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# Measurement of Genetic Parameters Within and Between Haimen Chicken Populations Using Microsatellite Markers

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**Abstract:** A total of 240 genomic DNAs were isolated from the four Haimen chicken populations: Rugao, Jiangchun, Wan-Nan and Cshiqishi, through a saturated salt procedure. The samples were used in a polymerase chain reaction (PCR) with 15 microsatellite markers. Amplified PCR-products with the markers were separated on a 12% polyacrylamide gel. Genetic parameters measured included allele number within locus per population, mean allele number across populations, mean allele number among loci for each population, effective allele number for each locus, mean across populations and among loci effective number of alleles ($\overline{Ne}$). Polymorphism information content (PIC) per locus and for all loci obtained as well as the average heterozygosity (H) among loci in the populations. The mean allele number for all loci ranged between 5.73±0.85 (Cshiqishi) to 6.00±0.74 (Rugao) and 6.00±0.84 (Jiangchun) with across populations for all loci equals 5.88±0.06; while (H) ranged from 0.6486±0.06 (Wan-Nan) to 0.7017±0.03 (Jiangchun) among loci and across populations, (H) was 0.6828±0.01. The ($\overline{Ne}$) ranged from 3.96±0.60 (Wan-Nan) to 4.11±0.47 (Rugao); and ($\overline{PIC}$) have values between 0.6068±0.06 (Wan-Nan) to 0.6509±0.04 (Jiangchun). The average heterozygosity among loci in each population was used further to obtained the cumulative power of discrimination (CPD) and was 99%. Angular genetic distances ($D_A$) calculated ranged between 0.1691 (Rugao vs Wan-Nan) to 0.3372 (Rugao vs Cshiqishi). Dendrogram developed linked Rugao and Jiangchun as closely related, Wan-Nan been intermediate and Cshiqishi distantly related. It was concluded that the markers were suitable for the measurement of all genetic parameters of Haimen chicken populations.

**Key words:** Haimen chicken populations, measurement, genetic parameters, microsatellites

## Introduction

Microsatellite markers are now been widely used in the genetic appraisal of several species populations including chickens. Because of the relative ease of scoring and ability to exhibits high level of polymorphisms as well as higher heterozygosities, its application as genetic appraisal tool is quite significant. Recent information in literature have revealed that microsatellite markers are useful in determining not only heterozygosity and estimating genetic distances among closely related species (Chen *et al*., 2004), it is also suitable for measurement of genetic parameters such as number of effective allele as well as the polymorphism information content in population and can detect rare alleles (Bartfai *et al*., 2003; Shen, 2004). 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 (Olowofeso *et al*., 2005). Haimen chickens are relatively in abundance in China and almost nothing is known about their genetic information. Because of the rich genetic diversity of the available chicken species in this region of China and for total conservation studies now and in the near future, it is therefore germane to measure the genetic indices of all the chicken populations whose information on them are scanty in literature with current markers in vogue in the avian industry so that generated results can be integrated to future poultry sector for conservation purposes. The objective of this study was therefore to measure all the genetic parameters both within and between Haimen chicken populations using microsatellite markers.

## Materials and Methods

**Blood collection, study location and sample size:** Blood samples were collected in Haimen Integrated Poultry Company, Jiangsu, China, by brachial venipuncture aseptically into haemotocrit tubes using heparinzed 13mm, 27 gauge needle with ethylenedi-amine-tetra acetic acid (EDTA) and heparin used as anticoagulants. Approximately 1ml of blood was collected from each of the bird into 1.5ml microfuge tubes, transferred to the laboratory and frozen at -80°C, before DNA extraction. Analyses were carried out at the research laboratory of

the Genetics and Breeding Unit, Yangzhou University, Jiangsu Province, China, between January, 2004 to December, 2004. The sample size by population were Rugao (60), Jiangchun (60), Wan-Nan (60) and Cshiqishi (60), respectively.

**DNA isolation, PCR protocol and electrophoresis:** Individual DNA was isolated from the 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 100ng/µl. Genotyping of DNA samples at the fifteen microsatellite markers were carried out using the isolated DNAs from chickens of different populations. The sequences of the microsatellite markers were replica of those used by Olowofeso (2005) and coded ADL 136, ADL166, ADL185, ADL201, ADL0226, ADL0292, LEI0066, LEI0094, LEI0166, MCW0039, MCW0058, MCW0085, MCW120, MCW145 and MCW0328, respectively. PCR reaction mixture with the final volume of 25µl included 1µl template DNA, 2.5µl of 10 x PCR Buffer, 1µl of 25mM dNTPs, 1µl of each (8pmol/µl) forward and reverse form of the primers and 0.2µl of 5U/µl Taq DNA polymerase (Sangon Company, Shanghai, China) with 2.2µl of 25mmol/l $MgCl_2$ and 16.1µl sterilized distilled water added. The reaction programme carried out in PCR Hybaid Touchdown Express System (PE 9600) was at (94°C, 300s), 35 cycles at (94°C, 60s), 60s at annealing temperatures ranged between 52-60°C and (at 72°C, 60s) with final extension step at (72°C, 600s). Thereafter, PCR-products were heat-denatured for another 600s in the PCR system and transferred to an ice-box, chilled at 0°C and loaded into the gel containing 12% polyacrylamide solution. Exactly 1µl of blue loading dye was placed on a tray and 10µl of the amplified product added, mixed and loaded to each lane of the glass trough with pBR322 DNA/MspI used as internal marker for sizing. The 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 2.0 software (EASTMAN KODAK DIGITAL SCIENCE DC120) was used for gel analyses.

**Statistical analysis:** The microsatellites data obtained with the four Haimen chicken populations were used for the measurement of genetic parameters of the populations. Allele frequencies (F), homozygosity ($H_i$), effective allele number ($N_e$) with its mean, heterozygosity of each locus ($h_i$), average heterozygosity among loci (H), polymorphism information content (PIC), its mean among loci and the cumulative power of discrimination (CPD) were the genetic parameters measured using the following formulae:

$$F = \frac{A}{2T}\ ;\ H_i = \sum_{i=1}^{n} Pi^2\ ;\ N_e = \frac{1}{H_i} = \frac{1}{\sum_{i=1}^{n} Pi^2} \text{ and } \overline{N}_e = \sum_{i=1}^{n} \frac{N_e}{r}$$

$$h_i = 1 - H_i = 1 - \sum_{i=1}^{n} Pi^2 \geq 1\ ;\ H = \sum_{i=1}^{n} \frac{1 - H_i}{r} = \sum_{i=1}^{n} \frac{1 - \sum_{i=1}^{n} Pi^2}{r} = \sum_{i=1}^{n} \frac{h_i}{r}$$

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - 2\sum_{i=1}^{n}\sum_{i=1}^{n} (P_i^2 P_j^2)\ :\ \overline{PIC} = \sum_{i=1}^{n} \frac{PIC}{r} \text{ and}$$

$$CPD = [1-(1-H_a)(1-H_b)(1-H_c)(1-H_d)] \times 100$$

where $H_a$---$H_d$, represents among loci average heterozygosity in each population and r, A, T and $P_i$ were loci number, allele number, population sample size and allele frequencies, respectively. A DISPAN (Ota, 1993) software was used to obtained the angular genetic distances ($D_A$) between population pairs. The results with this software were used to construct the dendrogram by Neighbour-joining (NJ) systematic clustering analysis.

## Results

**Allele number, heterozygosity, effective number of alleles and polymorphism information content:** The allele frequencies were the unpublished data obtained by Olowofeso (2005). The estimates of heterozygosity at different loci between populations showed large variation. The average heterozygosity (H) were obtained as among loci heterozygosities. Among loci heterozygosities were quite high and ranged from 0.6486±0.06 (Wan-Nan) to 0.7017±0.03 (Jiangchun). Across populations, the average heterozygosity was 0.6828±0.01. In all the microsatellite loci, there were distinct differences in the allele frequencies, which resulted in variable heterozygosity values. Effective number of alleles ($N_e$) and polymorphism information content (PIC) were obtained using the allele frequencies data for each locus in each population and across populations, respectively. The mean of these parameters calculated among loci for each population and across populations. The mean effective a llele number ($\overline{Ne}$), ranged from 3.96 ± 0.60 (Wan-Nan) to 4.11±0.47 (Rugao) among loci, and across populations, it was 4.05±0.03; while the mean polymorphism information content ($\overline{PIC}$) among loci was highest for Jiangchun population (0.6509±0.04), lowest for Wan-Nan population (0.6068±0.06) and across populations, it was 0.6355±0.01. The cumulative power of discrimination (CPD) across all populations was 99%. The generated results in each chicken population are summarized in Table 1.

**Genetic distances:** Using the allele frequencies data, the angular genetic distances ($D_A$) between population pairs were carried out and the results obtained with DISPAN software are presented in Table 2. The smallest genetic distance was obtained between Rugao vs

Table 1: Genetic parameters measured in the Haimen chicken populations with 15 microsatellite loci[a]

| *Locus | Observed features | Breeds/populations | | | | |
|---|---|---|---|---|---|---|
| | | Rugao (60) | Jiangchun (60) | Wan-Nan (60) | Cshiqishi (60) | Across populations mean± S.E |
| ADL 185 | Allele number | 7 | 7 | 8 | 7 | 7.25±0.22 |
| | Allele size range (bp) | 158-178 | 156-174 | 156-178 | 156-174 | - |
| | Range of frequencies | 0.03-0.28 | 0.04-0.28 | 0.02-0.28 | 0.01-0.27 | - |
| | $h_i$ | 0.8070 | 0.7978 | 0.7972 | 0.7875 | 0.7974±0.00 |
| | $N_e$ | 5.18 | 4.95 | 4.93 | 4.71 | 4.94±0.01 |
| | PIC | 0.7798 | 0.7698 | 0.7679 | 0.7545 | 0.7680±0.01 |
| ADL201 | Allele number | 3 | 4 | 3 | 4 | 3.50±0.25 |
| | Allele size range (bp) | 144-164 | 144-168 | 144-164 | 144-168 | - |
| | Range of frequencies | 0.03-0.54 | 0.05-0.54 | 0.17-0.61 | 0.03-0.52 | - |
| | $h_i$ | 0.5248 | 0.5873 | 0.5516 | 0.6270 | 0.5727±0.02 |
| | $N_e$ | 2.10 | 2.42 | 2.23 | 2.68 | 2.36±0.11 |
| | PIC | 0.4178 | 0.5175 | 0.4908 | 0.5649 | 0.4978±0.03 |
| ADL0292 | Allele number | 7 | 11 | 7 | 4 | 7.25±1.24 |
| | Allele size range (bp) | 140-160 | 130-158 | 140-160 | 133-140 | - |
| | Range of frequencies | 0.01-0.34 | 0.01-0.41 | 0.03-0.31 | 0.08-0.38 | - |
| | $h_i$ | 0.7701 | 0.7579 | 0.8033 | 0.6900 | 0.7553±0.02 |
| | $N_e$ | 4.35 | 4.13 | 5.08 | 3.23 | 4.20±0.33 |
| | PIC | 0.7355 | 0.7305 | 0.7769 | 0.6317 | 0.7187±0.03 |
| MCW0039 | Allele number | 4 | 4 | 2 | 5 | 3.75±0.54 |
| | Allele size range (bp) | 146-160 | 158-164 | 148-160 | 150-164 | - |
| | Range of frequencies | 0.02-0.41 | 0.13-0.38 | 0.08-0.92 | 0.03-0.35 | - |
| | $h_i$ | 0.6524 | 0.7153 | 0.1527 | 0.7340 | 0.5636±0.12 |
| | $N_e$ | 2.88 | 3.51 | 1.18 | 3.76 | 2.83±0.50 |
| | PIC | 0.5808 | 0.6633 | 0.1410 | 0.6866 | 0.5179±0.02 |
| MCW0058 | Allele number | 10 | 5 | 11 | 11 | 9.25±1.24 |
| | Allele size range (bp) | 185-217 | 205-218 | 177-213 | 179-215 | - |
| | Range of frequencies | 0.03-0.28 | 0.07-0.40 | 0.01-0.26 | 0.03-0.20 | - |
| | $h_i$ | 0.8399 | 0.7357 | 0.8566 | 0.8810 | 0.8283±0.03 |
| | $N_e$ | 6.25 | 3.78 | 6.97 | 8.40 | 6.35±0.84 |
| | PIC | 0.8218 | 0.6954 | 0.8417 | 0.8698 | 0.8072±0.03 |
| MCW0085 | Allele number | 7 | 9 | 7 | 7 | 7.50±0.43 |
| | Allele size range (bp) | 292-316 | 292-324 | 292-316 | 292-316 | - |
| | Range of frequencies | 0.04-0.29 | 0.01-0.18 | 0.05-0.24 | 0.01-0.33 | - |
| | $h_i$ | 0.8016 | 0.8672 | 0.8160 | 0.7485 | 0.8083±0.02 |
| | $N_e$ | 5.04 | 7.53 | 5.43 | 3.98 | 5.50±0.64 |
| | PIC | 0.7742 | 0.8524 | 0.7904 | 0.7083 | 0.7813±0.03 |
| MCW120 | Allele number | 13 | 15 | 13 | 15 | 14.00±0.50 |
| | Allele size range (bp) | 265-328 | 265-330 | 270-330 | 267-330 | - |
| | Range of frequencies | 0.01-0.18 | 0.01-0.16 | 0.01-0.15 | 0.01-0.16 | - |
| | $h_i$ | 0.8811 | 0.9017 | 0.9011 | 0.9092 | 0.8983±0.01 |
| | $N_e$ | 8.41 | 10.17 | 10.11 | 11.01 | 9.93±0.47 |
| | PIC | 0.8693 | 0.8936 | 0.8927 | 0.9022 | 0.8895±0.01 |
| MCW0328 | Allele number | 4 | 4 | 2 | 3 | 3.25±0.52 |
| | Allele size range (bp) | 256-303 | 256-303 | 229-232 | 232-238 | - |
| | Range of frequencies | 0.08-0.36 | 0.18-0.35 | 0.21-0.79 | 0.26-0.47 | - |
| | $h_i$ | 0.7055 | 0.7282 | 0.3298 | 0.6398 | 0.6008±0.08 |
| | $N_e$ | 3.40 | 3.68 | 1.49 | 2.78 | 2.84±0.42 |
| | PIC | 0.6481 | 0.6786 | 0.2754 | 0.5678 | 0.5425±0.08 |
| LEI0066 | Allele number | 4 | 4 | 4 | 4 | 4.00±0.00 |
| | Allele size range (bp) | 311-348 | 311-348 | 311-348 | 311-348 | - |

Contd.

| *Locus | Observed features | Breeds/populations | | | | |
|---|---|---|---|---|---|---|
| | | Rugao (60) | Jiangchun (60) | Wan-Nan (60) | Cshiqishi (60) | Across populations mean± S.E |
| Range of frequencies | | 0.06-0.49 | 0.03-0.48 | 0.02-0.48 | 0.08-0.40 | - |
| | $h_i$ | 0.6086 | 0.6098 | 0.5807 | 0.6828 | 0.6205±0.02 |
| | $N_e$ | 2.55 | 2.56 | 2.38 | 3.15 | 2.66±0.15 |
| | PIC | 0.5336 | 0.5320 | 0.4907 | 0.6236 | 0.5450±0.02 |
| LEI0094 | Allele number | 4 | 4 | 4 | 2 | 3.50±0.43 |
| | Allele size range (bp) | 216-240 | 216-240 | 216-240 | 209-212 | - |
| | Range of frequencies | 0.06-0.46 | 0.08-0.33 | 0.13-0.48 | 0.42-0.58 | - |
| | $h_i$ | 0.6559 | 0.7084 | 0.6699 | 0.4861 | 0.6301±0.04 |
| | $N_e$ | 2.91 | 3.43 | 3.03 | 1.95 | 2.83±0.27 |
| | PIC | 0.5933 | 0.6522 | 0.6173 | 0.3679 | 0.5577±0.06 |
| LEI0166 | Allele number | 7 | 5 | 6 | 5 | 5.75±0.41 |
| | Allele size range (bp) | 259-300 | 269-300 | 263-300 | 269-300 | - |
| | Range of frequencies | 0.03-0.33 | 0.02-0.47 | 0.03-0.39 | 0.02-0.47 | - |
| | $h_i$ | 0.7753 | 0.5907 | 0.6875 | 0.6443 | 0.6745±0.03 |
| | $N_e$ | 4.45 | 2.44 | 3.20 | 2.81 | 3.23±0.38 |
| | PIC | 0.7425 | 0.5055 | 0.6333 | 0.5805 | 0.6155±0.04 |
| ADL136 | Allele number | 7 | 7 | 6 | 6 | 6.50±0.25 |
| | Allele size range (bp) | 144-190 | 144-190 | 144-180 | 148-190 | - |
| | Range of frequencies | 0.08-0.23 | 0.03-0.34 | 0.08-0.30 | 0.07-0.34 | - |
| | $h_i$ | 0.8345 | 0.7836 | 0.7835 | 0.7691 | 0.7927±0.01 |
| | $N_e$ | 6.04 | 4.62 | 4.62 | 4.33 | 4.90±0.33 |
| | PIC | 0.8129 | 0.7538 | 0.7513 | 0.7355 | 0.7634±0.02 |
| MCW145 | Allele number | 7 | 5 | 7 | 6 | 6.25±0.41 |
| | Allele size range (bp) | 226-260 | 226-256 | 226-260 | 226-256 | - |
| | Range of frequencies | 0.03-0.39 | 0.02-0.41 | 0.03-0.33 | 0.06-0.38 | - |
| | $h_i$ | 0.7597 | 0.6893 | 0.7838 | 0.7596 | 0.7481±0.02 |
| | $N_e$ | 4.16 | 3.22 | 4.63 | 4.16 | 4.04±0.26 |
| | PIC | 0.7281 | 0.6311 | 0.7536 | 0.7266 | 0.7099±0.02 |
| ADL0226 | Allele number | 1 | 2 | 2 | 2 | 1.75±0.22 |
| | Allele size range (bp) | 202 | 198-202 | 198-202 | 198-202 | - |
| | Range of frequencies | 0.00-1.00 | 0.36-0.64 | 0.33-0.67 | 0.00-0.50 | - |
| | $h_i$ | 0.0000 | 0.4598 | 0.4444 | 0.5000 | 0.3511±0.10 |
| | $N_e$ | 1.00 | 1.85 | 1.80 | 2.00 | 1.66±0.19 |
| | PIC | 0.0000 | 0.3541 | 0.3456 | 0.3750 | 0.2687±0.08 |
| ADL166 | Allele number | 5 | 4 | 5 | 5 | 4.75±0.22 |
| | Allele size range (bp) | 149-157 | 151-157 | 149-157 | 149-157 | - |
| | Range of frequencies | 0.03-0.50 | 0.03-0.57 | 0.03-0.62 | 0.01-0.58 | - |
| | $h_i$ | 0.6615 | 0.5927 | 0.5711 | 0.5767 | 0.6005±0.02 |
| | $N_e$ | 2.95 | 2.40 | 2.33 | 2.36 | 2.51±0.13 |
| | PIC | 0.6133 | 0.5343 | 0.5333 | 0.5183 | 0.5498±0.02 |
| Among loci | Mean allele number ±S.E | 6.00±0.74 | 6.00±0.84 | 5.80±0.81 | 5.73±0.85 | 5.88±0.06 |
| | $\overline{Ne}$ ±S.E | 4.11±0.47 | 4.05±0.55 | 3.96±0.60 | 4.09±0.62 | 4.05±0.03 |
| | H±S.E | 0.6852±0.06 | 0.7017±0.03 | 0.6486±0.06 | 0.6957±0.03 | 0.6828±0.01 |
| | $\overline{PIC}$ ±S.E | 0.6434±0.06 | 0.6509±0.04 | 0.6068±0.06 | 0.6409±0.04 | 0.6355±0.01 |
| | CPD | | | | | 99.00%**[a] |

$h_i$, $N_e$, PIC, H, $\overline{Ne}$, $\overline{PIC}$ and CPD were as earlier defined; *Sample size in different chicken populations in parentheses.
**See Materials and Methods for the formula used for computation.

Jiangchun (0.1691) and the largest distance was found between Rugao vs Cshiqishi (0.3372). Using these distances obtained, the dendrogram (Fig. 1) was drawn. In the dendrogram, the Rugao and the Jiangchun chicken populations have the smallest measure of relatedness, Wan-Nan been intermediate and Cshiqishi

Table 2: Angular genetic distances ($D_A$) of the four Haimen chicken populations used in this study

| Population (s) | Rugao | Jiangchun | Wan-Nan | Cshiqishi |
|---|---|---|---|---|
| Rugao | **** | | | |
| Jiangchun | 0.1691 | **** | | |
| Wan-Nan | 0.1708 | 0.2502 | **** | |
| Cshiqishi | 0.3372 | 0.2596 | 0.2698 | **** |

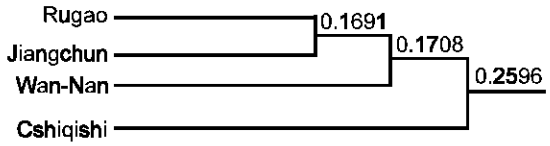


Fig. 1: Dendrogram based on $D_A$-values of microsatellites data (using the minimum distance clustering method) and the numbers at the branch points represents measure of relatedness.

appeared distantly related.

## Discussion

**Microsatellite allele frequency interpretation:** A total of 116 alleles were generated, the number of alleles per locus ranged from 2, produced by locus ADL0226 across all populations to 16 produced by locus MCW 120 and 1 to 15 per chicken population. The average allele number in population among loci ranged from 5.73 (Cshiqishi) to 6.00 observed in both (Rugao and Jiangchun) chicken populations, respectively.

This observed number of alleles for each locus revealed that all the fifteen loci were polymorphic with the Haimen chickens. The average allele number in populations were consistent with what was recommended (at least four alleles in population) for microsatellites to be used in the estimation of genetic diversity and genetic distances by Wimmers *et al.* (2000). The large allele size distribution and the number displayed per locus will be of immense importance in the use of these markers in further studies involving Haimen and other chicken populations. The size distribution of microsatellite alleles observed (Table 1), did not completely corresponds to that, that can be expected from a stepwise mutation model (SMM). According to SMM (Ohta and Kimura, 1973), allele frequencies of microsatellites should be strictly and normally distributed. In the present study, none of the distributions were normal. The distribution of the allele frequencies by the loci were quite irregular and complex in structure as earlier reported by Vanhala *et al.* (1998) and recently by Olowofeso *et al.* (2005) generally for microsatellites.

Allele range between 4 and 16, were common with most of the loci which is in agreement with previous researchers. Using MCW120 with Chinese chickens, Wu (2003) and Shen (2004) have obtained alleles between 9 and 10 and both asserted that MCW120 amplified DNA of Chinese chickens without difficulty. In this work, same locus was used and generated the highest number of alleles (16) observed in all the Haimen chickens. As a species-specific marker, MCW120 may therefore serve as a useful marker to detect population substructure in further studies of Chinese chickens. This observation was in good agreement with Russell *et al.* (2000) that suggested use of microsatellite locus generating highest allele for further population substructures. While LEI0066 produced 4 alleles in all the Haimen chicken populations, loci ADL136, LEI0166, MCW0039 and MCW 145, produced a total of 7 alleles across populations, respectively.

**Heterozygosity, effective number of allele and polymorphism information content:** Among loci heterozygosity ranged from 0.6486 (Wan-Nan) to 0.7017 (Jiangchun) chickens with across populations heterozygosity among loci equals 0.6828. This level of genetic diversity (heterozygosity) is similar to values reported in other Chinese chicken populations using microsatellites by Zhang *et al.* (2002) and Shen (2004), respectively. Romanov and Weigend (2001) using microsatellites with chickens have equally reported heterozygosity as high as above 0.60. While the results of the present study conformed to the results of the afore-mentioned authors, it was however slightly higher than the mean heterozygosities of 0.4492 to 0.6081 and 0.3514 to 0.5929 reported by Wu (2004) and Chen *et al.* (2004) with some Chinese chicken populations; the variation in results may be adduced to differences in location, different sample sizes, different experimental chickens and sources of the microsatellite markers used.

**Phylogenetic relationships:** The dendrogram constructed using the $D_A$ from the genetic distances data of the microsatellites showed that Rugao and Jiangchun formed the first group with the smallest genetic distance, followed by Wan-Nan and Cshiqishi was the most distantly related among the examined chicken populations.

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