Journal of Animal and Veterinary Advances 8 (7): 1381-1384, 2009
ISSN: 1680-5593

Genetic Differentiation among Four Chinese Sheep Breeds

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Abstract: Gene flow was analyzed among 4 Chinese sheep breeds of Mongolian group by structural loci and microsatellite markers. The results showed that genetic differentiation coefficient obtained from structural loci was 0.0164-0.0455, while, obtained from microsatellite loci was 0.0107-0.0239, indicating that genetic differentiation level was very low among 4 sheep breeds. Analysis of Molecular Variance (AMOVA) test indicated that most variations existed within breeds. Gene flow was fluent among breeds reflected from structural loci (Nm = 7.971) and microsatellite loci (Nm = 15.732). No obvious relevance existed between genetic difference and geographical distance. The findings demonstrated that genetic differentiation of sheep breeds of Mongolian group in China was mainly the impaction of natural selection (different environmental conditions).

Key words: Genetic differentiation, gene flow, microsatellite marker, Chinese sheep, Mongolian group

INTRODUCTION

Native sheep breeds in China can be divided into three groups, that is, Mongolian group, Hazake group and Tibetan group (Zhou *et al*., 1994). The Mongolian group is the dominant one, including several local breeds with special advantages as a result of selection, hybrid, natural environments and other factors. The timing of separation for the local breeds within the Mongolian group was about 1100 years ago, that was from Jin dynasty to Tang dynasty approximately (Geng, 2002). These local breeds were widely distributed in pasturing region, agricultural regionand interlaced region. They were domesticated in different way. Owing to wide geographical distribution and compatibility, these breeds hold some differentiation among them in morphological characters, ecological characters and genetic features (Sun *et al*., 2003; Yang *et al*., 2003; Lu *et al*., 2004, 2005). In this study, 2 genetic markers of structural loci and microsatellite loci were used to analyze gene flow among 4 sheep breeds of Mongolian group, revealing genetic differentiation and relationship between geographical distribution and genetic distance. It also provided evidences for phylogeny status of Mongolian sheep group.

MATERIALS AND METHODS

Applying simple random sampling methods in typical colony in the central area of habitat, 63 Hu sheep (Hu), 65 Tong sheep (Tong), 60 Small-tailed Han sheep (Han) and 73 Tan sheep (Tan) were selected from Huzhou of Zhejiang province, Baishui of Shanxi province, Liangshan of Shandong province and Yanchi of Ningxia province, respectively. Blood samples were collected and treated according to references (Geng *et al*., 2003). The genome DNA was extracted by common method described by Sun *et al*. (2004).

Experimentation: Starch gel electrophoresis was used to determine variations of 13 structural loci encoding Albumin (Al), Transferrin (Tf), Alkaline phosphatase (Alp), Arylesterase (Ary-Es), Leucine aminopeptidase (Lap), Hemoglobin-|β(Hb-|β), X-protein (X-p), Malate dehydrogenase (MDH), Catalase (Cat), Lysine (Ly), Esterase D (Es-D), Carbonic Anhydrase (CA) and Posassium (Ke) described by Tsunoda *et al*. (1990). Seven pairs of microsatellite primers were isolated from domestic sheep (*Ovis aries*) (Crawford *et al*., 1995). Primer synthesis and PCR amplification was performed according to reference (Sun *et al*., 2004). Amplified DNA fragments were subjected to electrophoresis on 8% polyacrylamide gel and visualized by silver stain.

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Statistical analysis: Gene diversity within population (H_S), total gene diversity (H_T), coefficient of genetic differentiation (G_{ST}), gene flow (Nm) and Nei's genetic distances were calculated by software of POPGENE32 (Raymond and Rousset, 1995) and TFPGA (Tajima, 1989). Analysis of Molecular Variance (AMOVA) test was also done by Arlequin 3.1 package (Excoffier *et al*., 2005). Mantel test model (Mantel, 1967) was used to detect the relativity between genetic distances and geographical distances.

RESULTS

Degree of genetic differentiation among breeds: As was shown in Table 1, Gene diversity within population (H_S) was between 0.3188-0.3650, on average of 0.3419, while, total gene diversity (H_T) was between 0.3332-0.3711, on average of 0.3540 according to structural loci. Gene diversity within population (H_S) was between 0.9106-0.9257, on average of 0.9181, while total gene diversity (H_T) was between 0.9236-0.9405, on average of 0.9340 according to microsatellite loci.

Coefficients of genetic differentiation were showed in Table 2. Coefficients of genetic differentiation from structural loci were between 0.0164-0.0455 and the average was 0.0343, while coefficients of genetic differentiation from microsatellite loci were between 0.0107-0.0239 and the average was 0.0169.

The results of AMOVA showed that 95.19% variations existed within breeds and only 4.81% variations existed among breeds according to structural loci. In addition, 97.43% variations existed within breeds and only 2.57% variations existed among breeds according to microsatellite loci.

The above mentioned results all revealed that genetic variations were very little and most variations existed within breeds. The degree of genetic differentiation was very low among 4 breeds estimated by both structural loci and microsatellite loci.

Level of gene flow: Gene flow between breeds were shown in Table 2, which was calculated according to Wright (1931).

Gene flow between Hu sheep and Tong sheep was the biggest both in structural loci and microsatellite loci. Gene flow between Hu sheep and Small-tailed Han sheep was the least in structural loci (Nm = 5.239), while gene flow between Hu sheep and Tan sheep was the least in microsatellite loci (Nm = 10.209). Although, gene flow estimated by 2 kinds of genetic markers was not totally in coincidence, gene flow between breeds was all >1. The average values of gene flow estimated by structural loci and microsatellite loci were 7.971 and 15.732, respectively,

Table 1: Gene diversity within populations (above diagonal) and total gene diversity (below diagonal)

Population	Hu	Tong	Han	Tan
Hu	-	0.3650	0.3353	0.3507
Tong	0.3711	-	0.3331	0.3485
Han	0.3513	0.3442	-	0.3188
Tan	0.3654	0.3586	0.3332	-
Hu	-	0.9137	0.9216	0.9106
Tong	0.9236	-	0.9257	0.9147
Han	0.9385	0.9405	-	0.9226
Tan	0.9329	0.9344	0.9340	-

*The left represents data from structural loci,while the right represents data from microsatellite loci

Table 2: Coefficients of genetic differentiation (above diagonal) and gene flow (below diagonal)

Population	Hu	Tong	Han	Tan
Hu	-	0.0164	0.0455	0.0402
Tong	14.959	-	0.0322	0.0282
Han	5.239	7.502	-	0.0432
Tan	5.964	8.626	5.535	-
Hu	-	0.0107	0.0180	0.0239
Tong	23.073	-	0.0157	0.0211
Han	13.633	15.637	-	0.0122
Tan	10.209	11.608	20.233	-

*The left represents data from structural loci and the right represents data from microsatellite loci

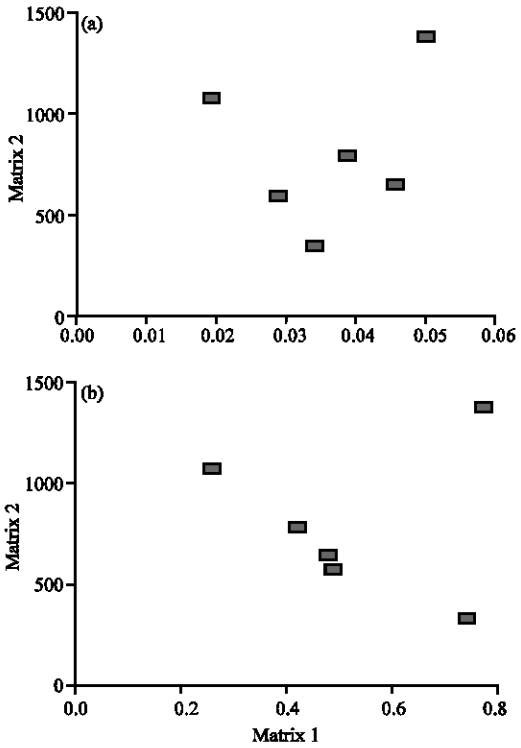

Fig. 1: Results of mantel test between matrixes of genetic distance and geographical distance, a): Represents result from structural loci and b): Represents result from microsatellite loci)

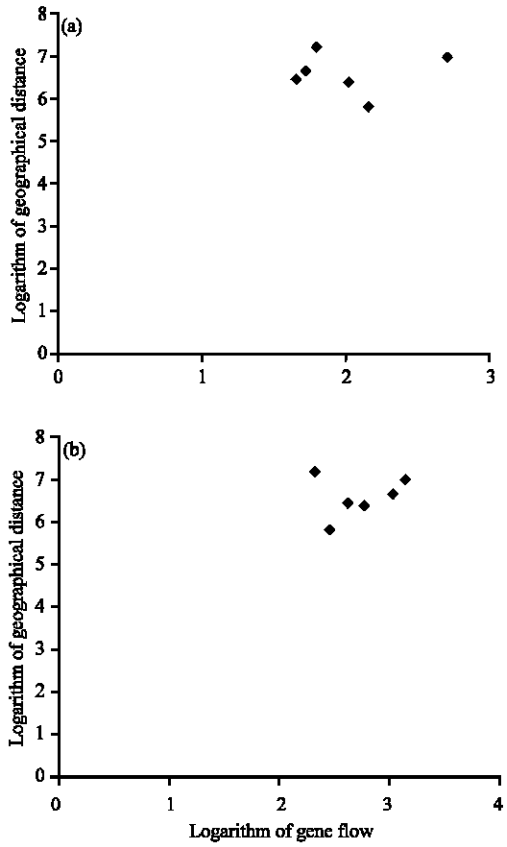

Fig. 2: Relevance between logarithm of gene flow and logarithm of geographical distance, a): Represents result from structural loci and b): Represents result from microsatellite loci)

which demonstrated that gene flow was very big between breeds. Big value of gene flow also showed that there was no high level of genetic differentiation between breeds.

Relativity between genetic distances and geographical distances: Mantel test results based on genetic distance and geographical distance were shown in Fig. 1. There was only faint positive relativity (r = 0.2161) between genetic distances and geographical distances among sheep breeds derived from structural loci. There was also faint negative relativity (r = -0.033) between genetic distances and geographical distances among sheep breeds derived from microsatellite loci.

A method indicating the relativity between genetic distances and geographical structure was developed by Slatkin (1987) that was, the ratio of logarithm between gene flow and geographical distance. There was no apparent relativity between genetic distances and geographical distances according to structural loci and microsatellite loci as was shown in Fig. 2.

The findings demonstrated that there was no direct relativity between genetic variations and geographical distances that was, geographical distances could not explain genetic distances among breeds.

DISCUSSION

Genetic variations within populations were very high among four sheep breeds of Mongolian group in China according to structural loci and microsatellite loci markers, while genetic variations among populations only possessed little proportion. Genetic variations within populations were over 19 times (data from structural loci) or 37 times (data from microsatellite loci) than those among populations according to AMOVA test. Coefficients of genetic differentiation were also quite small, revealing that most variations existed within breeds and the degree of genetic differentiation was very low.

Geographical isolation was a natural barrier to gene flow among populations which was easy to become an important factor affecting genetic differentiation (Tian *et al*., 2005). There was no obvious relativity between genetic distance and geographical distance among 4 sheep breeds of Mongolian group in China. Although, gene flow derived from 2 genetic markers was different, it was far >1. That was to say, gene flow among populations was not very low because of geographical isolation. On the contrary, wide gene flow was observed among populations. Genetic structure of Mongolian Group in China did not accord with the model of geographical isolation.

Genetic drift and natural selection were 2 main factors to give rise to genetic differentiation among populations (Zheng *et al*., 1997). Wright (1931) thought if gene flow was >1, it could play a uniform action, namely resisting the action of genetic drift and preventing the differentiation between populations. This study showed gene flow was far >1 among breeds. Hence, big gene flow was a main reason leading to low degree of genetic differentiation of Mongolian Group in China. There were great differences in environmental conditions for four sheep breeds, which indicated that each breed had different reactions to environmental pressure.

CONCLUSION

While, degree of genetic differentiation was very low for sheep breeds of Mongolian Group in China. Therefore, we could infer primarily that genetic differentiation among sheep breeds of Mongolian Group in China was mainly caused by the impaction of natural selection (different environmental conditions).

ACKNOWLEDGEMENTS

This research was sponsored by Qing Lan Project, the Project of the Basic Natural Science Foundation for Colleges and Universities in Jiangsu Province (08KJB230002, 06KJD230203) and the International Cooperation Project of the National Natural Science Foundation of P.R. China (30571323, 30213001, 30210103007 and 3041013150).

REFERENCES

Crawford, A.M., K.G. Dodds and A.J. Ede, 1995. An autosomal genetic linkage map of the sheep genome. Genetics, 140: 703-724. PMID: 7498748. http://genome.cshlp.org/content/11/7/1275.full.

Excoffier, Laval L.G. and S. Schneider, 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1: 47-50. http://www.zoores.ac.cn/qikan/Cpaper/wenxian.asp?bsid=4707.

Geng, R.Q., H. Chang, Z.P. Yang, W. Sun, L.P. Wang and S.X. Lu, 2003. Study on origin and phylogeny status of Hu sheep. Asian-Aust. J. Anim. Sci., 16 (5): 743-747. http://cat.inist.fr/?aModele=afficheN&cpsidt =15029461.

Geng, R.Q., 2002. Study on Origin and Phylogenetic Status of Hu Sheep and Tong Sheep. 1st Edn. M.A. Degree. Yangzhou University, pp: 27-28. http://acad.cnki.net/kns55/detail/detail.aspx?dbcode=CMFD&QueryID=23&CurRec=1.

Lu, S.X., H. Chang, L. Du, K. Tsunoda, W. Sun, Z.P. Yang, G.B. Chang and D.J. Ji, 2005. Phylogenetic Relationships of Sheep Populations from Coastal Areas in East Asia. Biochemical Genetics, 43 (5): 251-259. http://www.ingentaconnect.com/content/klu/bigi/2005/00000043/F0020005/00005217?crawler=true.

Lu, S.X., H. Chang, K. Tsunoda, Z.J. Ren, W. Sun, Z.P. Yang, X.L. Ren and G.B. Chang, 2004. The levels of genetic differentiation of small tailed Han sheep and Tan sheep populations using structural loci. Scientia Agricultura Sinica, 38 (9): 1890-1897. DOI: 10.1016/S1671-2927(06) 60137-5. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B82XG-4MGJPPY-B&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=5171eff6b0a2ae1b7dd87e668e7b0ca8.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220. PMID: 6018555 https://tspace.library.utoronto.ca/bitstream/1807/1698/1/as03003.pdf.

Raymond, M. and F. Rousset, 1995. An exact test for population differentiation. Evolution, 49: 1280-1283. http://crop.scijournals.org/cgi/content/full/40/6/1521.

Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. Science, 236: 787-792. DOI: 10.1126/science.3576198. PMID: 3576198. http://www.chinagene.cn/qikan/Cpaper/wenxian.asp?bsid=11949.

Sun, W., H. Chang, Z.J. Ren, Z.P. Yang, R.Q. Geng, S.X. Lu, L. Du and K. Tsunoda, 2004. Genetic differentiation between sheep and goats based on microsatellite DNA. Asian-Aust. J. Anim. Sci., 17 (5): 583-587. http://www.pjbs.org/ijps/fin561.pdf.

Sun, W., H. Chang, Z.J. Ren, Z.P. Yang, R.Q. Geng, S.X. Lu, L. Du and K. Tsunoda, 2003. Multivariate statistic analysis of morphology and ecology characters on some sheep populations in China. Agric. Sci. China, pp: 1271-1276. http://direct. bl.uk/bld/PlaceOrder.do?UIN=142860981&ETOC=RN&from=searchengine.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123: 585-595. http://www.cs.uni-duesseldorf.de/NewMA/Personen/entry_44/Dokumente/rosskopf2006.pdf.

Tian, F.G., Z.J. Ma and Z.H. Chen, 2005. The molecular ecology and biodiversity. J. Southwest University for Nationalities (Natural Science Edn.), 31 (1): 115-120. http://epub.cnki.net/grid2008/detail.aspx?filename=XNMZ200501028&dbname=CJFQ2005.

Tsunoda, K., T. Amano and K. Nozawa, 1990. Genetic characteristics of Bangladeshi sheep as based on biochemical variations. Japanese J. Zootechnical Sci., 61 (1): 54-66. http://www.chinaagrisci.com/V2/qikan/manage/wenzhang/030117.pdf.

Wright, S., 1931. Evolution in Mendelian population. Genetics, 16: 91-159. http://journal.shouxi.net/qikan/articledes.php?id=381542.

Yang, Z.P., H. Chang, W. Sun, R.Q. Geng and Y.J. Mao, 2003. The comparison of structural loci and microsatellite marker on genetic differentiation in sheep (goat) populations. Acta Veterinaria et Zootechnica Sinica, 34 (5): 427-433. http://www.xmsyxb.com/qikan/manage/wenzhang/03-05-3.pdf.

Zheng, X.Z., H.F. Xu and H.J. Lu, 1997. Research progress on population genetic heterogeneity. Biodiversity, 5 (3): 210-216. http://www.biodiversity-science.net/qikan/manage/wenzhang/1997-493.pdf.

Zhou, J.Z., M.N. Wang and J.F. Niu, 1994. Ancient History of Husbandry and Veterinaria in China. 1st Edn. Beijing. Agric. Sci. Press of China, pp: 113-118.