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Microsatellite DNA Typing for Assessment of Genetic Variability in Taihu Goose: A Major Breed of China

[1]Tang Qing-Ping, [1]Zhang Shuang-Jie, [1]Guo Jun, [1]Chen Kuan-Wei, [2]Lu Huo-Lin and [2]Su Jian-Dong
[1]Institute of Poultry Science, Chinese Academy of Agricultural Sciences,
Yangzhou, 225003, Jiangsu, P.R. China
[2]Xiang Yun Taihu Goose Co. Ltd., Su Zhou, 215111, Jiangsu, P.R. China

Abstract: The present study estimates genetic variability with a set of 26 microsatellite markers in a random sample of 60 animals of Taihu goose of China. Taihu goose is a dual-purpose breed, valued for its meat as well as down feather. It is famous for its good meat quality, high production and strong adaptability. Microsatellite genotypes were derived and allelic and genotypic frequencies, hererozygosities and gene diversity were estimated. A total of 87 alleles were distinguished. All the microsatellites were polymorphic, with mean allelic number of 3.1, ranging 2-6 locus^{-1}. Observed and expected heterozygosity ranged from 0.209-1.000 and from 0.276-0.769, respectively. Wide range of genetic variability supported the utility of these microsatellite loci in measurement of genetic diversity indices in other Chinese goose breeds too. Various average genetic variability measures, namely observed heterozygosity (0.829), expected heterozygosity (0.545) and mean polymorphism information content (0.464) values showed substantial within-breed genetic variability in this major breed of China, the F_{IS} values also supported low levels of inbreeding in the population (F_{IS} = -0.531). Genetic bottleneck hypotheses were also explored. The data suggest that the Taihu goose population has experienced a genetic bottleneck in the recent past.

Key words: Microsatellites, genetic diversity, genetic bottleneck, conservation priorities, Taihu goose

INTRODUCTION

The vast and varied poultry genetic resources of China are identified in the form of 133 documented breeds, with 81 chicken breeds, 26 duck breeds and 26 goose breeds (Xu and Chen, 2003) besides many populations still uncharacterized and undefined. Conservation of genetically unique breeds/populations is of top priority to prevent loss of genetic diversity within each domestic species. In view of the massive costs involved, it is not practicable to launch conservation and improvement programs for each livestock breed. In the absence of appropriate grouping of breeds, clustering derived from molecular characterization of general genetic variability will provide valuable evidence to rank/prioritize the breeds in terms of phylogenetic distinctness.

This will help breeders to implement rational decisions for conservation and improvement of valuable germplasm. There is a terrible risk that most breeds may peish before they have been exclusively recognized and exploited (FAO, 2000). Unique and genetically diverse populations should therefore be identified in order to cover the widest range of genetic variability. Accurate evaluation of populations with regard to their contribution to national and overall genetic diversity is an important step in determining priorities for conservation (Weigend *et al*., 1995).

Taihu goose is an important indigenous breed among them. It mainly distributes in Taihu area of the Yangtse River delta, Jiangsu province. Taihu goose is a special breed in Jiangsu province, famous for its good meat quality, high production and strong adaptability. It has been listed in the national protection list of China. However, Taihu geese have already suffered significant loss of economic importance and extensive decline in their population size primarily owing to indiscriminate crossbreeding. In these circumstances, it is necessary to design more efficient conversation strategies for Taihu geese.

In the process of developing strategies to conserve genetic diversity in domestic goose breeds, it is important to assess the genetic uniqueness of a given population, which may be deduced from genetic distances (Hillel *et al*., 2003). Molecular markers may serve as an important initial guide to evaluate breeds as genetic resources (Barker, 1994; Ruane, 1999; Teale *et al*., 1994;

Corresponding Author: Chen Kuan-Wei, Institute of Poultry Science, Chinese Academy of Agricultural Sciences, Yangzhou, 225003, Jiangsu, P.R. China

Weigend and Romanov, 2001). Within the framework of breed conservation, genetic characterization is important in guarding breed integrity and is a prerequisite for managing genetic resources. Among the currently used molecular marker systems for genetic characterization, microsatellites are widely adopted to quantify genetic variation within and among breeds because of their extremely informative polymorphic nature, their abundance in the genome and the ease of amplification and typing by PCR (Rosenberg *et al*., 2002).

In the present study, 26 microsatellite markers were used to investigate genetic diversity in Taihu geese. The population structure, genetic variability and genetic bottlenecks in Taihu geese were evaluated. The present study gives, an account of the existing within-breed genetic variability in Taihu geese and the genetated data can be used to determine genetic relationships with other indigenous as well as exotic goose breeds. The results may also contribute to a more efficient conversation effort on Taihu geese.

MATERIALS AND METHODS

Experimental population: A total 60 individuals from Taihu goose breed were genotyped. These individuals were randomly selected from a center of poultry resource in Xiangyun Taihu goose Co. Ltd, Suzhou city, Jiangsu Province, P.R. China.

DNA isolation: Per individual, 0.4 mL whole blood was collected from the ulnar vein with heparin as anticoagulant. Then, 4 mL of DNA lysate solution (2 m urea, 100 mM Tris-HCl (pH 8.0), 1 SDS%, 100 mM EDTA) was added and the mixture was stored at 4°C. DNA was isolated by using a phenol/chloroform based method (Sambrook *et al*., 2001).

Genotyping: The DNA polymorphism was assessed at 26 microsatellite loci (Table 1). Multiplex PCR including 2-5 pairs of primers per reaction were carried out according to FAO (2004) recommendations. Each PCR tube contained 20 ng of genomic DNA, 10 pmol of each forward primer labeled with either IRD 700 or IRD 800 (MWG-Biotech, Ebersberg, Germany), 10 pmol of each unlabeled reverse primer and 1 mM tetramethylammoniumchloride. The amplification protocol comprised initial denaturation and enzyme activation at 95°C (15 min), followed by 35 cycles of denaturation at 95°C (1 min), primer annealing at temperature varying between 55 and 65°C (1 min) and extension at 72°C (1 min) and a final extension at 72°C for 10 min. DNA fragments were visualized as bands on 8% polyacrylamide gel. Gel electrophoresis was performed on an ABI 310 DNA analyzer (Applied Biosystems Inc., USA). Electrophoregram processing and allele-size scoring was performed with the RFLPscan software package (Scanalytics, Division of CSP, Billerica, USA).

Table 1: The information of the 30 microsatellite markers

Markers	Total No. of alleles	Effective No. of alleles	Range allele sizes (bp)	Ho	He	PIC	F_{IS}
LEI0094	4	2.787	180-213	1.000	0.645	0.573	-0.542**
CANDA4	3	2.435	166-190	0.978	0.593	0.513	-0.642**
CANDA5	6	3.761	181-293	0.488	0.736	0.694	0.332**
CKW141	2	1.996	207-220	0.956	0.499	0.375	-0.915**
CKW10	3	2.546	135-156	1.000	0.606	0.528	-0.644**
CKW11	2	1.953	184-203	0.844	0.488	0.369	-0.731**
CKW12	3	1.611	205-227	0.489	0.373	0.318	-0.271*
CKW13	3	2.044	154-182	1.000	0.511	0.391	-0.957**
CKW16	3	1.519	215-244	0.422	0.336	0.292	-0.220
CKW17	3	2.776	210-236	0.600	0.638	0.561	0.091
CKW21	6	4.309	236-305	1.000	0.769	0.735	-0.290**
CKW22	3	2.167	185-230	0.750	0.541	0.483	-0.386**
CKW25	5	3.034	136-164	1.000	0.669	0.614	-0.488**
CKW26	2	2.000	190-238	1.000	0.500	0.375	-1.000**
CKW27	2	2.000	144-158	1.000	0.500	0.375	-1.000**
CKW28	2	2.000	202-218	1.000	0.500	0.375	-1.000**
ADL166	2	1.999	316-343	0.977	0.500	0.375	-0.955**
MCW134	2	1.990	130-144	0.698	0.498	0.374	-0.406**
MCW0264	3	2.431	264-313	0.296	0.595	0.526	0.523**
MCW104	2	1.999	262-314	0.977	0.500	0.375	-0.955**
MCW0085	3	2.117	254-296	0.861	0.527	0.420	-0.632**
MCW4	3	1.392	170-204	0.209	0.276	0.252	0.270*
MCW0014	3	2.100	266-310	1.000	0.523	0.410	-0.909**
G07	5	2.467	160-185	1.000	0.593	0.508	-0.681**
G09	4	3.293	100-116	1.000	0.697	0.642	-0.425**
G10	4	3.110	163-200	1.000	0.676	0.615	-0.471**
Mean	3.107	2.378	-	0.829	0.545	0.464	-0.531**

*p<0.05; **p<0.01

Statistical analysis: Genotypes were assigned for each individual based on allele size data. Allele frequencies and expected Heterozygosity (He) (Nei, 1987) for each locus were estimated with Microsatellite-Toolkit for Excel (Park, 2001). Genetic differentiation within breed was examined by F_{IS} for each locus, as implemented in FSTAT program (Version 2.9.3, Goudet, 2002). Significance of the F_{IS} was determined from permutation tests with the sequential benferroni procedure applied over all loci. Polymorphism Information Content (PIC) for each locus was obtained according to Botstein *et al.* (1980):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2$$

Where:

n = The number of alleles
p_i = Frequency of the allele I
p_j = Frequency of the allele j

To detect whether, the Taihu goose population has experienced a recent reduction in the effective population size or a genetic bottleneck, two different approaches were followed. In the first approach, based on heterozygosity excess, three different tests, namely a sign test, a standardized differences test and a Wilcoxon sign-rank test, were employed under different models of microsatellite evolution like the Infinite Allele (IAM), Stepwise Mutation (SMM) and Twophased (TPM) models of mutation. The second approach was the graphical representation of the mode-shift indicator proposed by Luikart and Cornuet (1997). These two approaches were conducted using Bottleneck v1.2.02 software (http://www.ensam.inra.fr/URLB; Cornuet and Luikart, 1996).

RESULTS AND DISCUSSION

All microsatellite loci typed were polymorphic. The number of alleles per locus, effective number of alleles, observed Heterozygosity (Ho), expected Heterozygosity (He) and Polymorphism Information Content (PIC) were shown in Table 1. Across the 26 microsatellites studied, a total of 83 alleles were observed in the Taihu goose breed. The allele frequency data revealed a reasonable amount of polymorphism (Table 1). The number of observed alleles varied between 2 and 9, with overall mean number of alleles per locus of 3.107. The overall effective number of alleles was less than the observed value across all the loci and ranged from 1.519-4.309, with mean of 2.378.

In this study, PIC values in the Taihu goose population ranged between 0.252 and 0.735, with mean of 0.464. Reasonably high PIC values observed for most of the markers are indicative of the usefulness of microsatellites for biodiversity evaluation in this breed. Mean observed heterozygosity, averaged over the 26 loci, was 0.829, which was higher than the expected heterozygosity (Table 1). Average expected heterozygosity (gene diversity) within the Taihu goose population ranged from 0.276 (MCW4) to 0.769 (CKW21), with overall mean of 0.545. This value exceeded the value reported in the 52 European chicken breeds (Hillel *et al.*, 2003), but lower than the value in the 14 Chinese indigenous goose breeds (Chen *et al.*, 2008). Taihu geese thus seem to harbour a good amount of genetic variation.

The F_{IS} estimates ranged between -1.000 and 0.523, with average of -0.531, at which three loci showed significant deficit of heterozygotes, while 22 loci showed excess of heterozygotes, with a negative F_{IS} value. On an average, there is a significant excess (53.1%) of heterozygotes in the Taihu goose population ($p<0.01$). About 21 microsatellite markers contributed to this result significantly. This suggests that the Taihu goose breed retains considerable genetic variability and low levels of inbreeding, despite its declining population in the breeding region.

The conclusion from the bottleneck analysis is the existence of bottleneck in Taihu goose breed in the recent past. The first approach based on heterozygosity excess works on the principle that in a recently bottlenecked population, the observed gene diversity is higher than the expected equilibrium gene diversity (Heq), which is computed from the observed number of alleles (k), under the assumption of a constant-size (equilibrium) population. All of the calculated p-values (Table 2) was significant ($p<0.05$), demonstrating that the null hypothesis of mutation-drift equilibrium is not fulfilled in this population. When a population goes through a bottleneck rare alleles tend to be lost and the average number of alleles per locus, or allelic diversity is reduced. Heterozygosity, however, is not reduced proportionally, because rare alleles contribute little to heterozygosity. The difference between allelic diversity and heterozygosity is used as the basis for statistical tests to detect presence of recent genetic bottleneck (Piry *et al.* 1999). The second approach, the allele frequency spectrum visualized by the qualitative graphical method is shown in Fig. 1. The microsatellite alleles were organized into 10 frequency classes, which permit checking whether the distribution followed the normal L-shaped form, where alleles with low frequencies (0.01-0.1) are the most numerous. The observed distribution suggests that the breed encountered a genetic bottleneck in the recent past. The concordance in

Table 2: Number of loci with heterozygosity excess/deficiency and probabilities obtained fromthree microsatellite evolution models for bottleneck test

Test	Exc. H exp.	Exc. H obs.	Def. H obs.	p-value
Sign test				
IAM	12.26	26	0	0.00000*
TPM	13.31	26	0	0.00000*
SMM	13.93	25	1	0.00000*
Standardized differences test (T2 value)				
IAM	7.643	-	-	0.00000*
TPM	6.372	-	-	0.00000*
SMM	5.222			0.00000*
Wilcoxon test (probabilities-one tail for H excess)				
IAM	-	-	-	0.00000*
TPM	-	-	-	0.00000*
SMM	-	-	-	0.00000*

Deviation from the mutational equilibrium $p<0.05$

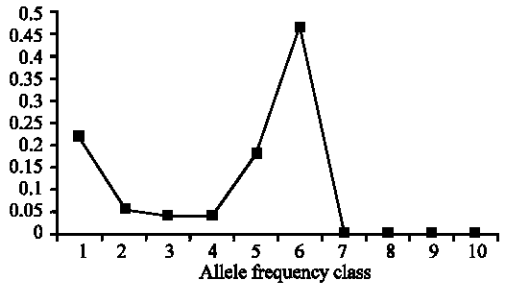

Fig. 1: Mode-shift analysis for test for genetic bottleneck in Taihu geese

the results of two approaches revealed the existence of a recent-past demographic reduction in the Taihu goose population.

The significant level of variability in Taihu goose breed is indicative of a valuable reservoir of genetic diversity in this breed. This fact, coupled with its evident environment adaptation and high economical value, emphasizes the importance of genetic regulation and conservation of this important indigenous breed as a valuable pure breed and its sustainable utilization. Nevertheless, according to our result, the breed encountered a genetic bottleneck in the recent past, which might be caused by its recent shrinking population size. Thus, it is now critical to initiate planned and organized breeding. To make a start, a Taihu goose breed society should be formed in advance, which should be educated and supported for comprehensive safeguarding and upgrading of the breed to make it economically sustainable.

CONCLUSION

In general, 26 microsatellite markers used in the present were polymorphic. Taihu goose breed has considerable genetic variation and low levels of inbreeding. However, the Taihu goose population has experienced a genetic bottleneck in the recent past, so it is now critical to initiate planned and organized breeding of this breed.

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REFERENCES

Barker, J.S.F., 1994. A global programme for determining genetic distances among domestic livestock breeds. In: Proceedings of the 5th World Congress on Genetic Applied to Livestock Production, Guelph and Ontario, Canada, 21: 501-508.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Chen, K.W., W.T. Song, W.J. Xu, H.F. Li and Q.P. Tang 2008. Study on genetic and genetic differentiation of indigenous geese breeds in eastern China. Acta Veterinaria et Zootechnica Sinica, 39 (3): 286-290.

Cornuet, J.M. and G. Luikart, 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144: 2001-2014.

FAO, 2000. World Watch List for Domestic Animal Diversity. 3rd Edn. Food and Agriculture Organization, Rome.

FAO, 2004. Guidelines for Development of National Management of Farm Animal Genetic Resources Plans. http://dad.fao.org/en/refer/library/guidelin/marker.pdf.

Goudet, J., 2002. FSTAT version 2.9.3.2. Department of Ecology and Evolution, University of Lausanne, LAUSANNE, Switzerland.

Hillel, J., A.M.M. Groenen, M., Tixier-Boichard and A.B. Korol, L. David, V.M. Kirzhner, T. Burke, A. Barre-Dirie, R.P.M.A. Crooijmans, K. Elo, M.W. Feldman, P.J. Freidlin, A. Mäki-Tanila, M. Oortwijn, P. Thomson, A. Vignal, K. Wimmers and S. Weigend, 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet. Sel. Evol., 35: 533-557.

Luikart, G. and J.M. Cornuet, 1997. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conserv. Biol., 12: 228-237.

Nei, M., 1987. Molecular evolutionary genetics. Columbia University Press, New York, USA.

Park, S.D.E., 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection Ph.D. Thesis) University of Dublin.

Piry, S., G. Luikart and J.M. Cornuet, 1999. Bottleneck: A computer program for detecting recent reductions in the effective population size using allele frequency data. J. Hered., 90: 502-503.

Rosenberg, N.A., J.K. Pritchard, J.L. Weber, H.M. Cann, K.K. Kidd, L.A. Zhivotovsky and M.W. Feldman, 2002. Genetic structure of human populations. Science, 298: 2981-2985.

Ruane, J., 1999. A critical review of genetic distance studies in conservation of animal genetic resources. J. Anim. Breed. Genet., 116: 317-323.

Sambrook, J., D.W. Russell and J. Sambrook, 2001. Molecular cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, New York, USA.

Teale, A.J., S.G. Tan and J.H. Tan, 1994 Applications of molecular genetic and reproductive technologies in the conservation of domestic animal diversity. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph and Ontario, Canada, 21: 493-500.

Weigend, S. and M.N. Romanov, 2001. Current strategies for the assessment and evaluation of genetic diversity in chicken resources. World's Poult. Sci. J., 57: 275-288.

Weigend, S., E. Vef, G. Wesch, E. Meckenstock, R. Seibold and F. Ellendorff, 1995. Conception for conserving genetic resources in poultry in Germany. Archiv für Gefügelkunde, 59: 327-334.

Xu, G.F. and K.W. Chen, 2003. Photograph Alloum of China Indigenous Poultry Breeds. 1st Edn. China Agricultural Press, Beijing.