Genetic Diversity of 14 Indigenous Grey Goose Breeds in China Based on Microsatellite Markers

Y. J. Tu*, K. W. Chen, S. J. Zhang, Q. P. Tang, Y. S. Gao and N. Yang[1]

Poultry Institute, Chinese academy of agriculture science, Yangzhou 225003, P. R. China

ABSTRACT : This experiment first cloned some microsatellite sequences for goose species by magnetic beads enriched method and studied the genetic structure research of 14 indigenous grey goose breeds using 19 developed and 12 searched microsatellite markers with middle polymorphism. According to the allele frequencies of 31 microsatellite sites, mean heterozygosity (H), polymorphism information content (PIC) and D_A genetic distances were calculated for 31-microsatellite sites. The results showed that 25 of 31microsatellite sites were middle polymorphic, so the 25 microsatellite markers were effective markers for analysis of genetic relationship among goose breeds. The mean heterozygosity was between 0.4985 and 0.6916. The highest was in the Xupu (0.6916), and in the Yan was the lowest (0.4985) which was consistent with that of PIC. The phylogenetic tree was completed through analysis of UPGMA. Fencheng Grey, Shoutou, Yangjiang and Magang were grouped firstly, then Xongguo Grey, Wugang Tong, Changle and Youjiang were the second group; Gang, Yan Xupu and Yili were the third group; Yongkang Grey and Wuzeng were the fourth group. The results could provide basic molecular data for the research on the characteristics of local breeds in the eastern China, and a scientific basis for the conservation and utilization of those breeds. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 1 : 1-6)***

Key Words : Microsatellite, Goose, Genetic Diversity

INTRODUCTION

Microsatellites, also known as simple sequence repeats, are small array of one to six tandemly arranged bases spread throughout the genomes. These polymorphism capture the repeat length variation associated with SSRs. A single primer specific to the conserved region flanking the repeat were the bases of primer design (Zeng et al., 2001). As co-dominant, highly polymorphic, highly abundant, inheritant, locus specific, analytically simple (Luo et al., 2001), they are widely used for DNA fingerprinting, paternity testing, construction of linkage maps, population genetic studies (Li et al., 2005; Osma et al., 2005), individual identification (Yoon et al., 2005), heterosis analysis, genetic bottleneck, estimation of the cultivative power of discrimination (Olowofeso et al., 2005; Pandy et al., 2005; Zhang et al., 2005) and in marker-assisted selection (Casacuberta et al., 2000). However, high cost is a major impediment to the routine application of SSRs in the genetic study of non-commercial species and for identifying markers located in chromosomal region of interest. Plenty of microsatellites have been used in chicken. However, there are very few SSRs markers designed in goose. Only Cathy designed five SSR primers for genetic diversity analysis in Canada.

China is one of the pioneer countries which have been breeding and raising goose for about more than 3,100 years. Breeding goose is a traditional vocation of peasant in China. About 7.6 hundred million geese were bred in 2001, which occupied 86% of the gross quantity in the world. There are 26 goose breeds in China, 14 of them are grey goose breeds which mostly distributed in the South of China (Xu, 2004). Because the Chinese people in the South dislike white colour and have the habits of breeding grey goose and like eating the meat of goose. Grey geese are meat breed with bigger body form. White goose breeds are mainly distributed in the North of China. Different economic and cultural background in different era and different aims of choosing and using goose, indigenous goose breeds which have different genetic characteristics and production performance come into being step by step. These indigenous goose breeds have better adaptability to extensive management, better immunity diseases, higher reproduction rate and better meat quality than those of the cultivated breeds in foreign country, which are natural gene reservoir and the good original material of crossbreed predominance and high yield. The goose breeds in China will have great effect on future goose breeding. However, these indigenous goose breeds were bred closedown and no scientific selecting system and cultivating methods, so they have no abundant genetic diversity, especially dominant gene of recessive were not protected perfectly, which restrict the development of goose breeding. How to protect and use these indigenous goose breed resources especially the breeds which have good traits is one of the hot research problems in genetics and breeding. So it is essential to study on the genetic diversity of goose breeds by using modern genetic methods and offer basic data for saving and using

* Corresponding Author: Yunjie Tu. Tel: +86-514-7233488, Fax: +86-514-7207327, E-mail: tyj3030@126.com

[1] College of Animal Science and Technology China Agricultrual University, Beijing 100001, P. R. China.

Received May 3, 2005; Accepted September 15, 2005

Table 1. Code, name, No. of sample and location of blood collection of 14 goose breeds

Code	Name	No. of sample	Location of blood collecting
G1	Fengchong Grey	60	Conservation farms in Fengcheng city Jiangxi province
G2	Xingguo Grey	60	Conservation farms in Xingguo county Jiangxi province
G3	Yongkang Grey	60	Conservation zone in Yongkang county Zhejiang province
G4	Changle	60	Conservation zone in Changle conty Fujian province
G5	Gang	60	Conservation zone in Yi clan Sichuan province
G6	Shitou	60	Conservation farms in Raoping county Guangdong province
G7	Wuzong	60	Conservation farms in Qingyuan county Guangdong province
G8	Yangjiang	60	Conservation farms in Yangjiang county Guangdong province
G9	Magang	60	Conservation farms in Kaiping county Guangdong province
G10	Youjiang	60	Conservation farms in Youjiang county Guangxi province
G11	Xupu	60	Conservation zone in Xupu county Hunan province
G12	Yan	60	Conservation farms in Langxi county Anhui province
G13	Wugang Tong	60	Conservation zone in Wugang county Hunan province
G14	Yili	60	Conservation zone in Kazakstan autonomy Sinkiang

these goose breeds in the future.

MATERIALS AND METHODS

Genomic DNA preparation

The blood samples of geese were collected randomly from their own conservation farms (Table 1). The ratio of male to female was 1:4 in every breed, i.e. 12 males and 48 females, which is consistent with the formula for evaluating genetic polymorphism and the sample number was quite reasonable. All samples were collected and anticoagulated in 70% ethanol at room temperature in the field. The genomic DNA was prepared from whole blood by a routine protocol and then stored at 4°C.

Microsatellite sites and PCR products

Microsatellite sites selection in goose breeds : This study cloned microsatellite sequences for goose breeds by magnetic beads gathering method, which involve: hybrid probe having biotin with genomic DNA fragments, mixed with avidin or chain avidin in a warm circumstance. Avidin or chain avidin can couple with biotin, DNA fragments hybrid with probe (these are DNA fragments including microsatellite sites) will adhere to the beads due to combination of avidin and antiavidin. When eluting filteration membrane or magnetic beads, it is important to control the magnitude. DNA fragments including microsatellite sites will be eluted if condition is controlled too strict and on the other hand, many fragments not required cannot be eluted. At last, eluted DNA fragments adhere to the beads by denature at high temperature for PCR and then clone PCR products. So cloned DNA fragments including abundant microsatellite sites were derived. In this study, we selected microsatellite sites in 5 goose breeds genomes (60 geese in each breed). Flanking sequences of microsatellite sites were designed by primer-primer 5.0. Taking 5-goose breeds microsatellite sites in Gen Bank: TTUCG1, TTUCG2, TTUCG4 and TTUCG5 into consideration and reference to 8-microsatellite sites: *ADL*166, *ADL*210, *MCW*4, *MCW*0014, *MCW*0264, *MCW*104, *MCW*0085 and *LEI*0094 in chicken as kindred poultry, we selected 19-microsatellite sites rich in polymorphism as experiment sites. They were *CKW*10, *CKW*11, *CKW*12, *CKW*13, *CKW*14, *CKW*15, *CKW*18, *CKW*19, *CKW*20, *CKW*21, *CKW*22, *CKW*41, *CKW*42, *CKW*43, *CKW*44, *CKW*45, *CKW*46, *CKW*47 and *CKW*48, respectively. All these sites were good in polymorphism, specificity and unlinked by one another. Microsatellite primer sequences were synthesized by Shanghai Sangon biological Engineering Technology and Services Company in China. Information about the microsatellite sites are in Table 4.

PCR protocol and electrophoresis : PCRs were carried out on 25 μl (about 100 ng) of genomic DNA in 25 μl mixture containing 1×reaction buffer, 1 unit of Taq DNA polymerase, 2.0 mmol/L of Mg^{2+}, 300 pmol of each dNTP, 2.5 pmol forward and reverse primers. The following PCR condition was used: 300 s at 95°C, 30 cycling of 50 s at 94°C, 50 s at annealing temperature of 50-60°C and 50 s at extension temperature of 72°C, and final extension step 300 s at 72°C in a Perkin-Elmer thermocycler 9600. PCR cycling was performed for 25 cycles at an annealing temperatures of 56°C in PCR System 9700. PCR products denatured for 5 min were loaded for PAGE, stained by silver nitrate and photographed by LCS.NO.00086. Taq DNA polymerase and dNTPs were sourced from Dinguo Biology Company in Beijing,China. PBR322DNA/Msp I Markers was used.

Statistical analysis

Based on the microsatellite and the generated allele frequencies, Polymorphism Information Content (PIC), homozygosity (H), and genetic distances (D_A) were

Table 2. Estimation of mean genetic variability of 14 goose breeds based on microsatellite sites

Breeds	PIC	H	Breeds	PIC	H
G1	0.355	0.5976	G9	0.361	0.6512
G2	0.387	0.6869	G10	0.353	0.5611
G3	0.364	0.6612	G11	0.389	0.6916
G4	0.379	0.6713	G12	0.323	0.4985
G5	0.375	0.6508	G13	0.351	0.5639
G6	0.358	0.6727	G14	0.352	0.5670
G7	0.364	0.6119			
G8	0.378	0.6515			

calculated (Nei, 1978). The 14 grey goose breeds was clustered by using UPGMA on DISPAN software.

RESULTS

SSR isolation and genetic variability within population

100 clones were sequenced, 49 of which were not designed primers because either the clones lacked SSR repeats, or the SSR repeats were interrupted by cloning sites or the sequence data were poor. We designed PCR primers for the remaining 51clones, 32 of which were not used, because 8 gave no amplification product, 10 produced monopolymorphic in our initial screening population of six breeds, and 14 gave complex multibanded patterns.

This study calculated D_A among 14 grey goose breeds. D_A between Gang and Yan was the nearest, which was 0.2028 and D_A between Yongkang Grey and Magang was the farthest, which was 0.6749. Similarly, we calculated mean heterozygosity (*H*) and mean *PIC* (Table 2) in 14 grey goose breeds according to allelic gene frequency at microsatellite sites, and D_A (Table 3). The mean heterozygosity of 14 goose breeds was ranged from 0.4985 to 0.6916. The highest was in the Xupu (0.6916), and in the Yan was the lowest (0.4985). The results of the heterozygosity were consistent with that of *PIC*. 25 of the 31 microsatellite markers were effective markers for analysis of genetic relationship among goose breeds and were the bases in the construction of highly saturated maps, and in some cases, in marker-assited selection in goose.

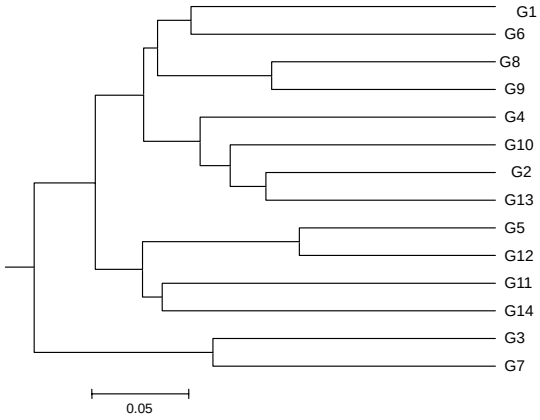

Figure 1. The dendrogram of 14 grey goose breeds using UPGMA method of clustering.

Hardy-Weinberg equilibrium tests

No systematic deviations of all the locus pairs with respect to linkage equilibrium across all populations were detected.

Population structure

The phylogenetic tree was completed through analysis of UPGMA. Fencheng Grey, Shoutou, Yangjiang and Magang were grouped firstly, then xingguo Grey, Wugang Tong, Changle and Youjiang were the second group; Gang, Yan, Xupu and Yili were the third group; Yongkang Grey and Wuzeng were the fourth group.

DISCUSSION

Efficiency of SSR enrichment from AFLP DNA by affinity capture

SSR was traditionally developed by screening the genomic library but very low percentage of clones was found to contain SSR fragments (Zane et al., 2002). In this study the use of AFLP and affinity capture technologies can obviate the tedious work of library construction which can

Table 3. D_A genetic distances between 14 goose breeds

Breeds	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.2501												
3	0.4920	0.5353											
4	0.3303	0.2469	0.6656										
5	0.3575	0.2928	0.3937	0.4436									
6	0.3148	0.3680	0.4706	0.3716	0.3791								
7	0.3230	0.4028	0.2916	0.5866	0.2609	0.3515							
8	0.3308	0.3216	0.5069	0.3254	0.4665	0.3280	0.4595						
9	0.3449	0.2672	0.6749	0.3895	0.4256	0.3932	0.4557	0.2312					
10	0.3575	0.2462	0.7253	0.3588	0.4189	0.4847	0.5105	0.4333	0.4750				
11	0.3826	0.3326	0.4624	0.4513	0.3005	0.3680	0.3960	0.3716	0.4132	0.3949			
12	0.4357	0.3721	0.5352	0.3899	0.2028	0.5366	0.3623	0.4722	0.5395	0.4219	0.4197		
13	0.3758	0.2369	0.4897	0.3096	0.3968	0.3131	0.3941	0.3337	0.4202	0.3024	0.3314	0.3252	
14	0.3891	0.4171	0.5959	0.5567	0.3446	0.4490	0.3861	0.4140	0.5112	0.3983	0.3446	0.3943	0.3638

Table 4. The information of 31 pairs of microsatellite primers in goose

Sites	Primer(5'-3')	Sequence	Accession numbers	Temperature (°C)
*CKW*10	F:ACATCCAGTTTGTGCTGCATAC	(A)18	AY720923	52
	R: R:CAAAGCCCCCATTCAAATAATA			
*CKW*11	F:CTGAGTTGAACCTGATGCAGAC	(A)16	AY720924	55
	R:AACACCAAAGGAGAGCAGAGAC			
*CKW*12	F:CATAAGTTCTCCCAAACAAGAGTG	(A)25	AY720925	53
	R: AGAAAGGGACACACAGCTAACC			
*CKW*13	F: AGGCTGAGGTGGGAGAATTTAT	(AAAC)5	AY720926	58
	R: TTCTTCCACTTCTCCCAAAGAA			
*CKW*14	F: AACTGATCCGGCAGAAAACTAA	(CCT)5	AY720927	59
	R: ACTTAGCATGCAGCTTCACAAA			
*CKW*15	F: AGGCATGATATCTGTCCCTGAT	(AG)5	AY720926	55
	R: TTTCAGTGCAATTACCCATTCA			
*CKW*18	F: AATGTGCTGTGTCACATTCTCC	(CAAAA)7	AY720929	52
	R: CATCATCCAACGATTCAGACAT			
*CKW*19	F: ACATGTCCTGAAGCATTTTCCT	(GAAA)5	AY720930	59
	R: TTCCTTTTCGCCTATGATGTCT			
*CKW*20	F: GATCAGAAATGAAGTGCAGACG	(TG)12	AY720931	55
	R: TGCTCCATTAATTATGCAACCTT			
*CKW*21	F: CCCAGAACAGTGCTAGAAGAGG	(TTA)10	AY722649	53
	R: AGCGAGTCACTCCAGTACCTTC			
*CKW*22	F: CCAAACAAGAGTGTTGGGAGGG	(A)14	AY722650	58
	R:CAGCTAACCCAAAGATACCTACCAG			
*CKW*41	F: CTAAGGTAGATTGTACATCAC	(TA)33	AY787855	55
	R: GCAGGTTAAACACGTTGTTCTG			
*CKW*42	F: TTTGCACCAGATTACACTCCT	(TA)8	AY787856	56
	R: GCAGGTTCTTAAGGAGGGATG			
*CKW*43	F: CAGAAGACAGGCCTGCAAAT	(CA)11	AY790340	56
	R: TCCAAGGCTTACTTCCCAAG			
*CKW*44	F: TCTTCTACTTCCTGACAGATGAGG	(CAA)7	AY790332	58
	R: TTGAATTGATGCCCTTTTTCTT			
*CKW*45	F: TGAAACCAATTTTTCCCATTC	(TA)13	AY790333	55
	R: TCCTGGCCAATCCCATAGTA			
*CKW*46	F: GCAGCTGATGAGAAGCAGAA	(CA)58	AY790334	60
	R: GAGTGTGTGTGTGCGTCTGTT			
*CKW*47	F: AACTTCTGCACCTAAAAACTGTCA	(T)8(TG)7	AY790335	58°C
	R: TGCTGAGGTAACAGGAATTAAAA			
*CKW*48	F: AAATTGGGCCTAAGTTGCTACA	(AAAAAG)7	AY790336	56
	R: CAACTGGCTGTGGTTCTCCT			
TTUCG1	F: CCCTGCTGGTATACCTGA	(CA)13	U66089	54
	R: GTGTCTACACAACAGC			
TTUCG2	F: GAGAGCGTTACTCAGCAAA	(GT)11	U66090	55
	R: TCACTCTGAGCTGCTACAACA			
TTUCG4	F: GGTGTATACTTGCTGAGTGT	(GT)10	U66092	56
	R: CTAGAACTAGTGGATCTCTC			
TTUCG5	F: GGGTGTTTTCCAACTCAG	(TCTAT)8	U66093	58
	R: CACTTTCCTTACCTCATCTT			
*ADL*166	F: TGCCAGCCCGTAATCATAGG	(TG)15	G01588	55
	R: AAGCACCACGACCCAATCTA			
*ADL*210	F: ACAGGAGGATAGTCACACAT	(CA)14	G01630	55
	R: GCCAAAAAGATGAATGAGTA			
*MCW*4	F: GGATTACAGCACCTGAAGCCACTA	(CA)27	L40038	63
	R: AAACCAGCCATGGGTGCAGATTGG			
*MCW*14	F: AAAATATTGGCTCTAGGAACTGTC	(CA)10	L40040	63
	R: ACCGGAAATGAAGGTAAGACTAG			
*MCW*0085	F: GTGCAGTTATATGAAGTCTCTC	(GT)11	G54426	56
	R: GGTATACAGGGCTTCTGAAACA			
*MCW*120	F: CTATGTAAAGCTTGAATCTTCA	(GT)14	L43644	57
	R: ATTCCTGGGTGCTAATTTACC			
*MCW*264	F: AGACTGAGTCACACTCGTAAG	(CA)19	G32032	55
	R: CTTACTTTTCACGACAGAAGC			
*MCW*104	F: TAGCACAACTCAAGCTGTGAG	(GT)17	L43640	60
	R: AGACTTGCACAGCTGTGACC			

save not only time but also expense. We had successfully developed 31 primers from six china indigenous goose breeds, nineteen of which were middle polymorphic specific and did not link each other. It is a rapid, non-radioactive and inexpensive method for the isolation of microsatellites from genomic DNA libraries based on the principles of streptavidin-coated magnetic bead capture of biotin-labeled probe hybridized to DNA. The experiment can be employed as a reliable option for any molecular laboratory to develop SSR markers.

Variability within the 14 chinese goose populations

It can be difficult and time-consuming to distinguish indigenous goose breeds on morphological characteristics alone, and for this reason it is important to develop molecular markers to aid goose breeds identification.

Several marker systems have been applied to goose breeds including RAPD (HAO et al., 1999), RFLP (Shi et al., 1998) and SSR (Cathy et al., 2001). We focus on SSRs because of polymorphism and because they are codominant, which makes them particularly useful for genetic diversity, genetic mapping.

In this study, we reported 19 new SSR loci and used SSRs to estimate genetic diversity in 14 goose breeds. Heterozygosity, called genetic diversity, reflects genetic variation on tested locus among population. Low heterozygosity indicted high genetic uniformity thus poor genetic diversity. With the microsatellite markers used in this study, genetic diversity estimates were relatively high. The mean heterozygosity across all populations in the present work was ranged from 0.4985 to 0.6916. The highest was in the Xupu goose (0.6916), and in the Yan was the lowest (0.4985). The results of the heterozygosity were consistent with that of PIC. Observation of excess heterozygosity are not uncommon in goose (HAO et al., 1999; Cathy et al., 2001), but it is not clear what promotes heterozygosity in goose breeds. There might be inbreeding in population because of the relatively small group of breeding farm in Langxi Yan goose breeding farm in Anhui Province. The people in Langxi and Guangde county in Anhui province eliminate plenty of Yan goose, however, breed WanXi White goose, because the price of white down is higher in recent years. The low diversity of the Yan also suggested that the population bottleneck after the dramatic reduction of the population size because of the closed system and limited number of sires. The existence of the recent bottleneck effect was supported by the analysis using BOTTLENECK program (Sasazaki et al., 2004). Thus, it is necessary to properly adjust the structure of the breeding group and reproduction according to proportionally preserve this valuable resource.

Analysis genetic relationship in 14 Chinese indigenous grey goose breeds

The structure and evolution relationship are evaluated by analyzing genetic distance, and different breeds can be distinguished. The project of breed protection and the plan of breeding can be made by analyzing genetic distance. Genetic distance is the basis for genetic diversity research. Genetic distance should be an index for group structure and breeds diversity when breeds conservation decision are being made. Microsatellite allelic gene frequency analysis was one of the best methods at present. Genetic distance derived from microsatellite can reflect the time of diversity as well as genetics and variation among breeds. The clustering based on UPGMA and Nei's distance are better method for analyzing genetic diversity in different breed population on the research of varied genetic distance by Takahashi and Nei. Small estimation values of distance may indicate population substructure (i.e., subpopulation in which there was random mating but there was reduced amount of gene flow) (Rana et al., 2004).

UPGMA tree was completed through analysis of D_A genetic distances in this research. 14 goose breeds were clustered four groups, which reflected their relationship on geography distributing and origination in some degree. Shitou, Yangjiang, Magang in Guangdong province were in the first group, however, Wuzeng were clustered with Yongkang Grey in the second group, because Wuzeng had earlier differentiation. According to "records in Qingyuan county", Wuzeng were the fond breed for the indigenous people from Song dynasty; The people in Shandong Fujian and Zhejiang province migrated in Qingyuan county in Guangdong province, Yongkang Grey in Zhejiang were brought in Qingyuan county and were crossed with indigenous goose, so Wuzeng were cultivated for a long time. Yan and Gang were clustered in the third group, which both were medium body and had nearer geography distributing. The origination of goose was not restricted in one place or one time, the culture relic of goose domestication was found in China Europe and Africa. Most goose breeds in China were considered as originating from *anser cygnoides*, while Yili in Sinkiang and most goose breeds in Europe were considered originated from *anser anser*. *Anser cygnoides* and *anser anser* are same category and different genus. So the domesticated goose breeds from them were crossed and have good offspring and better heterosis. Yili were grouped with Xupu in this research, which reflect that they were likely to be crossed and Yili had consanguinity of Xupu. The results of cluster can conjecture consanguinity of these goose breeds, and support the hypothesis that geographical distance is an important factor influencing the genetic relatedness of populations (Islam et al., 2005). Microsatellite marker are more credible for the research of breeds origination because the evolution of breeds in the form of breed and in nature and artificial selection have little effect on the structure of Microsatellite locus.

ACKNOWLEDGEMENTS

We wish to express our thanks to anonymous reviewers for the revision of our manuscript. This work was supported by the program of important animal resource protection of Ministry of Agriculture in P. R. China (No.2001AA243081).

REFERENCES

Appannavar, M. M., M. G. Govindiaiah and K. P. Ramesha. 2003. Genetic Distance Study among Deoni Breed of cattle using Random Amplified DNA Markers. Asian-Aust. J. Anim. Sci. 16(3):315-319.

Barker, J. S. F. 1994. A global protocol for determining genetic distance among domestic livestock breeds. In: Proceeding of 5th world congress on genetic Application of Livestock Production. 21:501-508.

Brown, S. M., M. S. Hopking and S. E. Mitchell. 1996. Multiple methods for the identification of polymophic simple sequence repeats (SSRs) in sorghum. [*Sorghum bicolor* (L) Moench]. Theor. Appl. Genet. 93:190-198.

Casacuberta, E., P. Puigdomenech and A. Monfort. 2000. Distribution of Microsatellite in relation to coding sequences within the Araabidopsis thaliana genome. Plant Sci. 157:97-104.

Cathy, J. C. 2001. Microsatellite markers in Canada geese (Branta canadensis), brief communication. 173-175.

Chen, G. H., X. S. Wu, D. Q. Wang, J. Qin, S. L. Wu, Q. L. Zhou, F. Xie, R. Cheng, Q. Xu, X. Y. Zhang and O. Olwofeso. 2004. Cluster Analysis of 12 Chinese Native Chicken Populations using Microsatellite Markers. Asian-Aust. J. Anim. Sci. 17(8):1047-1052.

Crawford, A. M. and R. P. Littlejohn. 1998. The use of DNA marker in deciding conservation priorities in sheep and other livestock. Anim. Gen. Res. Inform. 23:21-26.

Fischer, D. and K. Bachmann. 1998. Microsatellite Enrichment in organisms with large genomes (Allium cepa L). Bio Techniques. 394:796-802.

Gao, G. Q., G. H. He and Y. R. Li. 2003. Microsatellite Enrichment from AFLP fragments by megnetic beads, Acta Botanica sinica. 45(11):1266-1269.

Goldstein, D. B., A. R. Linares, L. L. Cavalli-Sforza and M. W. Feldman. 1995. An evaluation of genetic distances for use with microsatellite loci. Gene. 139:463-471.

Hak, Yoon D., S. Kong and Jae-Don Oh. 2005. Establishment of an Individual Identification System Based on Microsatellite Polymorphisms in Korean Cattle (Hanwoo). Asian-Aust. J. Anim. Sci. 18(6):762-766.

Islam, M. S, A. S. I. Ahmed, M. S. Azam and M. S. Alam. 2005. Genetic Analysis of Three River Populations of *catla catla* (HAMILTON) using Randomly Amplyfied Polymorphic DNA Markers. Asian-Aust. J. Anim. Sci. 18(4):453-457.

Kijas, J. M. H., J. C. S. Fowler and C. A. Garbett. 1994. Microsatellite Enrichment from the citrus genome using biotinylated oligonucleotide sequences bounds to streptavidin-coated gnetic particles. Bio Techniques. 16:657-662.

Li, M. H., E. Nogovitsina, Z. Ivanova, G. Erhardt, J. Vilkki, R. Popv, I. Ammosov, T. Kiselyova and J. Kantanen. 2005. Genetic Contribution of Indigenous Rakutian Cattle to Two Hybrid Populations Revealed by Microsatellite Variation. Asian-Aust. J. Anim. Sci. 18(5):613-619.

Nei, M. 1972. Genetic distance between populations. Am. Nature. 106:283-291.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Gene. 89:583-590.

Noah, A., B. Terry, E. Kari and Marcus W. Feldman. 2004. Empirical Evaluation of Genetic Clustering Methods Using Multilocus Genotypes From 20 chicken Breeds. Genetics 159:699-713.

Olowofeso, O., J. Y. Wang, J. C. Shen, K. W. Chen, H. W. Sheng, P. Zhang and R. Wu. 2005. Estimation of the cultivative power of discrimination in Haimen chicken populations with ten microsatellite markers. Asian-Aust. J. Anim. Sci. 18(8):1066-1070.

Osma, S. A. M., M. Sekino, M. Nishibori, Y. Yamamoto and M. Tsudzuki. 2005. Genetic Variabity and Relationships of native Japanese Chickens Assessed by Microsatellite DNA Profiling. Asian-Aust. J. Anim. Sci. 18(6):755-761.

Pandy, A. K., D. Kumar, R. Sharma, I. Sharma, R. K. Vijh and S. P. S. Ahlawat.2005. Population structure and genetic bottleneck analysis of Ankleshwar poultry breed by Microsatellite makers. Asian-Aust. J. Anim. Sci. 18(7):915-916.

Rana, R. S, K. V. Bhat, S. Lakhanpal and W. S. Lakra. 2004. Comparative Genetic Diversity in Natural and Hatchery Populations of Indian Major Carps. Asian-Aust. J. Anim. Sci. 17(9):1119-1203.

Sasazaki, S., T. Honda, M. fukushima, K. Oyama, H. Mannen, F. Mukai and S. Tsuji. 2004. Geneaalogical Relationship between pedigree and Microsatellite Information and Analysis of Genetic Structure of a Highly Inbred Japanese Black Cattle Strain. Asian-Aust. J. Anim. Sci. 17(10):1355-1359.

Selvi, P. K., J. M. Panandam, K. Yusoff and S. G. Tan. 2004. Molecular Characterization of the Mafriwal Diary cattle of Malaysia using Microsatellite Markers, Asian-Aust. J. Anim. Sci. 17(10):1366-1368.

Thevenon, L. T., L. Thur, V. Ly, F. Maudet, A. Bonnet, P. Jarne and J. C. Maillard. 2004. Microsatellite Analysis of Genetic diversity of the Vietnamese Sika Deer. Heredity 95(1):11-18.

Xu, G. F. and K. W. Chen. 2004. “Photograph Album of China Indigenous Poultry Breeds”. 216-265.

Wang, X., H. H. Gao, S. M. Geng and H. B. Li. 2004. Genetic diversity of 10 indigenous pig breeds in Chinaby using microsatellite markers. Asian-Aust. J. Anim. Sci. 17(9):1219-1222.

Zhang, J. H., Y. Z. Xiong and C. Y. Deng. 2005. Correlations of Genic Heterozygosity and Variances with Heterosis in a Pig population Revealed by Microsatellite DNA Marker. Asian-Aust. J. Anim. Sci. 18(5):620-625.