Estimation of the Cumulative Power of Discrimination in Haimen Chicken Populations with Ten Microsatellite Markers

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ABSTRACT : To estimate the cumulative power of discrimination (CPD) existing within Haimen chicken populations in China, we isolated a total of 252 genomic DNAs from four chicken populations (Rugao, Jiangchun, Wan-Nan and Cshiqishi) through a saturated salt procedure. All the genomic DNAs were used in a polymerase chain reaction (PCR) with ten microsatellite markers. Amplified PCR-products with the selected markers were separated on a 12% polyacrylamide gel with pBR322DNA/MspI used as internal standard marker. Genetic diversity indices including mean allele number among loci, unbiased heterozygosity (h_i) within locus, effective number of alleles (N_e) and polymorphism information content (PIC) as well as the unbiased average heterozygosity (H) among loci in the populations were calculated using the generated allele frequencies by each marker. The mean allele number for all loci ranged between 4.00±0.33 (Rugao) to 4.90±0.48 (Cshiqishi) and across populations for all loci was 4.60±0.20, while (H) ranged from 0.65±0.03 (Rugao) to 0.69±0.03 (Jiangchun) among loci and across populations, (H) was 0.67±0.01. The generated unbiased average heterozygosity among loci in each population was integrated to the global formula of CPD and the result demonstrated that the CPD within the four Haimen chicken populations was 98.75%. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1066-1070)***

Key Words : Estimation, Cumulative Power of Discrimination, Haimen Chickens, Markers

INTRODUCTION

Genetic markers in chickens are mostly molecular DNA markers, which have been classified into type I and type II, (Emara and Kim, 2003). While type I are genes with well established functions, type II are anonymous DNA segments. The use of type I nowadays is not as high as the most commonly used type II anonymous DNA markers. Included in the type II are the highly repetitive and polymorphic microsatellites, retrotransposon elements, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs) and many others. Among the type II markers, microsatellites have been widely used in examining population genetic structures of several species. Because of the relative ease of scoring and ability to exhibits high level of polymorphisms as well as higher heterozygosities, its application in population genetic structure is quite significant.

With chickens, several authors including Ponsuksili et al. (1999); Zhang et al. (2002) and Chen et al. (2002) have tested the usefulness of these microsatellite markers. Today, determination of heterozygosity and genetic distances based on microsatellite analysis is regarded as the most convenient method and can be used with any species population. Recent information in literature have revealed that microsatellites are useful in determining not only heterozygosity and estimating genetic distances among closely related species (Chen et al., 2004), it is also suitable for determining genetic indices such as number of effective allele as well as the polymorphism information content in population. Bartfai et al. (2003), Shen (2004) and Wu (2004) have equally used the microsatellite markers to determine the rare alleles in many populations including chickens. These markers can in addition be used to generate data suitable for the estimation of cumulative power of discrimination of any population including the avian species, though, information on this are still scanty in literature. In the current investigation however, we have amplified chicken genomic DNAs, determine the genetic diversity indices in the chicken populations under consideration and integrate the unbiased average heterozygosity value in each population into the global formula of cumulative power of discrimination (CPD) to generate this important parameter. The objective of this study was therefore to estimate the CPD in Haimen chicken populations using ten microsatellite markers.

MATERIALS AND METHODS

Study location

The sample collection was carried out in Haimen Integrated Poultry Company and analyses were carried out at the research laboratory of the Genetics and Breeding Unit, Yangzhou University, Jiangsu Province, East China, between April, 2003 to June, 2004. The sample size by population were Rugao yellow chicken (RYC) 62, Jiangchun yellow chicken (JYC) 62, Wan-Nan yellow chicken (WYC) 63 and Cshiqishi yellow chicken (CYC) 65,

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respectively. The birds were caught from cages, placed on flat table and the veins within their wings were searched for and using heparinzed 13 mm, 27 gauge needle, blood from each chicken was collected into 10 ml haemotocrit tubes containing sequestering agent (EDTA), transferred to laboratory and frozen at -80°C.

DNA isolation, PCR protocol and electrophoresis

Individual DNA was isolated from 2 ml of chicken blood collected using exactly the saturated salt method previously described by Miller et al. (1988). Concentration of the DNA solution was measured based on the micro-gel method (Sambrook et al., 1989) and each DNA was adjusted to 100 ng/µl. Genotyping of DNA samples at the ten selected microsatellite markers were carried out using the isolated DNA from chickens of different populations. The composition of the PCR reaction mixture with the final volume of 25 µl included 1 µl template DNA, 2.5 µl of 10×PCR Buffer, 1 µl of 25 mM dNTPs, 1 µl of each (8 pmol/µl) forward and reverse form of the primers and 0.2 µl of 5 U/µl Taq DNA polymerase (Sangon Company, Shanghai, China) with 2.2 µl of 25 mmol/L $MgCl_2$ and 16.1 µl sterilized distilled water added. The reaction programme carried out in Perkin Elmer-PCR Hybaid Express System (PE 9600) was at (94 °C, 300 s), 35 cycles at (94°C, 60 s), 60 s at annealing temperatures ranged between 56-65°C and (at 72°C, 60 s) with final extension step at (72°C, 600 s). Following complete cycles, the PCR-products were heat-denatured for another 600 s in the PCR system and thereafter transferred to the ice-box, chilled at 0°C and loaded into the gel containing 12% polyacrylamide solution (7 M urea, acrylamide and N'N'methylene bisacrylamide) and 25 µl of 30% TEMED [$C_6H_{16}N_2$] and 450 µl of 10% ammonium tetraoxosulphate (vi) [$(NH4)_2SO_4$], were added to the mixture and carefully poured into the gel cassettes made up of 20×20 cm double-glass plates clamped together by iron-clips. Before loading of the amplified products, 1 µl of blue loading dye was placed on a tray and 10 µl of the amplified product added, mixed carefully and loaded to each lane of the glass trough with pBR322DNA/Msp I used as internal marker to determine the size of the amplified products. PCR-products were separated on polyacrylamide gel electrophoresis which lasted for 06:00 h 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 genotype 2.0 software (EASTMAN KODAK DIGITAL SCIENCE) was used for all analyses.

Statistical analyses

Based on the microsatellite markers selected and the generated allele frequencies, the proportion of homozygosity was depicted as $H_o = \sum P_i^2$, where P_i represents allele frequencies per locus. Effective allele number (N_e) was obtained as inverse of H_o and $N_{em} = \sum N_e/r$, where, N_{em} is the among loci average effective allele number and 'r' represents loci number. The unbiased heterozygosity of each locus was determined as $h_i = 1-H_o$. The unbiased average heterozygosity, according to Zhang et al. (2002) and Chenyambuga et al. (2004) was expressed as: $H = \sum h_i/r = (1-\sum P_i^2)/r$, where 'r' is as earlier defined. Cumulative power of discrimination (CPD) according to Fan et al. (2002) was examined as : $CPD = (1-[(1-H_a)(1-H_b)(1-H_c)(1-H_d)])\times 100\%$, where H_a-----H_d, represents among loci unbiased average heterozygosity in each population, while mean polymorphism information content of loci (PIC_m) was determined using Botstein et al. (1980) relation: $PIC_m = \sum[(1-\sum P_i^2)- \sum\sum 2P_i^2 P_j^2]/r$.

RESULTS

The allele frequencies (data unpublished) obtained by the microsatellite markers were used to determine the within locus (h_i) heterozygosity, effective number of alleles (N_e) and polymorphism information content (PIC) among the respective populations. The mean allele number among loci ranged from 4.00±0.33 (Rugao) to 4.90±0.48 (Cshiqishi) and it was 4.60±0.20 across populations (Table 1).

Among the loci used, only two loci (MCW150 and MCW0067) produced same number of alleles (4 each) in different chicken populations, while others produced same allele numbers in two or three populations but not completely same in all the populations. Across populations, the highest mean number of alleles 6.25±1.03 was produced by locus MCW0183 and the lowest value of 2.50±0.50 was produced by locus MCW0294. In all the populations, the among loci heterozygosities were very high. The highest unbiased average heterozygosity of 0.69±0.03 among loci was observed in Jiangchun and the least 0.65±0.03 was recorded for Rugao chicken population. Across populations, the unbiased average heterozygosity was 0.67±0.01. In all the ten markers used, there were distinct differences in the allele frequencies, which resulted in variable heterozygosity values. Integrating the calculated unbiased average heterozygosity in each chicken population to the general formula of cumulative power of discrimination (CPD) gave a value of 98.75%.

DISCUSSION

Several authors have reported the beneficial values of microsatellite markers in several population studies. The main interest of this work was to appraise further the

additional usefulness of microsatellite markers with chickens in China whose genetic information have not been included in the common chicken populations. Based on the results of this investigation, the generated mean allele number across populations ranged from 2.50 (MCW0294) to 6.25 (MCW0183) indicating that the microsatellite markers used were variable and polymorphic with Haimen chicken populations. The observed mean allele number among loci in each of the chicken population as well as the unbiased average heterozygosities in this work were consistent with what was reported by Barker (1994) and Wimmers et al. (2000), respectively. Apart from two loci MCW150 and MCW0067 that produced regular allele number in all the populations, others used in this work produced irregular allele distribution indicating the markers have complex structure as earlier reported by Vanhala et al. (1998). Number of alleles per locus and heterozygosity measures provide good information about genetic variation in population (Nei, 1987; Yang et al., 2004) and heterozygosity in any population can be estimated using microsatellite markers (Ponsuksili et al., 1999; Bartfai et al., 2003). In this work, the estimated unbiased average heterozygosities produced by the markers were very high and ranged from 0.65 to 0.69, a similarly very high heterozygosities in Chinese chicken populations with microsatellites had equally been reported by Zhang et al. (2002). With the same markers used in this study and ten additional ones with nine other Chinese chicken populations. Shen (2004) had reported average heterozygosities ranged between 0.64 to 0.70, this work does not differ much from his results, however, results of the unbiased average heterozygosities in this work were higher than what was reported by Wu (2004), the variation in results may be as a result of differences in location, different sample size and completely different experimental chickens.

A cumulative power of discrimination calculated in any species population and goes beyond 50% is an indication of high level of variation in populations (Sano et al., 1997; Fan et al., 2002). In this work, we observed a CPD of 98.75% showing clearly that there is high genetic variations among the Haimen chicken populations tested.

Table 1a. Genetic information in the Haimen chicken populations with the ten microsatellite markers

		Breeds/populations*				
Marker(s)	Observed features	RYC (62)	JYC (62)	WYC (63)	CYC (65)	Across populations mean±SE
MCW0295	Allele number	5	5	4	5	4.75±0.13
	Allele size range (bp)	80-112	80-96	80-101	80-112	-
	Range of frequencies	0.04-0.40	0.02-0.40	0.15-0.35	0.01-0.38	-
	h_j	0.69	0.72	0.73	0.73	0.72±0.01
	N_e	3.23	3.57	3.70	3.70	3.55±0.03
	PIC	0.64	0.67	0.67	0.68	0.67±0.00
MCW0294	Allele number	2	4	2	2	2.50±0.50
	Allele size range (bp)	318-326	303-345	323-366	323-366	-
	Range of frequencies	0.50	0.04-0.46	0.50	0.50	-
	h_j	0.50	0.58	0.50	0.50	0.52±0.02
	N_e	2.00	2.38	2.00	2.00	2.10±0.10
	PIC	0.38	0.49	0.38	0.38	0.41±0.03
MCW0183	Allele number	4	6	9	6	6.25±1.03
	Allele size range (bp)	281-346	281-346	278-356	281-346	-
	Range of frequencies	0.03-0.52	0.01-0.42	0.01-0.33	0.01-0.37	-
	h_j	0 .55	0.72	0.74	0.69	0.68±0.04
	N_e	2.22	3.57	3.85	3.23	3.22±0.36
	PIC	0.45	0.68	0.70	0.63	0.62±0.06
MCW150	Allele number	4	4	4	4	4.00±0.00
	Allele size range (bp)	211-267	211-267	211-267	211-267	-
	Range of frequencies	0.23-0.27	0.22-0.28	0.18-0.32	0.24-0.25	-
	h_j	0.75	0.75	0.73	0.75	0.75±0.01
	N_e	4	4	3.70	4	3.93±0.08
	PIC	0.70	0.70	0.68	0.70	0.70±0.01
MCW145	Allele number	4	5	6	5	5.00±0.41
	Allele size range (bp)	186-214	186-214	186-218	186-218	-
	Range of frequencies	0.15-0.35	0.02-0.43	0.02-0.44	0.05-0.33	-
	h_j	0.71	0.64	0.61	0.73	0.67±0.03
	N_e	3.45	2.78	2.56	3.70	3.12±0.27
	PIC	0.66	0.57	0.53	0.69	0.61±0.04

Table 1b. Genetic information in the Haimen chicken populations with the ten microsatellite markers

		Breeds/populations*				
Marker(s)	Observed features	RYC (62)	JYC (62)	WYC (63)	CYC (65)	Across populations mean±SE
MCW134	Allele number	4	4	6	4	4.50±0.50
	Allele size range (bp)	275-311	275-311	275-329	275-311	-
	Range of frequencies	0.19-0.31	0.19-0.31	0.01-0.44	0.01-0.49	-
	h_j	0.74	0.74	0.61	0.52	0.65±0.05
	N_e	3.85	3.85	2.56	2.08	3.09±0.45
	PIC	0.69	0.69	0.53	0.40	0.58±0.07
MCW120	Allele number		6	5	7	6.00±0.41
	Allele size range (bp)	265-305	265-305	261-312	261-312	-
	Range of frequencies	0.02-0.46	0.11-0.22	0.05-0.44	0.01-0.38	-
	h_j	0.57	0.82	0.64	0.70	0.68±0.05
	N_e	2.33	5.56	2.78	3.33	3.50±0.72
	PIC	0.47	0.80	0.57	0.65	0.62±0.07
MCW104	Allele number	3	4	3	7	4.25±0.95
	Allele size range (bp)	115-209	115-245	115-209	115-209	-
	Range of frequencies	0.04-0.46	0.01-0.49	0.07-0.43	0.01-0.37	-
	h_j	0.53	0.60	0.56	0.71	0.60±0.04
	N_e	2.13	2.50	2.27	3.45	2.59±0.30
	PIC	0.42	0.52	0.46	0.66	0.52±0.05
MCW0067	Allele number	4	4	4	4	4.00±0.00
	Allele size range (bp)	164-193	164-193	164-193	159-187	-
	Range of frequencies	0.12-0.38	0.05-0.45	0.16-0.34	0.11-0.39	-
	h_j	0.68	0.59	0.73	0.67	0.67±0.03
	N_e	3.13	2.44	3.70	3.03	3.08±0.26
	PIC	0.62	0.50	0.68	0.61	0.60±0.04
MCW32	Allele number	4	5	5	5	4.75±0.13
	Allele size range (bp)	269-334	269-354	269-354	269-354	-
	Range of frequencies	0.20-0.30	0.12-0.27	0.11-0.37	0.03-0.44	-
	h_j	0.74	0.78	0.75	0.61	0.72±0.04
	N_e	3.85	4.55	4.00	2.56	3.74±0.42
	PIC	0.69	0.75	0.71	0.50	0.66±0.06
Among loci	Mean allele number±SE	4.00±0.33	4.70±0.26	4.80±0.61	4.90±0.48	4.60±0.20
	N_{em}±SE	3.02±0.25	3.52±0.33	3.11±0.24	3.11±0.22	3.19±0.11
	H±SE	0.65±0.03	0.69±0.03	0.66±0.03	0.66±0.02	0.67±0.01
	PIC_m±SE	0.57±0.04	0.64±0.03	0.59±0.04	0.59±0.04	0.60±0.02
	CPD (%)	-	-	-	-	98.75%**

h_j, N_e, PIC, H, N_{em}, PIC_m, CPD, RYC, JYC, WYC and CYC were as earlier defined.

* Sample size in different populations in parentheses.

** See Materials and Methods for the formula used for computation.

CONCLUSION

The results of this experiment revealed that microsatellite markers is/are sufficient enough as molecular marker to estimate all the genetic information in species populations. This survey apart from showing the within and among chicken population genetic information, it revealed the cumulative power of discrimination among the four Haimen chicken populations in China to be exactly 98.75%.

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