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

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Phylogenetic Scenario of Port-City Chickens in China Based on Two-Marker Types

O. Olowofeso[1,2], J.Y. Wang[1], K.Z. Xie[1] and G.Q. Liu[1]
[1]Department of Animal Genetics, Breeding and Reproduction,
Yangzhou University, Yangzhou, 225009, P. R. China
[2]Department of Agricultural Science, Adeyemi College of Education,
P.M.B. 520, Ondo, Nigeria
E-mail: jideolowofeso@yahoo.ca

Abstract: To detect the genetic lineage of four ubiquitous port-city (Haimen) chicken populations in China, a genetic study involving two-marker types viz: random and microsatellite markers were used with the genomic DNAs isolated from the chicken populations. A total of two hundred and forty genomic DNAs with 60 samples from each of Rugao, Jiangchun, Wan-Nan and Cshiqishi populations were used. All samples were subjected to both 45 and 35 cycles of amplifications with five random and fifteen microsatellite markers in polymerase chain reactions (PCRs), respectively. Average band-sharing coefficient (BSC) and the Nei standard genetic distances (D_{ij}) obtained using the data generated by the random markers with the genomic DNAs. Using the microsatellite markers with the same set of genomic DNAs, allele frequencies were obtained through direct gene counting approach. The generated allele frequencies were used to compute the (D_{ij}) between populations. With the random markers, the (D_{ij}) obtained ranged from 0.0083 (Jiangchun and Cshiqishi) to 0.2789 (Rugao and Wan-Nan); while that of microsatellite markers ranged from 0.2360 (Rugao and Jiangchun) to 0.5072 (Rugao and Cshiqishi), respectively. Phylogenetic trees developed using both standard (D_{ij}'s) revealed significant variations in the level of divergence between populations. Phylogenetic linkage of these chicken populations based on the random markers showed Jiangchun and Cshiqishi to be well related, whereas Rugao and Wan-Nan distantly related. For the microsatellite markers, Rugao and Jiangchun appeared very close, while Rugao and Cshiqishi populations appeared remote between the populations. The results emanated from the application of these markers with these chicken populations suggests that Jiangchun and Cshiqishi and or Rugao and Jiangchun are likely to have originated from the same source and Wan-Nan intermediate in both cases of the phylogeny trees, might had been developed through crossbreeding activities of these populations.

Key words: China, phylogenetic study, port, chickens, markers

Introduction

China is a country with heavy human population and this increase had lead to the high demand for the available animal and poultry products in all parts of the country. Among the cheapest and highly affordable protein source for this teeming population is mainly the poultry products. Poultry production equally contributes significant proportion to the recent growing economy of China. Poultry particularly chickens are very important and had been recognized as important genetic resource among the avian species. Presently in China, 47 breeds comprising 27 original native breeds, 9 developed and 11 introduced exotic breeds abound in the country (Zhang *et al*., 2002). All these breeds/populations of chickens have been recognized and efforts are made daily to conserve them. However, the exact total of chicken populations still remains obscured, as there are several ubiquitous chicken populations whose genetic information on them have not been well documented in literature. A recent survey by Olowofeso *et al*. (2005a) revealed that almost nothing is known about the common port-city chickens in Haimen, China. Information on Rugao, Jiangchun, Wan-Nan and Cshiqishi chicken populations in this region of the country are completely not available. A detail genetic study of these chicken populations is therefore imperative so as to integrate the resource into the poultry sector. The genetic variation of these populations with other chicken populations in the country need investigation so as to be able to identify populations of particular merit. The phylogenetic linkage of these populations needs to be well developed, as these chicken populations may be good source of research material now and in the near future. Genetic variations and phylogenetic relationships between various species are now been examined using type II markers of which random and microsatellite markers are inclusive (Emara and Kim, 2003). The use of these markers with common carp brood-stocks had been reported by Bartfai *et al*. (2003) and with chickens by Zhang *et al*. (2002),

respectively. The efficacies of microsatellite markers with several chicken populations have equally been reported by Vanhala *et al.* (1998); Ponsuksili *et al.* (1999); Romanov and Weigend (2001). The advantages of these markers are manifold. Random markers have been used by (Plotsky *et al.*, 1995; Smith *et al.*, 1996) in random amplified polymorphic DNA (RAPD) technique to evaluate genetic variation and relatedness within and among poultry species and microsatellite markers have proved useful in genome mapping, parentage tests of several species and had been found suitable for the determination of genetic variation in chicken populations. Recently, the genetic parameters both within and between chicken populations were measured using microsatellite markers by Olowofeso *et al.* (2005a). Based on the wide significance of these markers, we therefore employed them with the genome of the port-city chickens in China so as to obtain the divergence level among the populations. The objective of this study reported herein was to know the standard genetic distances between the ubiquitous port-city chicken populations and reconstruct acceptable phylogenetic trees for the populations using data generated from two alternative markers.

Materials and Methods

Experimental stocks, sample size and genomic DNA isolation: Four ubiquitous chicken populations: Rugao (RC), Jiangchun (JC), Wan-Nan (WC) and Cshiqishi (CC) maintained at the Vertically Integrated Poultry Company, Port-city (Haimen), China; were sampled for the present study. Total of (n = 240) blood samples were collected with sixty from each of the chicken population from which DNAs were isolated and used for this study. Collected blood were centrifuged and genomic DNAs were purified by the salting out procedure described by Miller *et al.* (1988) with little modifications as reported elsewhere by Olowofeso *et al.* (2005a). The DNA isolation flowchart used in the present study is depicted in Fig. 1. Concentrations and quantification of the DNAs after reconstituted in moisture were carried out with the aid of laboratory spectrophotometer.

Fig. 1: DNA isolation flowchart used in this study.
Source: DNA isolation flowchart adapted from Olowofeso (2005).

RAPD and Microsatellite PCR programme and conditions: The RAPD-PCR conditions used with the final volume of 25 µl included 2.5 µl of 10 x PCR Buffer, 2.2 µl of 25 mmol/L $MgCl_2$, 1 µl of dNTPs (25 mM dNTPs), 1.5 µl of each random primer, 0.2 µl Taq DNA polymerase (5U/µl) (Sangon Company, Shanghai, China), 16.6 µl sterilized distilled water and 1 µl of each template DNA from individual chicken was used. PCR

Table1: Five informative random markers selected for the amplification of port-city chicken DNAs in this study

Marker's code	Sequences (5'-3' flanking region)	Molecular weight (g)
S246	ACCTTTGCGG	3008
S249	CCACATCGGT	2977
S1083	CCCACCCTTG	2913
S1088	GTCGCCCTCA	2953
S1092	CCCAGGCTAC	2962

was performed in a Hybaid Express system 9600 (Perkin Elmer) programmable incubator with the following setting: initial denaturation (94°C, 60 s), 45 cycles of denaturation (94°C, 30 s), annealing (37°C, 120 s) and extension (72°C, 120 s). The ramping time from denaturation to annealing and that from annealing to extension were programmed for equal time of 300 s, respectively (Murayama *et al.*, 1999; Dai, 2004). Genotyping of DNA samples with the fifteen microsatellite markers were carried out using 100 ng/µl of individual genomic DNA isolated from the four port-city chicken populations. The composition of the PCR reaction mixture with the final volume of 25 µl in each micro-PCR tube, contained 1 µl template DNA, 2.5 µl of 10 x Buffer, 1 µl of 25 mM dNTPs, 2 µl (1 µl forward plus 1 µl reverse form) of each pair of primer, 0.2 µl of (5U/µl) Taq DNA polymerase, 2.2 µl of 25 mm/mol Mg^{2+} (Sangon Company, Shanghai, China) with 16.1 µl sterilized distilled water added. The thermo cycling MS-PCR reaction programme was carried out in the same PCR system used for the RAPD-PCR with setting changed to: 94°C, 300 s (initial denaturation), 35 cycles of denaturation at (94°C, 60 s), annealing temperatures of the microsatellites used ranged between 52-60°C (Table 5) for 60 s, and extension at (72°C, 60 s), followed by final extension at (72°C, 600 s).

Random sampling and initial random primer/marker screening: A total of forty samples from the four chicken populations (10 from each population DNA samples) were randomly selected and used in the primary screening with the forty arbitrary 10-mer random primers, all within the kits S241-S260 and S1081-S1100, respectively. Five of the random markers (Table 1) were quite informative with the random DNA samples and were selected and used as criterion markers in subsequent analyses. These selected markers were used for the amplification of both individual and pooled population DNAs in the RAPD-PCR protocol. For the microsatellite markers, the code and some of the results generated are presented in Table 5.

Submerged agarose and Polyacrylamide gel electrophoresis (SAGE/PAGE): Amplified RAPD-PCR products were detected by using Smith *et al.* (1996) method. The RAPD fragments amplified by the

Table 2: Band-sharing coefficient (BSC) of each marker, the mean across markers, average frequency of band (q) and heterozygosity (H) in the four port-city chicken populations

Population (s)	BSC of each RAPD marker					$\overline{BSC}$ ±S.E	q	H
	S246	S249	S1083	S1088	S1092			
Rugao	0.9354	0.7943	0.8808	0.9422	0.9112	0.8928±0.02	0.6726	0.4933
Jiangchun	0.9566	0.9678	0.9587	0.9263	0.9391	0.9497±0.01	0.7757	0.3664
Wan-Nan	0.9345	0.9512	0.8556	0.8830	0.8701	0.8989±0.02	0.6820	0.4825
Cshiqishi	0.9212	0.8987	0.9373	0.9520	0.9338	0.9286±0.01	0.7328	0.4217

Table 3: Distribution of bands in the port-city chicken population pooled DNAs: Rugao (A), Jiangchun (B), Wan-Nan (C) and Cshiqishi (D)

S/No.	Bands (bp)	Markers	A	B	C	D	S/No.	Bands (bp)	Markers	A	B	C	D
1	4983	S246	1	1	1	1	28	1569	S1083	1	1	1	1
2	3755	S246	1	1	1	1	29	1522	S1083	1	1	1	1
3	2759	S246	1	1	1	1	30	1302	S1083	1	1	1	1
4	2314	S246	0	1	0	1	31	1218	S1083	1	1	1	1
5	2028	S246	1	0	0	0	32	1168	S1083	1	0	0	0
6	1818	S246	1	1	1	1	33	1078	S1083	1	1	0	1
7	1670	S246	0	1	0	0	34	1017	S1083	1	1	0	1
8	1555	S246	1	1	1	1	35	950	S1083	1	1	1	1
9	1467	S246	1	1	0	1	36	840	S1083	1	1	1	1
10	1374	S246	1	1	0	0	37	2623	S1088	1	1	1	1
11	1322	S246	1	1	0	0	38	1966	S1088	0	1	0	1
12	1196	S246	1	1	0	0	39	1500	S1088	1	1	1	1
13	1054	S246	1	1	1	1	40	1469	S1088	0	0	0	1
14	4011	S249	0	1	1	1	41	1391	S1088	0	1	1	1
15	3548	S249	0	1	1	1	42	1302	S1088	1	1	1	1
16	2800	S249	0	1	1	1	43	1235	S1088	1	1	1	1
17	1879	S249	0	0	1	0	44	1078	S1088	1	1	1	1
18	1719	S249	0	1	1	1	45	975	S1088	1	1	1	1
19	1579	S249	1	1	1	1	46	890	S1088	1	1	1	1
20	1522	S249	1	1	1	1	47	840	S1088	0	0	1	0
21	1364	S249	1	1	1	1	48	1807	S1092	1	1	0	1
22	1164	S249	1	1	1	1	49	1603	S1092	1	1	0	1
23	1078	S249	0	0	1	0	50	1483	S1092	1	1	1	1
24	3501	S1083	0	1	0	1	51	1437	S1092	0	1	1	1
25	2671	S1083	0	1	1	1	52	1382	S1092	1	1	1	1
26	2151	S1083	0	1	1	1	53	1342	S1092	0	0	1	1
27	1733	S1083	1	1	1	1							

polymerase chain reaction were separated by high-resolution gel electrophoresis using 1.4% agarose gels with a drop (0.5 µg/ml) of ethidium bromide (EB) used as staining agent. The gels were run in Submarine Agarose Unit containing a 15 x 10-cm tray. Initially, 1 µl of mobility marker was placed on cellophane paper and 20 µl of the amplified products added, mixed and loaded into each capillary well of the prepared gel. The electrophoresis gadget was connected to a direct current (D. C) source and lasted for 90 minutes at 100 V and 20 mA. The buffer used in the gel was 0.5 x TBE while 15 µl λ DNA/Hind III + EcoR I was used in the same gel as internal molecular marker to estimate the size of the amplified products. Following electrophoresis, gels were visualized under UV trans-illuminator and the RAPD profiles were examined with the Kodak Digital Camera (DC120). Scoring of band of each individual in all the populations as well as in pooled population DNAs were performed based on the presence/absence of the amplified bands. Bands present were classified as 1, absence of band denoted with zero and unclear bands were assumed negligible. After complete cycles, the MS-PCR products were denatured at 94^{o}C for 600 s in the PCR Hybaid system, transferred to ice-box, chilled at 0^{o}C and loaded into prepared gel containing 12% polyacrylamide solution (PAGE) made up of 210 g of 6 M urea, acrylamide and N'N'-methylene bisacrylamide (19:1) added together

Table 4: Band-sharing coefficient on marker basis, their means and heterozygosity (H) among four port-city chicken population pooled DNAs using random markers

Pairwise populations	BSC of each marker					$\overline{BSC}$ ± S.E	q	H
	S246	S249	S1083	S1088	S1092			
Rugao vs Jiangchun	0.8696	0.6667	0.8182	0.8750	0.8889	0.8237±0.04	0.5801	0.5915
Rugao vs Wan-Nan	0.7059	0.5714	0.7368	0.8750	0.5000	0.6778±0.06	0.4324	0.7242
Rugao vs Cshiqishi	0.7368	0.6667	0.8182	0.8235	0.8000	0.7690±0.03	0.5194	0.6492
Jiangchun vs Wan-Nan	0.6667	0.8889	0.8571	0.8889	0.6667	0.7937±0.05	0.5458	0.6247
Jiangchun vs Cshiqishi	0.8000	1.0000	1.0000	0.9474	0.9091	0.9313±0.03	0.7379	0.4153
Wan-Nan vs Cshiqishi	0.8571	0.8889	0.8571	0.8421	0.8000	0.8490±0.01	0.6114	0.5597

Table 5: Microsatellite markers selected for the present study (annealing temperature (^{0}C), allele size range and total number of alleles in the whole populations)

Marker (s)	Annealing temperature	Allele size range (bp) observed	Rare alleles	Total alleles across populations
ADL 185	52	156-178 (22 bp)	0	8
ADL 201	55	144-168 (24 bp)	0	4
ADL 0292	60	130-160 (30 bp)	2	12
MCW0039	58	146-164 (18 bp)	2	7
MCW0058	60	177-218 (41 bp)	2	14
MCW0085	56	292-324 (32 bp)	2	9
MCW120	55	265-330 (65 bp)	0	16
MCW0328	55	229-303 (74 bp)	3	8
LEI0066	55	311-348 (37 bp)	0	4
LEI0094	58	209-240 (31 bp)	2	6
LEI0166	55	259-300 (41 bp)	1	7
ADL136	52	144-190 (46 bp)	0	7
MCW145	58	226-260 (34 bp)	0	7
ADL0226	58	198-202 (4 bp)	0	2
ADL166	55	149-157 (8 bp)	0	5
Total	-	(507 bp)	14	116
Mean	-	(33.80±4.68 bp)	0.93±0.28	7.73±0.95

with TBE buffer containing Tris and boric acid (2:1) together with 2 ml EDTA and were standardized with sterilized water. Exactly 25 µl of 30% TEMED ($C_6H_{16}N_2$) and 450 µl of 10% ammonium persulphate (AP) were added to each 60 ml of PAGE solution to serves as cross-links, then mixed and carefully poured into the gel cassettes made up of 20 x 20-cm double-glass plates clamped together by iron-clips. Thereafter, two plastic combs, each with 26 flat-teeth wells were inserted into the upper part of the glass. The thickness of the gel used was 0.4 mm. Before loading the amplified PCR-products, 1 µl of loading dye was placed on a tray and 10 µl each of the product was added, mixed and loaded to each lane in each of the glass trough. The middle lane in each of the trough was loaded with commercial internal size standard containing 4 µl loading dye mixed with 1 µl of pBR322 DNA/MspI marker to determine the size of the amplified products. Buffer 1 x TBE was placed in the glass trough to run the gel and electrophoresis lasted for 6 hours at 100 V, 10 mA, with a drop of ethidium bromide (EB) used as staining agent before visualization of the products under UV trans-illuminator, photographed and Genotyper (version 2.0) DNA fragment analysis software (Eastman Kodak) was used for the gel analyses.

Statistical analysis: Band-sharing coefficient (BSC) in each chicken population and between populations were determined considering all the informative random markers. Standard genetic distance (D_{ij}) between populations estimated. Allele frequencies of the microsatellite markers were obtained by direct gene counting method and used to compute the Shannon information index (P) of each locus in each chicken population as well as the (D_{ij}) between populations. Phylogenetic trees using the D_{ij} estimates for combinations of populations were constructed using Sandra (2003) method.

Results

The five informative random markers selected (S246, S249, S1083, S1088 and S1092) produced satisfactory results with individual template DNAs with fragments ranged from 835 to 5033 base pairs (bp); and ranged

Table 6: Shannon information index (P) for each locus in each of the four port-city chicken populations

Locus	Rugao	Jiangchun	Wan-Nan	Cshiqishi
ADL185	2.5285	2.5144	2.5123	2.3796
ADL201	1.1672	1.5038	1.3513	1.5985
ADL0292	2.3237	2.5948	2.5394	1.8077
MCW0039	1.6213	1.8956	0.4137	2.0292
MCW0058	2.9255	2.0972	3.0589	3.2441
MCW0085	2.5236	2.9878	2.5857	2.2225
MCW120	3.2719	3.5579	5.1488	3.6445
MCW0328	1.8418	1.9373	0.7382	1.5297
LEI0066	1.5535	1.5354	1.4118	1.7884
LEI0094	1.7029	1.8553	1.7812	0.9799
LEI0166	2.3882	1.5168	1.9622	1.7282
ADL136	2.6956	2.4535	2.3780	2.3254
MCW145	2.3600	1.8550	2.4392	2.2949
ADL0226	0.0000	0.9413	0.9183	1.0000
ADL166	1.8341	1.5383	1.6395	1.5235
Mean±S.E	2.0492±0.20	2.0523±0.17	2.0586±0.29	2.0064±0.18

from 840 to 4983 bp with pooled DNAs of the four port-city chicken populations. In the band patterns produced both in the individual and pooled DNAs amplifications, there were distinct differences in the population in number and sizes of the amplified fragments with the markers. Using the amplified bands (data unpublished), band-sharing coefficient (BSC) were calculated on marker basis for each population considering all comparisons between individuals in same population and the mean of this parameter for each population obtained. The BSC of the population ranged from 0.8928±0.02 (Rugao) to 0.9497±0.01 (Jiangchun), respectively. Using the generated mean band-sharing coefficients, the average frequency of band denoted as 'q' was determined for each population. With the known value of 'q' in each population, the heterozygosity (H) for each chicken population estimated. Heterozygosity was highest (0.4933) in Rugao chicken population and the lowest (0.3664) recorded in Jiangchun chicken population, respectively. The results of these are summarized in Table 2. Using the same informative markers with the pooled population DNAs and scoring of bands in manner similar to those of the individual DNA samples, a total of 53 bands were detected. The highest number of band detected was 13, produced by markers S246 and S1083; while marker S1092 produced 6 bands, being the least number of bands generated by the informative markers. Table 3, summarizes the 53 bands obtained with the pooled DNAs.

With these bands, computed average band-sharing coefficient between population pairs ranged from 0.6778±0.06 (Rugao vs Wan-Nan) to 0.9313±0.03 (Jiangchun vs Cshiqishi) and heterozygosities in both cases were 0.4153 (Jiangchun vs Cshiqishi) and 0.7242 (Rugao vs Wan-Nan), respectively (Table 4). With the fifteen microsatellite markers, a total of 116 alleles across the four port-city chicken populations were obtained, 55 (47.41%) alleles were owned in every population and a total of fourteen uncommon (rare) alleles were observed across all the populations with an average of 0.93±0.28 per locus. The number of alleles per locus ranged from 2 produced by locus ADL0226 across all populations to 16 produced by locus MCW120. The mean number of alleles per locus calculated was 7.73±0.95. The size difference between the alleles observed within the loci ranged from 4 bp (ADL 0226) to 74 bp (MCW0328) with an average of 33.80±4.68 bp per locus (Table 5). The allele frequencies of each locus in each of the examined chicken population were used to compute the Shannon information index (P) and the mean of this among loci in each population was between 2.0064±0.18 (Cshiqishi) to 2.0586±0.29 (Wan-Nan) (Table 6).

The standard genetic distances (D_{ij}) based on the two-marker types differed from each other and varies between populations. Using the average band-sharing coefficient in population and in pairwise populations respectively, the D_{ij} calculated ranged from 0.0083 (Jiangchun vs Cshiqishi) to 0.2789 (Rugao vs Wan-Nan) chickens. The D_{ij} calculated from the microsatellite allele frequencies ranged from 0.2360 to 0.5072. The smallest distance was between Rugao and Jiangchun and the largest was found between Rugao and Cshiqishi, respectively. The D_{ij} of the random markers with the population pairs are presented below the diagonal, while that of the microsatellite markers are presented above the diagonal (Table 7). Using these values, the phylogenetic trees (Fig. 2: a, b) were constructed by using the minimum distance value between population pairs.

Discussion

The genetic information about chicken populations and

Table 7: Genetic distances (D_{ij}) based on random markers (below) and based on microsatellite markers (above) the diagonal obtained between the four port-city chicken populations

Population (s)	RC	JC	WC	CC
RC	0.0000	0.2360	0.2709	0.5072
JC	0.1115	0.0000	0.3785	0.3510
WC	0.2789	0.1520	0.0000	0.4600
CC	0.1689	0.0083	0.0733	0.0000

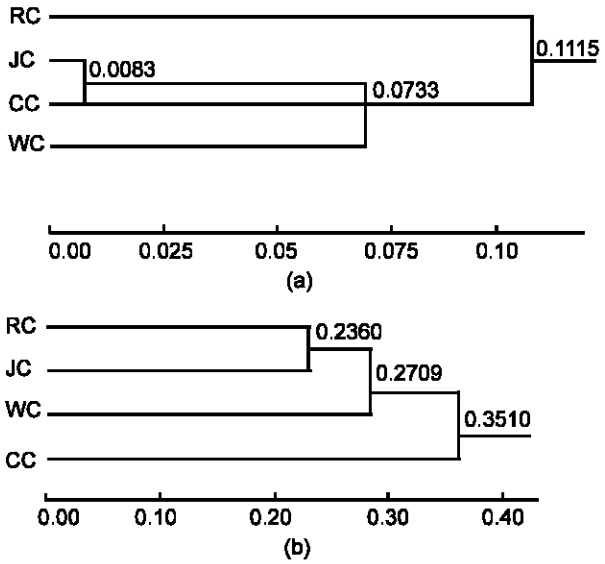

Fig. 2a,b: Phylogenetic trees based on (D_{ij}) of five random and fifteen microsatellite markers used with four port-city chickens in China

population relationships estimated by use of both random and microsatellite markers are not only useful as guide in defining objectives for designing future investigations of the genetic variations, but also suitable for conservation strategies. Data generated from the application of these two alternative markers can be used easily to develop phylogenies that allow farmers to perform more targeted breeding programs. The five random markers selected for this study (S246, S249, S1083, S1088 and S1092) generated reproducible bands that were quite informative about the genetic background of the port-city chicken populations, indicating that the conditions used in this study may be employed in future screening of random markers for use with chicken populations.

The informative markers produced amplifiable bands in both individual and pooled population DNAs. The band range of 840 to 4983 bp in the pooled DNAs were within those of the range 835 to 5033 bp obtained with the individual DNAs, confirming that the primers/markers selected were suitable and capable of revealing the status of both individual and pooled population DNAs. Murayama *et al.* (1999) using random markers in RAPD technique reiterated that the significance of random marker is insufficient until it is capable of amplifying individual and pooled DNA samples from same population. Based on the results of this study, the selected random markers were suitable for complete amplification of port-city chicken individual and pooled DNA samples, respectively. The patterns displayed by the markers in this work with the examined chickens could be significant source of DNA markers for the development of gene maps for the chickens as previously utilized by Levin *et al.* (1993); Cheng and Levin (1995) with chickens, respectively.

In this work, the band-sharing coefficients were high in both within (0.8928 to 0.9497) and between populations (0.6778 to 0.9313), respectively; and this may be the major reason responsible for the lower heterozygosities for both within and between populations. Higher band-sharing coefficients bring about lower heterozygosities or vice-versa, a similar phenomenon of this nature had previously been reported by Zhang *et al.* (2002) with chickens and Chen *et al.* (2002) using random markers with six other animal species. The highest genetic distance obtained with the use of the random markers in this work was 0.2789 (Rugao vs Wan-Nan), this is quite consistent with the highest genetic distance of 0.28 reported by Smith *et al.* (1996) using random markers in RAPD technique with chicken and turkey populations.

The fifteen microsatellite markers used were all highly polymorphic and the total allele produced by each locus across all populations ranged from 2 (ADL 0226) to 16 produced by MCW 120, with mean among loci across the four port-city chickens equals 7.73±0.95 (Table 5). The additive inverse of mean band-sharing coefficient to obtained genetic distances (Smith *et al.*, 1996; Chen *et al.*, 2002) with random markers data was used in this study and for the microsatellite markers, the Nei (1972) genetic distance approach was employed, because they were frequently used distance measures in population genetic studies. The present study revealed significant differences in the genetic distances between populations in the two alternative markers used leading to variations in the reconstructed phylogenies. Using allozymes, RAPD and microsatellites with chickens, Zhang *et al.* (2002) obtained different phylogenetic trees between populations. In a related work, Ponsuksili *et al.* (1999) used DNA fingerprinting and microsatellites with chickens and equally obtained different phylogenetic trees. In this work, the phylogenetic trees obtained from the use of both random and microsatellite markers with port-city chickens differed and population relationships deviate accordingly, confirming that the use of these markers (random and microsatellite) conformed to the observations of previous authors.

Generally when constructing phylogenetic tree, it is difficult to judge succinctly which is the best one with

regard to the genetic relationship of the examined populations. With the use of different criteria-morphological, biochemical and type II markers to determine genetic relationships and or distances, one has to expect to end up with different phylogenetic trees. However, the phylogenetic trees developed from the two-marker types used in this study provided enough information to predict the likely originator and the genetic distances existing between the four port-city chicken populations. The phylogenetic trees developed based on the data of the two-marker types differed, however, Jiangchun appeared to be likely the originator of the source of the examined chicken populations and Wan-Nan chicken was found to be intermediate among the populations in both genetic trees, indicating that this chicken population may had probably been derived from Rugao-Jiangchun or Jiangchun-Cshiqishi cross-breeding activities, and comparing the two types of the marker used, microsatellite markers produced allelism, allele frequencies and detect even rare alleles unlike random markers, therefore, microsatellite markers may be suitable molecular markers to appraise the genetic background of port-city chicken populations in the near future.

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