ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 5 (4): 365-369, 2006
ISSN 1682-8356

Genetic Differentiation Degree and Time of Divergence Between Chinese Chicken Populations Inferred from Micro Satellite Data

G.J. Dai[1], O. Olowofeso[1,2] and J.Y. Wang[1]*
[1]College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, P. R. China
[2]Department of Animal Production and Health, Federal University of Technology,
P. M. B. 704, Akure, Nigeria

Abstract: Five chicken populations in Yangzhou and Haimen cities, both in Jiangsu Province, East China that have recently arose the attention of poultry researchers are the New Yangzhou (NY-1), Rugao (HR-1), Jiangchun (HJ-2), Wan-Nan (HW-3) and the Cshiqishi (HC-4) chickens, respectively. Genetic differentiation degree, genetic distances and the actual time of divergence between these chicken populations were obtained by employing a suite of marker panel containing five carefully selected Micro satellite loci with 81 genomic DNAs isolated from the chicken's blood samples. The isolated genomic DNAs were subjected to polymerase chain reaction (PCR); with all the loci involving 35 cycles carried out in a ready-to-go-thermo cycler and amplified products analyzed. Allele frequencies data were generated in the initial analyses using Pop Gene 32 software and used to compute multi-populations expected heterozygosity (H), across population's genetic differentiation degree (F_{ST}) for each locus and the genetic distances (D_{ij}) between population pairs considering all loci. The F_{ST} obtained ranged from 0.0082 (MCW4) to 0.0415 (ADL 176). The D_{ij} between population pairs computed were used to calculate the divergence time (t) in years. The estimated time of divergence between these chicken populations oscillates between 167 (Wan-Nan vs. Cshiqishi) and 1954 years (Rugao vs. Jiangchun), respectively.

Key words: Chinese chickens, genetic differentiation, divergence time, marker panel

Introduction

Evidence in literature have now revealed that Micro satellite markers have heuristic values and the widespread usage of these molecular markers by the scientific community are providing reliable results on population species that need to be conserved. Micro satellite markers are now regarded as marker genes. They are appropriate tools for the study of the relationship between breeds and for breed development. Apart from facilitating the investigation of breed mixtures and quantification of components of a given hybrid in population, they also help in revealing breed/population admixture that had long been forgotten through lack of known records. In the poultry sector, it had been regarded as marker of high utility (Kaiser *et al.,* 2000; Emara and Kim, 2003; Olowofeso *et al*., 2005b). The usage of these markers with chickens of several origins have been reported by Vanhala *et al.* (1998); Romanov and Weigend (2001a) and Williams *et al.* (2004) have examined the benefits of micro satellites with other birds including the red-winged blackbirds (*Agelaius phoeniceus*). Quellar *et al.* (1993); Avise (1994); Dawson *et al.* (1997) have described micro satellites as useful tools for addressing a wide range of important issues in population biology. Data generated from application of micro satellites to species populations are suitable for estimating population differentiation, gene flow and kinship studies (Quellar *et al*., 1993; Morera *et al.,* 1999). Use of micro satellites to detect genetic differentiation and the estimated divergence time of five chicken populations called New Yangzhou, Rugao, Jiangchun, Wan-Nan, and Cshiqishi maintained largely in both Yangzhou and Haimen cities, East-China, are lacking in searched literature. These chicken populations produced jumbo-type eggs and they can all thrive well in harsh environmental conditions. While extensive genetic studies have been carried out in nearly all the other chicken populations in China with several markers including the micro satellites, genetic information of these five chicken populations have not been adequately studied. In order to detect population of particular merits and which population of these chickens that deserve conservation, it is important to carry out a precise research ahead of a breeding program. The present study was therefore carried out to assess the degree of genetic differentiation between populations, the genetic lineage and the exact time these chicken populations diverge from one another with the aid of high utility Micro satellite markers.

Materials and Methods

Sample size and sample collection: A total of 81 chicken bloods were collected from the five chicken populations: New Yangzhou (NY-1), Rugao (HR-1), Jiangchun (HJ-2), Wan-Nan (HW-3) and Cshiqishi (HC-4). Details of the chicken populations investigated in this

Table 1: Name and sample size of the five chicken populations used in this study

Population	City of collection	Date of sample collection	Sample size (n)
New Yangzhou	Yangzhou	Nov/Dec. 2003	15
Rugao	Haimen	January 2004	10
Jiangchun	Ditto	Ditto	14
Wan-Nan	Ditto	Ditto	21
Cshiqishi	Ditto	Ditto	21

Table 2: Summary of overall allele frequencies generated with the selected loci*

Allele/Locus	ADL166 (55°C)	ADL176 (52°C)	MCW4 (63°C)	MCW0014 (62°C)	MCW134 (58°C)
A_i	0.8025	0.8951	0.8704	0.7531	0.4012
B_i	0.0802	0.0123	0.0556	0.1790	0.3457
C_i	0.0309	0.0247	0.0494	0.0679	0.1914
D_i	0.0432	0.0432	0.0123	0.0000	0.0432
E_i	0.0247	0.0062	0.0062	0.0000	0.0185
F_i	0.0185	0.0185	0.0062	0.0000	0.0000

*Number in parentheses represents the annealing temperature of each locus

study are summarized in Table 1. Blood sample was the material collected and used in all the populations from which genomic DNAs were extracted. Blood sample of 200 µL were taken by wing venipuncture of the brachial vein using needles immersed in illicit gin and stored at 4°C in prepared lysis buffer containing (10 mM Tris-HCl, 10 mM EDTA, 10 mM common salt (NaCl), PH 7.8).

DNA extraction: All samples collected were centrifuged for 120 s and DNAs were extracted by method earlier described by Ardern *et al*. (1997). Total genomic DNA was extracted from the blood starting with cell lysis and 150 µL whole blood was pipette into a 1.5 mL miniature tube and re-suspended in 400 µL lysing buffer (44 mM NH_4Cl, 10 mM NH_4HCO_3) with centrifugation at 1000 g for 600 s to pellet cells, 20 µL RNase A stock solution synthesized by (Sangon Biotech. Company, Shanghai, China) was added to the samples and shaked. The resultant supernatant was removed and replaced with 1 mL SET buffer containing 10 mM Tris-HCl, PH = 8.0, 200 mM common salt (NaCl), 0.1 M EDTA and 0.5% sodium dodecyl sulphate (SDS) and samples were digested with 0.5 µg/µL proteinase K kept dried at 4°C. Samples and buffer were mixed thoroughly and vortexed for 15 s to ensure efficient lysis and perfectly mixed solution. The resultant mixtures were placed in hot water-bath, incubated overnight at 55°C with slight shaking. Following incubation, high molecular weight DNA was extracted twice with phenol-chloroform-isoamyl (25:24:1 v/v)– (phenol chloromethane and isoamyl alcohol). In each case, phenol-chloroform-isoamyl was added to the resultant filtrate collected into new tubes after repeated centrifugations and eventually the DNA samples were precipitated by 70% ethyl alcohol absolute. Tubes containing the resultant DNA samples were air-dried for 600 s so that it can easily re-dissolve. The air-dried DNA pellets were dissolved in 300 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, PH = 8.0) to prevent protein and contaminants that may inhibit PCR reactions and the mixtures were vortexed. The content of each miniature tube containing the DNA was diluted with sterilized water before the purity and the concentration of each DNA sample was carried out. Following the determination of the $A_{260}/_{280}$ value of the DNA samples fluorimetrically, the concentration of the total DNA was adjusted to 100 ng/µL with sterilized distilled water and 1 µL of the DNA samples were used as template for polymerase chain reaction.

Polymerase chain reaction and electrophoresis: The five marker panels used with the five chicken populations were sourced from Sangon Biotech. Company, Shanghai, China. The PCR employed involved 35 cycles of amplification with total volume of 20 µL made up of 1 µL template DNA, 2.5 µL of 10 x PCR Buffer, 1 µL of 25 mM dNTPs, 1 µL of each (8 pmol/µL) forward (F) and reverse (R) of the primers, 2.2 µL of 25 mmol/L $MgCl_2$, 11.1 µL sterilized distilled water and 0.2 µL of 5 U/µL *Taq* DNA polymerase (Sangon Biotech. Company, Shanghai, China). Reaction programmes were carried out in a ready-to-go PCR Hybaid Express System (PE 9600) with the following settings: initial denaturation (94°C, 300 s), denaturation (94°C, 60 s), 60 s at annealing temperatures of the five loci ranged between 52 to 63°C and (72°C, 60 s) with final extension at (72°C, 600 s). Miniature tubes containing amplified PCR-products were carefully opened and equal volume of loading dye made up of *xylene cyanol, bromophenol blue and formamide* (1:1:1000) added to each tube, covered and further denatured by heating at 94°C for 600 s in the same PCR system, then 10 µL of the denatured samples were allowed to run on 12% denaturing sequencing gel with incorporation of pBR322 DNA/MspI as internal standard for sizing (Sangon Biotech. Company, Shanghai, China). Electrophoresis was allowed to run for 2.16 x 10^4 s at 1 W, 10,000Ω. The gel

Table 3: Polymorphism information content, heterozygosity and genic variation statistics for all loci*

Locus	2n	PIC	Heterozygosity statistics			Genic variation statistics		
			Obs-Hom.	Obs-Het.	H	n_e	E_{NA}	I
ADL166	162	0.3327	0.6667	0.3333	0.3458	6	1.5286	0.7874
ADL176	162	0.1911	0.7901	0.2099	0.1959	6	1.2436	0.4859
MCW4	162	0.2279	0.7407	0.2593	0.2367	6	1.3101	0.5470
MCW0014	162	0.3752	0.6049	0.3951	0.3962	3	1.6562	0.7041
MCW134	162	0.6464	0.4568	0.5432	0.6807	5	3.1319	1.2597
Mean±S.E	162	0.3547±0.80	0.6519±0.16	0.3481±0.16	0.3711±0.20	5.2000±0.51	1.7741±0.39	0.7568±0.25

*Standard error (S.E) and (n) is as earlier defined in Table 1.

Table 4: Multi-populations F-Statistics and the gene flow for all loci

Locus	F_{IS}	F_{IT}	F_{ST}	N_m
ADL166	-0.0097	0.0070	0.0166	14.8102
ADL176	-0.1172	-0.0708	0.0415	5.7741
MCW4	-0.0998	-0.0908	0.0082	30.2378
MCW0014	-0.0833	-0.0533	0.0277	8.7753
MCW134	0.1815	0.2111	0.0362	6.6561

was then carefully transferred into an open tray containing 10 cm^3 of pure water and a drop of ethidium bromide (EB) was added to the content of the tray and allowed to settle for 180 s before visualization of the gel result under UV trans-illuminator, photographed and genotype data were determined using Genotyper (ver. 2.0) Eastman Kodak Digital Science-DC120.

Statistical analysis: Multi-populations descriptive statistics were carried out on all data. Allele frequencies were obtained using Pop Gene 32 software. Effective number of alleles (E_{NA}) and Shannon's information index (I) were obtained using the formula suggested by Kimura and Crow (1964) and embedded in Pop Gene 32 software. Multi-populations polymorphism information content (PIC) for each locus was calculated using Cervus software (Marshall *et al.*, 1998). Multi-populations expected heterozygosity (H) for each locus was obtained using the formula:

$$H = 1 - \sum_{i=1}^{n} P_i^2$$

suggested by Nei (1973). The F-statistics (fixation indexes-F_{IS} and F_{IT}) and multi-populations genetic differentiation degree assessed as F_{ST} for each locus was generated with the aid of the Pop Gene 32 software. Multi-populations gene flow (N_m) for each locus was calculated from F_{ST} by employing Wright (1951) formula designated as:

$$Nm = 0.2500 \frac{[1-F_{ST}]}{F_{ST}}$$

Genetic distances (D_{ij}) among populations were computed from allele frequencies data using Nei (1972) approach. Estimated divergence time (t) in years between the chicken populations were generated with the relation, $t = 100D_{ij}t_o / 2\alpha$ where D_{ij} is the genetic distance obtained from the Micro satellite data (Nei, 1972) and $\alpha = 0.0012$, is the assumed mutation rates of Micro satellite loci (Weber and Wong, 1993) and a generation interval (t_o) of 1 year was assumed.

Results

The five marker panels used were highly informative with the samples of the five chicken populations generating adequate data for all the analyses. A total of 26 alleles were observed across populations considering all marker panels (Table 2), with overall mean of 5.2000 ± 0.51 per locus. Multi-populations expected heterozygosity (H) for each locus ranged from low (ADL176) to high (MCW134) and was (0.1959 to 0.6807) and mean (H) across all loci was 0.3711 ± 0.20. The observed homozygosity (Obs-Hom.) and observed heterozygosity (Obs-Het.) generated with each locus across the chicken populations as well as the multi-populations expected heterozygosity (H) for each locus, PIC, observed number of alleles across populations for each locus (n_e), E_{NA} and I-values generated are summarized in Table 3. The F_{IS} and F_{IT}-values generated varied for each locus across populations. They were – 0.1172 to 0.1815 and – 0.0908 to 0.2111 for F_{IS} and F_{IT}, respectively. The multi-populations F_{ST} ranged from 0.0082 (MCW4) to 0.0415 (ADL176). Using simple substitution common to population genetic studies, the gene flow depicted as N_m was between 5.7741 (ADL176) and 30.2378 (MCW4). Results of all these parameters are summarized in Table 4. Genetic distances D_{ij} between populations based on Nei's standard ranged between 0.0040 (Wan-Nan vs. Cshiqishi) and 0.0469 (Rugao vs. Jiangchun), and the estimated divergence time (t) in years (a dependable variable on the calculated genetic distances between populations) oscillates between 167 and 1954 years in these chickens (Wan-Nan vs. Cshiqishi) and (Rugao vs. Jiangchun), respectively. Table 5, summarized the

Table 5: Nei's original measures of genetic identity (GI) above and genetic distance (D_{ij}) below the diagonal*

Population	NY-1	HR-1	HJ-2	HW-3	HC-4
NY-1	0.0000	0.9710	0.9788	0.9936	0.9868
HR-1	0.0294 (1225)	0.0000	0.9541	0.9810	0.9811
HJ-2	0.0214 (892)	0.0469 (1954)	0.0000	0.9816	0.9831
HW-3	0.0064 (267)	0.0192 (800)	0.0186 (775)	0.0000	0.9960
HC-4	0.0133 (554)	0.0191 (796)	0.0171 (713)	0.0040 (167)	0.0000

*Abbreviations are as defined within the text and numbers in parentheses represents t-values in years.

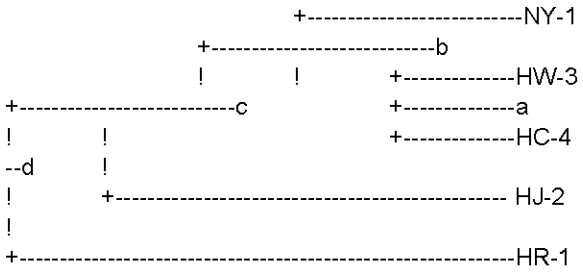

Fig. 1: Dendrogram based on the estimated divergence time among five Chinese chicken populations inferred from Micro satellite data

generated results of both the pairwise genetic identity, pairwise genetic distances and the estimated divergence time between these chicken populations. Using the estimated divergence time (t), the dendrogram common to these chicken populations was developed and represented in (Fig. 1).

Discussion

Results of this study revealed that genetic parameters in populations could be derived from Micro satellite markers. With Micro satellite markers, Morera *et al.* (1999); Sun *et al.* (2004) and Chang *et al.* (2005) have succeeded in determining the genetic differentiation in dog breeds, sheep and quail populations. In a related development and earlier research, Dawson *et al.* (1997) have obtained the genetic differentiation existing between passerine birds (*Dendroica petechia*) using Micro satellite DNA markers. It can therefore be concluded that these markers are the most effective tools for determining population genetic differentiation. With the five marker panels selected for the present study, the multi-populations genetic differentiation degree depicted as F_{ST} was obtained for each locus (Table 4). The PIC, the heterozygosity, allele number and the effective allele number obtained in this study and presented in Table 3 were however consistent with the results of Chen *et al.* (2004) and Olowofeso *et al.* (2005b) using Micro satellite markers with Chinese chicken populations. The mean Shannon information index in this work was 0.7568±0.25 (Table 3) across all loci and across chicken populations. This value was however lower than the Shannon information index range of 2.0064±0.18 to 2.0586±0.29 obtained by Olowofeso (2005). The variation in this parameter may be coupled with number of marker panels used and the difference in population size as well as variation in the PCR programme and conditions adopted. Gene flow (N_m) depends mainly on the F_{ST} values and a lower value of the F_{ST} brings about higher value of N_m or vice versa as revealed by the current investigation (Table 4). Similar results of this nature had been reported by Mukesh *et al.* (2004). Using Micro satellite data, the genetic distances of species populations have been obtained by several research workers including Li *et al.* (2000); Mukesh *et al.* (2004) and Olowofeso *et al.* (2005b) etc. Generated results of this parameter (genetic distance) provide suitable data to develop a consensus dendrogram between populations. It also provides a direct guide of obtaining the divergence time between several populations when integrated into appropriate formula common to population genetic studies (See Mukesh *et al.*, 2004). Integrating the genetic distances obtained with Micro satellite data, Li *et al.* (2000), obtained the divergence time between pig populations as values ranging from 653 to 1856 years and submitted that Micro satellite revealed the variation and divergence among breeds more objectively than other molecular markers. Mukesh *et al.* (2004) had reported estimated divergence time of three Indian native cattle breeds to be between 776 and 1296 years using Micro satellite data. In a recent survey of four of the chicken populations under consideration, Olowofeso (2005) had reported estimated divergence time between populations to be oscillating between 98 and 211 years. In this work, the estimated divergence time between these five chicken populations was oscillating between 167 and 1954 years. Therefore, diversity between two or more populations can be determined more accurately by divergent time. The easy determination of estimated divergence time between populations can therefore be regarded as further applicability of Micro satellite markers in population genetics.

References

Ardern, S.L., D.M. Lambert, A.G. Rodrigo and I.G. McLean, 1997. The effects of population bottlenecks on multilocus DNA variation in robins. J. Hered., 88: 179-186.

Avise, J.C., 1994. Molecular Markers, Natural History and Evolution, Chapman and Hall, London.

Chang, G.B., H. Chang, X.P. Liu, W. Xu, H.Y. Wang, W.M. Zhao and O. Olowofeso, 2005. Developmental research on the origin and phylogeny of quails. World's Poult. Sci. J., 61: 105-112.

Chen, G.H., X.S. Wu, D.Q. Wang, J. Qin, S.L. Wu, Q.L. Zhou, F. Xie, R. Cheng, Q. Xu, B. Liu, X.Y. Zhang and O. Olowofeso, 2004. Cluster analysis of 12 Chinese native chicken populations using microsatellite markers. Asian-Aust. J. Anim. Sci., 17: 1047-1052.

Dawson, R.J.G., H. Lisle-Gibbs, K.A. Hobson and S.M. Yezerinac, 1997. Isolation of microsatellite DNA markers from a passerine bird, *Dendroica petechia* (the yellow warbler), and their use in population studies. Heredity, 79: 506-514.

Emara, M.G. and H. Kim, 2003. Genetic markers and their application in poultry breeding. Poult. Sci., 82: 952-957.

Kaiser, M.G., N. Yonash, A. Cahaner and S.J. Lamont, 2000. Microsatellite polymorphism between and within broiler populations. Poult. Sci., 79: 626-628.

Kimura, M. and J.F. Crow, 1964. The number of alleles that can be maintained in a finite population. Genetics 68: 47.

Li, X., K. Li, B. Fan, Y. Gong, S. Zhao, Z. Peng and B. Liu, 2000. The genetic diversity of seven pig breeds in China, estimated by means of microsatellites. Asian-Aust. J. Anim. Sci., 13: 1193-1195.

Marshall, T.C., J. Slate, L.E.B. Kruuk and J.M. Pemberton, 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol., 7: 639-655.

Morera, L., C.J. Barba, J.J. Garrido, M. Barbancho and D.F. de Andres, 1999. Genetic variation detected by microsatellites in five Spanish dog breeds. J. Hered., 90: 654-656.

Mukesh, M., M. Sodhi, S. Bhatia and B.P. Mishra, 2004. Genetic diversity of Indian native cattle breeds as analysed with 20 microsatellite loci. J. Anim. Breed. Genet., 121: 416-424.

Nei, M., 1972. Genetic distance between populations. Am. Nat., 106: 283-292.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci., USA, 70: 3321-3323.

Olowofeso, O., 2005. Estimation of genetic diversity and genetic distances of chicken populations in Haimen-China with two-fold techniques. Ph.D. Thesis, Yangzhou University, Yangzhou, China.

Olowofeso, O., J.Y. Wang, K.Z. Xie and G.Q. Liu, 2005b. Phylogenetic scenario of port-city chickens in China based on two-marker types. Int. J. Poult. Sci., 4: 206-212.

Quellar, D.C., J.E. Strassmann and C.R. Hughes, 1993. Microsatellite and kinship. Trends Ecol. Evol., 8: 285-288.

Romanov, M.N. and S. Weigend, 2001a. Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. Poult. Sci., 80: 1057-1063.

Sun, W., H. Chang, Z.J. Ren, Z.P. Yang, R.Q. Geng, S.X. Lu, L. Du and K. Tsunoda, 2004. Genetic differentiation between sheep and goats based on microsatellite DNA. Asian-Aust. J. Anim. Sci., 17: 583-587.

Vanhala, T., M. Tuiskula-Haavisto, K. Elo, J. Vilkki and A. Maki-Tanila, 1998. Evaluation of genetic variability and genetic distances between eight chicken lines using microsatellite markers. Poult. Sci., 77: 783-790.

Weber, J.L. and C. Wong, 1993. Mutation of human short tandem repeats. Hum. Mol. Genet., 2: 1123-1128.

Williams, C.L., H.J. Homan, J.J. Johnston and G.M. Linz, 2004. Microsatellite variation in red-winged blackbirds (*Agelaius phoeniceus*). Biochem. Genet., 42: 35-41.

Wright, S., 1951. The genetic structure of populations. Ann. Eugen., 15: 323-354.