, 0.25 mM of each dNTP, 2.5 U of Taq DNA polymerase and 5 µl of 10 × PCR reaction buffer. The PCR was performed by an initial denaturation at 95℃ for 5 min followed by 30 cycles of denaturation at 94℃ for 1 min, annealing at 67℃ for 1 min and extension at 72℃ for 2 min, with a final extension step at 72℃ for 10 min. The products were subjected to electrophoresis in a 0.8% agarose gel and examined after ethidium bromide staining. The resulting PCR fragments were then inserted into a vector (TA-cloning Kit, Takara).

Sequencing and handling of MHC class I gene sequences

All sequences were obtained from randomly chosen clones. Nucleotide sequencing was performed by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The nucleotide data were obtained by sequencing the forward and reverse strand. This might remove some results of sequencing artefact. Sequence analysis was aligned using ClustalX 1.83 package (Thompson et al., 1997). Wu-kabat index system (Wu and Kabat, 1970) was applied to identify polymorphism in α1 and α2 domains. We modeled three-dimensional model of MHC class I structure using the SWISSMODEL web server (http://www.expasy.ch/ swissmod/ SWISS-MODEL.html), which could search for similar templates of available structures from the protein data bank (PDB), while SWISS-Pdb viewer 4.01 was used to generate a three-dimensional image (Arnold et al., 2006; Schwede et al., 2003; Guex and Peitsch, 1997). In addition, phylogenetic tree of amino acid sequences were created using MEGA3.1 program (Kumar et al., 2004) by neighbor-joining method (Saitou and Nei, 1987) and the bootstrap test was carried out with 1000 iterations.

RESULTS

Alignment of MHC class Iα sequences of two Chinese chicken breeds

We cloned 17 and 15 of MHC class Iα sequences of HBP and WC breeds, respectively. All the full-length cDNAs contained a complete coding sequence (CDS) including 1068 or 1035 bp, and were composed of 7 or 8 exons. Some sequences were shorter by 33 bp by sequencing, which revealed a lack of exon 7. The full-length sequences deduced encoded a 355 or 344 amino acid polypeptide including 21 amino acid residues of a leader peptide, followed by 88 residues of the α1 domain, 91 residues of the α2 domain, 91 residues of the α3 domain and 53 or 64 residues of the TM/CY domain. Alignment of the amino acid sequences revealed 112 variable amino acid sites in α chain, of which 5, 36, 40 and 31 sites were located in the signal peptide, α1, α2, α3 and TM/CY domains respectively. There were 76 variable sites in the α1 and α2 domains (Figure 1). The result show that mutations were located mainly in the two domains, and this was similar to other chicken breeds such as Leghorn, Silky chicken and *N. meleagris*.

Polymorphism of the α1 and α2 domains

Mutation frequency was divided into three levels by Wu-kabat index analysis: score = 1 indicated no mutation, 1 <score <4 indicated a low degree of variation, while score ≥ 4 indicated a high degree of variation. As shown in Figure 2, a total of 68 amino acid positions were all replaced in α1 and α2 domains of MHC class Iα in both chicken breeds, accounting for 38% of the total residues in two domains. Moreover, 23 sites had high mutation frequency (score ≥4) in two domains of HBP, and 10 and 13 of polymorphic sites were in α1 and α2 domains, respectively. There were 25 sites with high mutation frequency (score ≥4) in the two domains of WC, 13 of polymorphic sites in the α1 domain, and others in the α2 domain. Further analysis of sequences revealed four and six main peaks (score ≥8) in the two domains of HBP and WC, respectively; with four main sites (score ≥8) and all were located at the residues 9, 111, 113 and 153 in both chicken breeds.

High polymorphic sites in α1 and α2 domains among different species

Comparison of high polymorphic sites of the α chain revealed a structural similarity between chicken breeds and other species (Table 1). There were six highly variable sites (9, 69, 111, 113, 149 and 153) with scores (≥8) in α1 and α2 domains of chicken by Wu-kabat index analysis. Similarly, the sites (9, 66, 97, 111 and 113) appeared in duck with high mutation frequency (score≥8), which had 28 polymorphic residues(score≥4) in α1 and α2 domains, 11 of them had scores ≥8 (Xia et al. 2004). Moreover, the α1 and α2 domains of HLA-A and H-D in human and mouse (Bjorkman and Parham 1990; Pullen et al. 1992) revealed nine and 19 sites with highly variable sites (score ≥8), respectively, including the sites (9,114, 116 and 156) in human corresponding to the sites (9, 111, 113 and 153) in chicken.

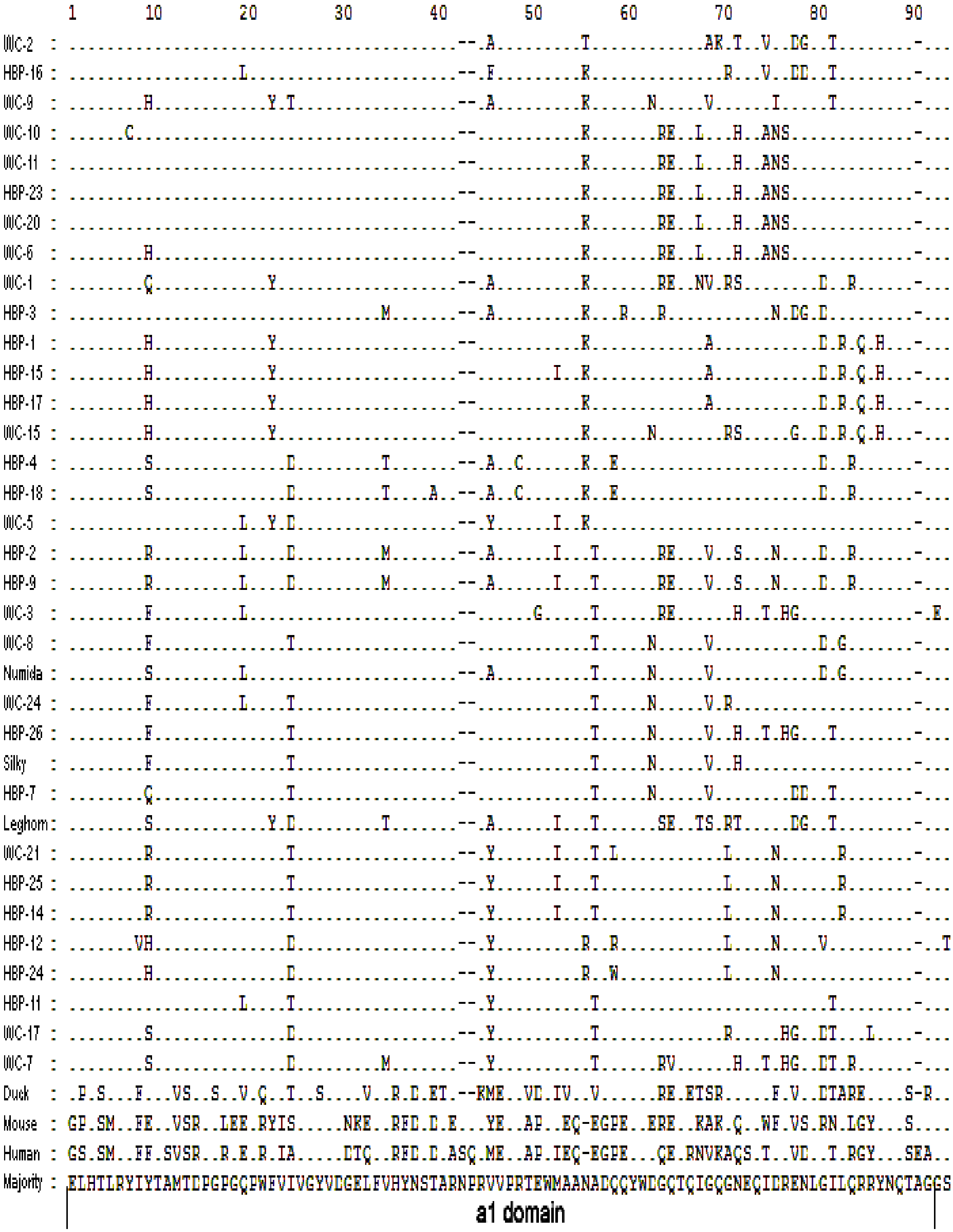

Figure 1. Alignment of amino acid sequences of α1 and α2 domains of MHC class I in chickens, duck, human and mouse. The numbers above the sequences represent amino acid positions. Dots represent identity in amino acid residues. Dashes represent the indel positions in the sequence alignment.

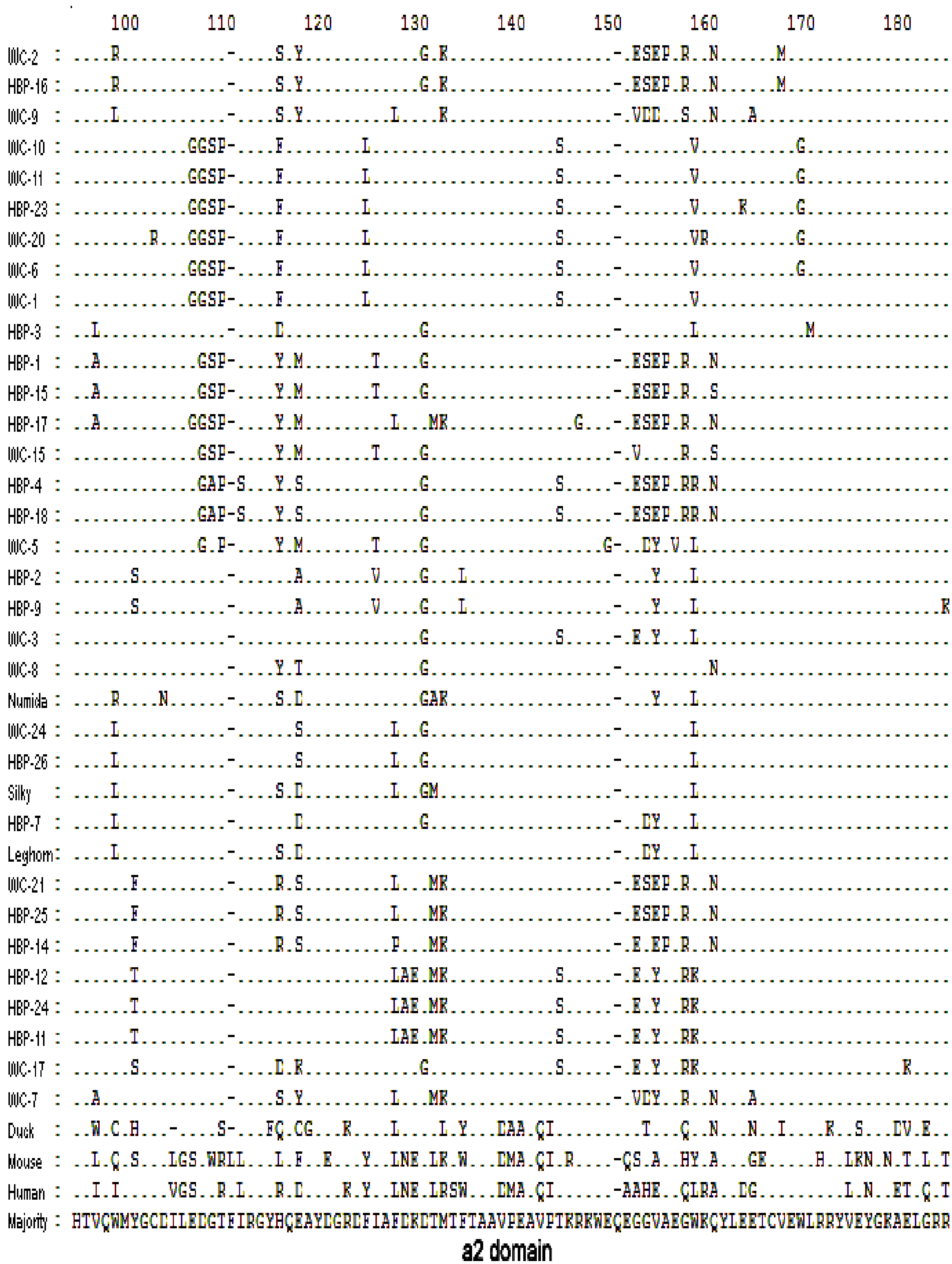

Figure 1. Contd.

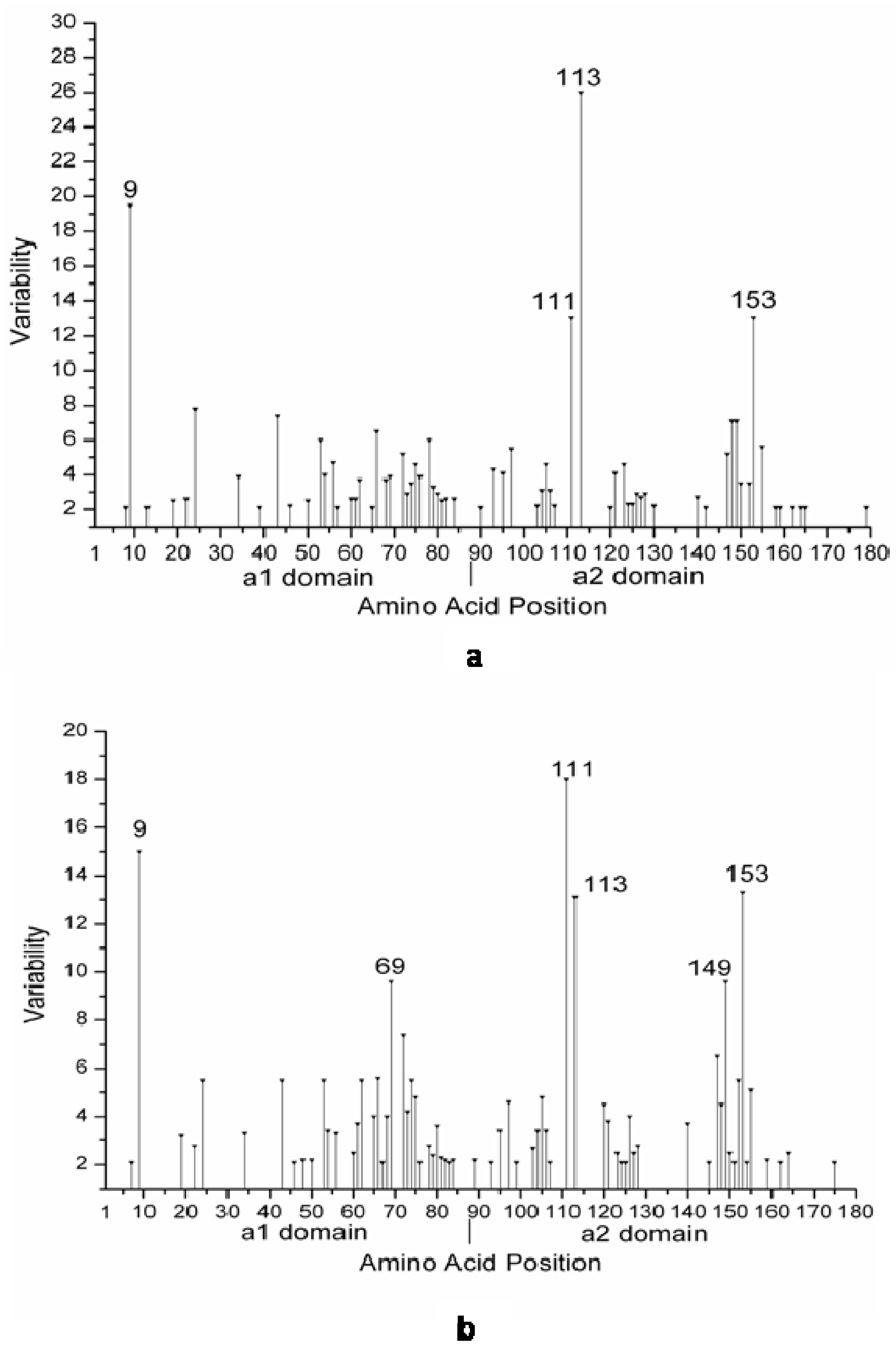

Figure 2. Wu-Kabat plot of amino acid variability in the α1 and α2 domains of MHC class I of the Haibei Partridge chicken (a) and Wenchuang chicken (b). Polymorphic sites had a Wu–Kabat score ≥4. The Wu-Kabat scores ≥8 are marked with their positions.

Table 1. Amino acid positions with high variability (Wu-kabat index ≥4.0) in MHC class I α1 and α2 domains of chicken (HBP and WC), duck, mouse (H-D) and human (HLA-A).

Position		Chicken (HBP)	Chicken (WC)	Duck	Human	Mouse
Bird	Mammal					
α1 domain						
9	9	19.5	15	12.1	9.1	6.5
24	24	7.8	5.5	8		6.5
27	45			4.5		7.4
32	62			6	13.9	5.2
43	63	7.4	5.5		4.2	10.4
51	66			4.5		6.8
52	67			4.5		
53	69	6.	5.5	6		
54	70	4		8		4.3
56	73	4.7				6.5
60	77			4.5		
61	80			12.1		4.3
62	81		5.5	6		
65	82		4	6		
66	89	6.5	5.6	9		4.3
68			4	12.1		
69			9.6	12.1		
72		5.2	7.4			
73			4.2			
74			5.5			
75		4.6	4.8	6		
76				4.5		
78		6				
α2 domain						
93	95	4.3		6	5.4	8.7
95	97	4.1		15	5.8	10.8
97	99	5.5	4.6	8		13
105	114	4.6	4.8		8.3	8.1
111	116	13	18	15	6.3	8.7
113	150	26	13.1	10		4.9
116	152			6	5.9	4.3
120	155		4.5			10.4
121	156	4.1			8.3	9.3
123		4.6				
126			4			
127						
128						
146				4.5		
147		5.2	6.5			
148		7.1	4.5			
149		7.1	9.6			
150				4.5		
152			5.5			
153		13	13.3	4.5		
154				4.5		
155		5.6	5.1			
156				4.5		

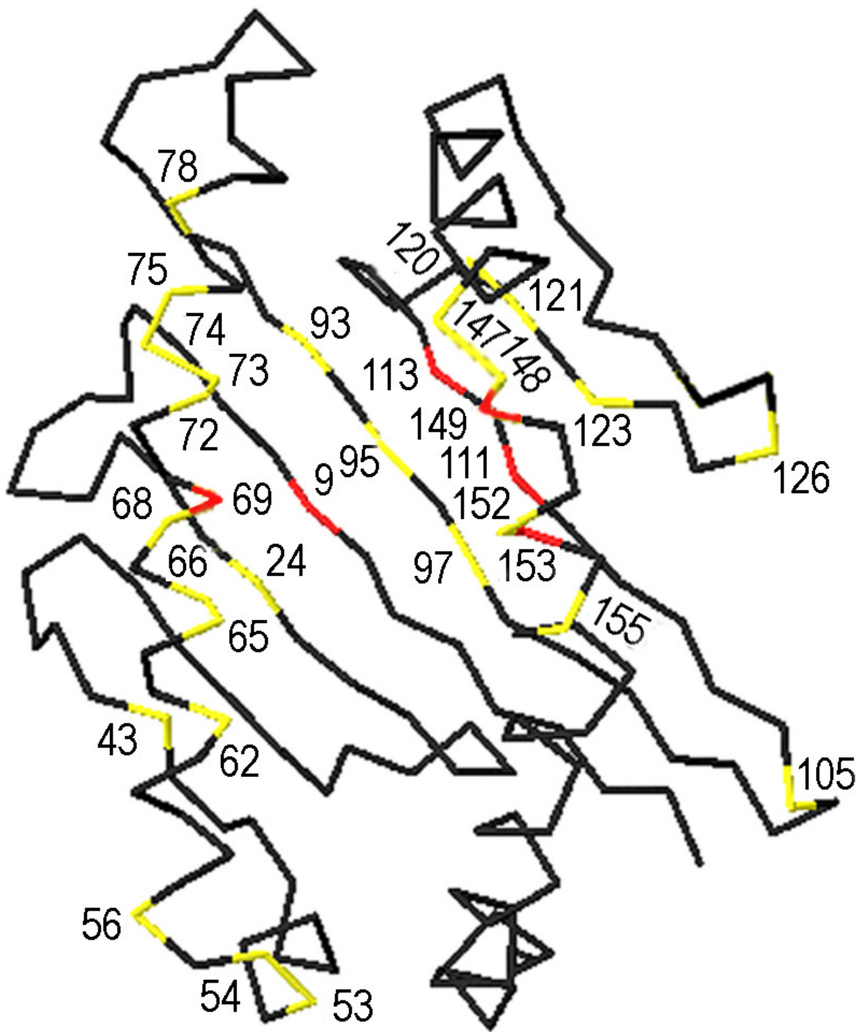

Figure 3. The backbone trace of α1 and α2 regions in MHC class I of chicken based on the crystal structures reported, sequences and sites in Figure 1 and Table 1. Amino acid residues in the model are colored individually (Wu-Kabat score ≥4 – yellow, Wu-Kabat score ≥8 – red).

Spatial orientation of highly polymorphic sites in α1 and α2 domains

To understand the role of high variable amino acid residues in the spatial structure of MHC class I α chain, we simulated the three-dimensional structure of α1 and α2 domains according to the crystal structure reported (Koch et al., 2007). As shown in the Figure 3, the α1 and α2 domains appeared as a configuration based on two α helices and eight β sheets, which formed antigen peptide binding groove in the intramolecular structure. Most of the highly variable residues were located in the α helices or β sheets, especially the sites (62, 65, 66, 68, 69, 72, 73, 74, 75 and 78) in the α1 helix and the sites (152, 153 and 155) in the α2 helix of the molecular middle domain. In addition, three amino acid residues with mutation frequency (score ≥ 8) were located in the β sheet, with only one (site 153) in the α helix.

Phylogenetic analysis of a1 and a2 domains

Furthermore, we constructed phylogenetic trees based on 38 amino acid sequences of α1 and α2 domains in MHC class Iα as aligned by MEGA software (Figure 4). The homology of all alleles among the four breeds ranged from 77.7 to 99.5%, and these sequences were divided into several groups. The evolution of MHC class Iα among duck, mouse and human had familiar way, but the sequences of MHC class Iα in chicken breeds were distributed in different branches without rules, which revealed no correlation among breeds. This result suggests that MHC class Iα chains of different chicken breeds retain similar genetic characters from ancestral

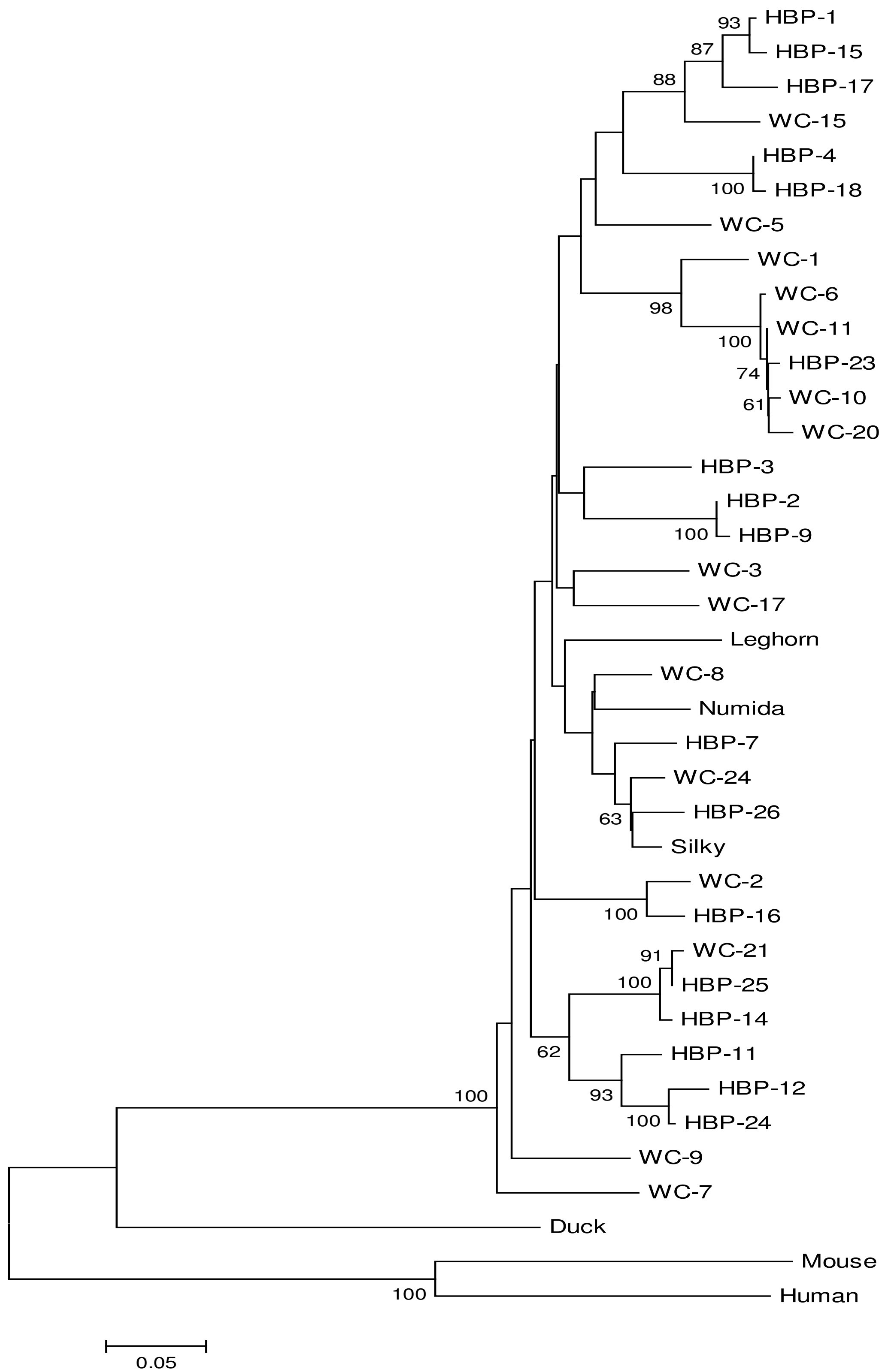

Figure 4. Phylogenetic tree of chicken MHCI created by the neighbor-joining method based on the amino acid sequences aligned in Figure 1 (see Figure 1 for sequence references). Genetic distance is indicated at the bottom. The reliability of the cluster analyses are tested by bootstrap confidence limits and indicated as percent success per 1,000 bootstrap trials with values above 50% presented on nodes.

MHC gene, though they present some distinct diversity in the character and production during long-term breeding.

DISCUSSION

Comparison of predicted structure of these MHC class Iα chain indicated obvious features: first the diversity of these polymorphic amino acid residues, whose score of mutation frequency was higher than or equal to 4 on the Wu-kabat index occurred in a1 and a2 domains, especially in the side chain that contacted with the antigen in PBD. This was similar to the mammalian MHC class II DRB, even as Furlong and Yang (2008) found that almost all amino acid residues inferred to be under positive selection were in the PBD and in contact with the antigen side chains, although residues outside of but close to the PBD. In the evolution process, mutation of the amino acid sequence occurs in the non-PBD, destroying PBD structure integrity which makes MHC molecules fail in binding antigen peptides, while some mutations within the PBD increase the potential of MHC binding antigen peptides. The polymorphic genes of MHC are regarded as essential genes for individual fitness under conditions of selection (Eizaguirre et al., 2009), and it is just these negative or positive selections that drive MHC allelic diversity at loci for antigen presentation (Goto et al., 2009).

We all know that MHC gene descended from a common ancestry and came into being polymorphism under the environmental pressure. In birds, a comparison of MHC class I gene sequences between the quail and chicken in phylogenetic analysis showed that the quail MHC genes were duplicated after the separation of these two species from their common ancestor (Shiina et al., 2004). The two breeds analysed in our study were bred in two Chinese areas that were thousands of miles apart. No relationship was found between breeds feature and MHC class Iα gene in phylogenetic trees. Similarly, Koutsogiannouli et al. (2009) surveyed the level of MHC genetic diversity of European and Asia brown hares (*Lepus europaeus*) in natural populations, and did not find a strong phylogeographic signal in the full-length exon 2eeu-DQA alleles' phylogeny. Xu et al. (2009) found that three MHC alleles (DQB, DRA and MHC-I) of cetaceans showed similarity and identity when compared with the phylogenetic trees. Moreover, Gu and Nei (1999) thought that the MHC evolution in mammals is in agreement with the birth-and-death model of evolution. Under conventional breeding conditions, the animal MHC are subjected to likely pathogen-mediated selection as adaptive processes, rather than breed and geographical differences, although further studies still need to be implemented.

ACKNOWLEDEGMENTS

We are grateful to Dr. Yansheng Zhang (Natural Product Department, The Plant Biotechnology Institute, National Research Council of Canada) for the modification and comments on this manuscript. The research was financially supported by a grant from the National Natural Science Foundation of China under award number 30671537.

REFERENCES

Arnold K, Bordoli L, Kopp J, Schwede T (2006). The SWISS- MODEL Workspace: A web-based environment for protein structure homology modelling. Bioinformatics, 22(2): 195-201.

Becker L, Nieberg C, Jahreis K, Peters E (2009). MHC class II variation in the endangered European mink *Mustela lutreola* (L. 1761)-consequences for species conservation. Immunogenetics, 61(4): 281-288.

Bjorkman PJ, Parham P (1990). Structure, function, and diversity of class I major histocompatibility complex molecules. Annu. Rev. Biochem. 59: 253-288.

Chaves LD, Krueth SB, Reed KM (2009). Defining the turkey MHC: sequence and genes of the B locus. J. Immunol. 183(10): 6530-6537.

Eizaguirre C, Yeates SE, Lenz TL, Kalbe M, Milinski M (2009). MHC-based mate choice combines good genes and maintenance of MHC polymorphism. Mol. Ecol. 18(15): 3316-3329.

Ewald SJ, Livant EJ (2004). Distinctive polymorphism of chicken B-FI (major histocompatibility complex class I) molecules. Poult. Sci. 83(4): 600-605.

Furlong RF, Yang Z (2008). Diversifying and purifying selection in the peptide binding domain of *DRB* in mammals. J. Mol. Evol. 66(4): 384-394.

Goto RM, Wang Y, Taylor RL, Jr, Wakenell PS, Hosomichi K, Shiina T, Blackmore CS, Briles WE, Miller MM (2009). BG1 has a major role in MHC-linked resistance to malignant lymphoma in the chicken. Proc. Natil. Acad. Sci. USA. 106(39): 16740-16745.

Gu X, Nei M (1999). Locus specificity of polymorphic alleles and evolution by a birth-and-death process in mammalian MHC genes. Mol. Biol. Evol. 16(2): 147-156.

Guex N, Peitsch MC (1997). SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modelling. Electrophoresis, 18(15): 2714-2723.

Hunt HD, Fulton JE (1998). Analysis of polymorphisms in the major expressed class I locus (*B-FIV*) of the chicken. Immunogenetics, 47(6):456-467.

Kaufman J, Milne S, Gobel TWF, Walker BA, Jacob JP, Auffray C, Zoorob R, Beck S (1999). The chicken B locus is a minimal essential major histocompatibility complex. Nature, 401: 923-925.

Koch M, Camp S, Collen T, Avila D, Salomonsen J., Wallny H., van Hateren A, Hunt L, Jacob JP, Johnston F, Marston DA, Shaw L, Dunbar PR, Cerundolo V, Jones EY, Kaufman J (2007). Structures of an MHC class I molecule from B21 chickens illustrate promiscuous peptide binding. Immunity, 27(6): 885-899.

Koutsogiannouli EA, Moutou KA, Sarafidou T, Stamatis C, Spyrou V, Mamuris Z (2009). Major histocompatibility complex variation at class II DQA locus in the brown hare (*Lepus europaeus*). Mol. Ecol. 18: 4631-4649.

Kumar S, Tamura K, Nei M (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. 5(2): 150-163.

O'Leary CE, Wiseman RW, Karl JA, Bimber BN, Lank SM, Tuscher JJ, O'Connor DH (2009). Identification of novel MHC classIsequences in pig-tailed macaques by amplicon pyrosequencing and full-length cDNA cloning and sequencing. Immunogenetics, 61(10): 689-701.

Pullen JK, Horton RM, Cai ZL, Pease LR (1992). Sturucture diversity of the classical H-2 genes: K, D and L. J. Immunol. 148(3): 953-967.

Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4(4): 406-425.

Schwede T, Kopp J, Guex N, Peitsch MC (2003). SWISS- MODEL: An automated protein homology-modeling server. Nucleic Acids Res. 31(13): 3381-3385.

Shiina T, Shimizu S, Hosomichi K, Kohara S, Watanabe S, Hanzawa K, Beck S, Kulski JK, Inoko H (2004). Comparative genomic analysis of two avian (quail and chicken) MHC domains. J. Immunol. 172(11): 6751-6763.
Silva MC, Edwards SV (2009). Structure and evolution of a new avian MHC class II B gene in a sub-Antarctic seabird, the thin-billed prion (Procellariiformes: *Pachyptila belcheri*). J. Mol. Evol. 68(3): 279-291.
Takeshima SN, Sarai Y, Saitou N, Aida Y (2009). MHC class II DR classification based on antigen-binding groove natural selection. Biochem. Biophys. Res. Commun. 385(2): 137-142.
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25(24): 4876-4882.
Westerdahl H, Wittzell H, von Schantz T (2000). Mhc diversity in two passerine birds: no evidence for a minimal essential Mhc. Immunogenetics, 52(12): 92-100.
Wu TT, Kabat EA (1970). An analysis of the sequences of the variable domains of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. J. Exp. Med. 132: 211-250.
Xia C, Lin CY, Xu GX, Hu TJ, Yang TY (2004). cDNA cloning and genomic structure of the duck (*Anas platyrhynchos*) MHC classIgene. Immunogenetics, 56: 304-309.
Xu SX, Ren WH, Li SZ, Wei FW, Zhou KY, Yang G (2009). Sequence polymorphism and evolution of three cetacean MHC genes. J. Mol. Evol. 69(3): 260-275.