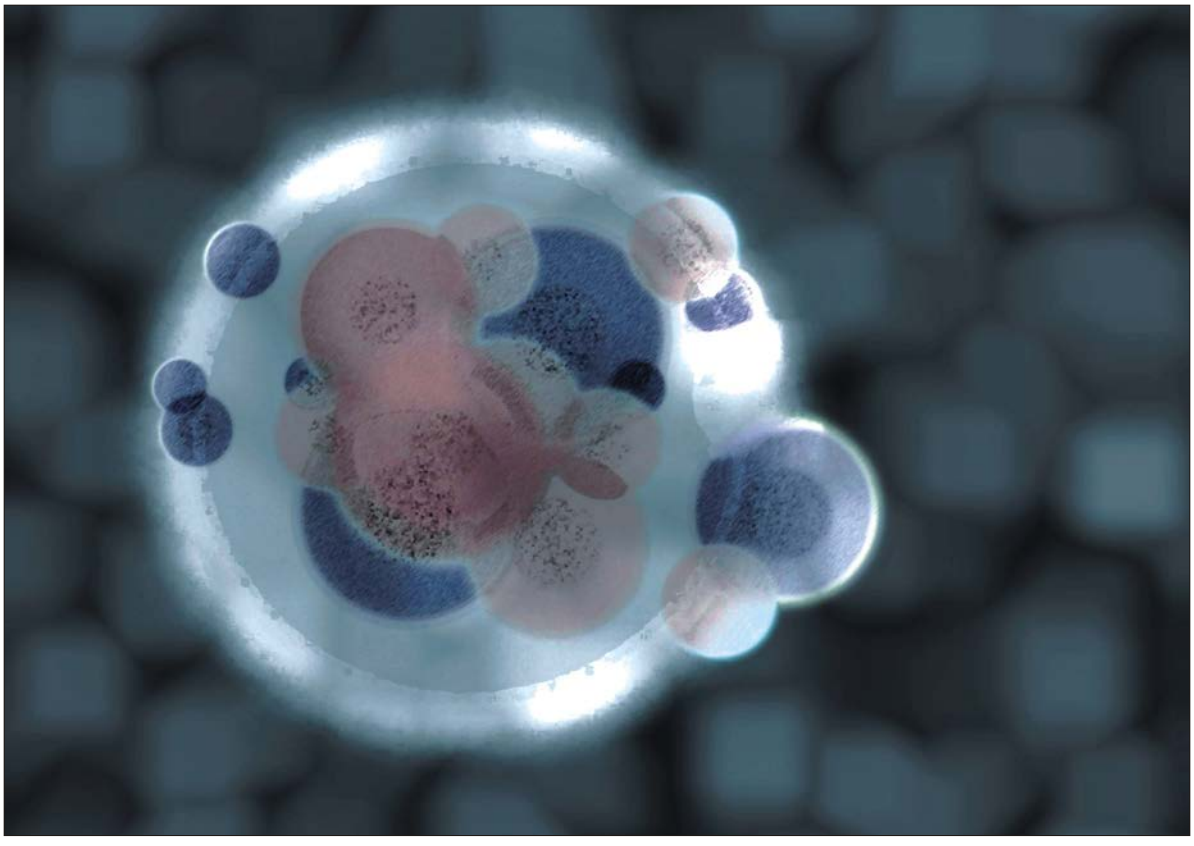

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Assessment of Genetic Diversity and Distance of Lipoprotein Lipase Between Red Jungle Fowl and Domestic Chicken Breeds

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Abstract: Polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) was used to analyze lipoprotein lipase gene polymorphism in Red Jungle Fowl (Gallus gallus spadiceus), Rugao, Anka, Wenchang and Slikies chicken populations. The results shows high lipoprotein lipase gene diversity, effective number of allele and Shannon information index in Silikes population. The average total genetic diversity (H_T), genetic diversity within population (H_S) and coefficient of genetic differentiation (G_{ST}) across all loci of lipoprotein lipase gene were 0.577±0.013, 0.535±0.011 and 0.073, respectively. The average gene flow across all loci in five chicken populations was 6.332. in addition, the highest lipoprotein lipase genetic distance was 0.220 between Rugao and Anka populations, while the lower was 0.013 between Wenchang and Silikes populations The constructed lipoprotein lipase phylogeny tree shows that Anka and Red jungle fowl were genetically related than the other breeds with 74.4% bootstrapping percentages and then both are related by 74.4% bootstrapping percentages with Wenchang followed by Rugao and Silikes breeds.

Key words: Lipoprotein lipase, genetic diversity, genetic distance, phylogeny tree

INTRODUCTION

Conservation of animal genetic resources is a topic of discussion since the 1950s (Simon, 1984). Biological, economical, cultural and emotional reasons are some of the arguments that support this initiative. It is essential to avoid the loss of genetic variability since these resources may be valuable for future breeding requirements (Hodges, 1984). Loss of genetic variation has been of particular concern in captive populations, whose sizes tend to be small over many generations out of practical necessity. Populations of endangered species also tend to be small and loss of genetic variation seriously threatens their abilities to persist and recover. A large number of studies, particularly during the 1970's, have documented the characterization of blood group and allozyme systems of livestock (Baker and Manwell, 1980). However, the level of polymorphism observed in proteins is often low which has reduced the general applicability of protein typing in diversity studies. With the development of Polymerase Chain Reaction (PCR) and sequencing technologies associated with automatic and/or semi-automatic large scale screening system, DNA based polymorphisms are now the markers of choice for molecular based surveys of genetic variation. Importantly, polymorphic DNA markers showing different patterns of Mendelian inheritances can now be studied in nearly all of our major livestock species. Typically, they include D-loop and cytochrome B mitochondrial DNA (mtDNA) sequences (maternal inheritance), Y chromosome specific Single Nucleotide Polymorphism (SNP) and microsatellites (paternal inheritance), autosomal microsatellite (bi-parental inheritance) (Avise, 1994). Genetic markers are powerful tools in genetic diversity of domestic animals (Zhang *et al*., 2002). Different categories of genetic markers may provide widely varying interpretations of the genetic structure of populations. For questions relating to the roles played by population size, mating systems and gene flow, selectively neutral genetic markers are preferred. However, for questions relating to the effects of selection, selected markers should be used (McDonald, 1997). For most questions in population genetics, it is best to use genetic markers that are selectively neutral, highly informative, reproducible and relatively easy (inexpensive) to assay. Lipoprotein lipase is a primary enzyme required for chylomicrons catabolism. In birds, LPL regulation in the adipose tissue seems to be less sensitive to the nutritional state (Hermier *et al*., 1984). Reduction of LPL activity should lead to increased triglycerides, decreased HDL and therefore premature atherosclerosis. Comparative studies in lean and fat lines of chickens show that in avian species, triglycerides accumulation in adipocytes depend manly on the availability of plasma substrate VLDL rather than the activity of LPL (Hermier *et al*., 1989). In this study chicken populations were varied from higher fat breed Anka to lean Red jungle fowl, to understand whether LPL effect is genetically or physiologically our aim was to estimate the diversity and distance of lipoprotein lipase gene between Red jungle fowl and domestics chicken populations.

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MATERIALS AND METHODS

Population, DNA extraction and primers design: Sample of blood from Anka, Rugao and Wenchang populations were collected from Jiangsu Poultry Institute, Yangzhou, China in September 2005. However, samples of Silkies and Red jungle fowl were the DNA used before and kept where our study was conducted in lab of animal genetic resource, College of Animal Science and Technology, Yangzhou University, China. DNA was isolated by saturated salt method previously described by (Sambrook *et al*., 1989). Lipoprotein lipase gene primers were designed by Oligo 6.0 deposited in (Table 1). Polymerase chain reaction single strand conformation polymorphism technique (PCR-SSCP) was developed for polymorphism analysis.

PCR-SSCP analysis: PCR-SSCP analysis was carried out in total volume 20 μL of PCR reaction, contain 100 ng of template DNA, 13.3 μL of sterilized distilled water, 0.5 μL (5 pmol) of each primer, 1.5 μL 10X PCR Buffer (Mg2plus), 2.5 μL of 2.5 mM dNTP Mixture and (5U uL^{-1}) of Taq polymerase (TakaRa Biotechnology Dalian Co., Ltd.). The PCR conditions determined was initially denatured at 94°C for 3 min, followed by 30 cycles at 94°C for 30 sec and annealed for 30 sec and 72°C for 30 sec, finally PCR product was extended at 72°C for 8 min. PCR products were loaded in to 12% (39:1) polyacrylamide under 150 V for 9 h. Electrophoresis and silver stained, the gel was visualized under ultraviolet light using trans-illuminator photographed by (Eastman Kodak Digital Science DC120).

Statistical analysis: The genetic diversity, effective number of allele, Shannon's information index and genetic diversity statistics, total genetic diversity (H_T), genetic diversity within population (H_S), coefficient of gene differentiation (G_{ST}) performed using the Popgene version 1.31. Polymorphism Information Content (PIC) estimated according to the following formula (Botstein *et al*., 1980).

$$PIC = 1 - \sum_{I=1}^{} Pi^2 - \sum_{I=1}^{} \sum_{j=I+1} Pi^2 Pj^2$$

N = number of alleles, Pi = gene frequency of the allele I, Pj = gene frequency of allele j.

The Neighbor Joining (NJ) method implemented by Phylip version 3.63 was used to construct the phylogenetic tree and reliability of tree Topology was examined by bootstrap re-sampling.

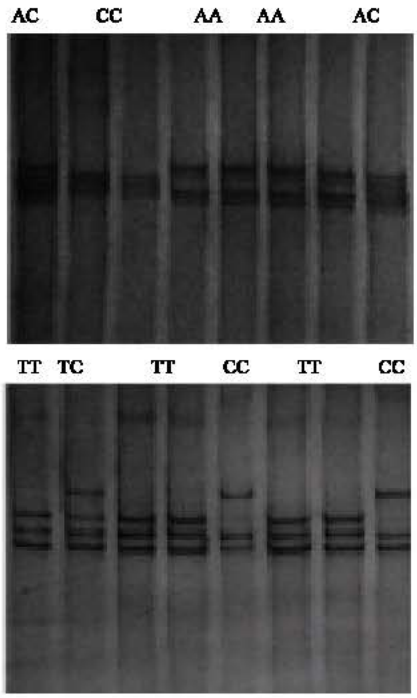

Fig. 1: Polymorphisms of LPL10 primer in intron8 of lipoprotein lipase gene, (A) PCR-SSCP genotype, (B) Sequence analysis

RESULTS

Genetic diversity: The gene diversity a proportion of individuals heterozygous at a locus was a measurement unit for population heterozygosity. The level of mean population heterozygosity reflected the degree of population genetic consistency. The lower of population heterozygosity, was related with the higher of the population genetic consistency and vice versa. In the present study genetic diversity was detected by PCR-SSCP techniques (Fig. 1). Lipoprotein lipase gene was high diverse in Silikes and low diverse in Red jungle fowl, while, LPL10 locus showed high diversity with average 0.571±0.107 of the gene. The effective allele number was estimate the reciprocal of homozygosity it is ranged from 2.682±0.345 to 1.845±0.915 for Silikes and Red jungle fowl, respectively (Table 2). LPL10 locus observed higher estimates 2.827, with average 2.405±0.597 of the gene. On the other hand, Shannon's Information Index was ranged from 1.036±0.071 to 0.654±0.494 for Silikes and Red jungle fowl, respectively, with average 0.953±0.162 across loci (Table 3). In addition, the mean Polymorphism Information

Table 1: Primers sequences, location, PCR product and annealing temperature of chicken lipoprotein lipase gene

Primers	Sequences (5-3 flanking region)	Direction	Location	PCR product (bp)	Annealing name temperature (°C)
LPL -9	GACGAAACATGGAAAACAG	Upstream	14486-14843	357	54.4
LPL -9	CAACTCCCAAGAAAACTCA	Downstream			
LPL-10	GCTGAGTTTTCTTGGGAGTTGGG	Upstream	14822-15217	395	59.8
LPL-10	GCCTTGCTCCCTTGAATGTTTG	Downstream			

Table 2: Genetic variation statistics of lipoprotein lipase in populations

Population	Sample size	Na	Ne	He	I	PIC
Rugao	89	3.000±0.000	1.849±0.171	0.457±0.050	0.796±0.087	0.299±0.038
Anka	59	3.000 ±0.000	2.639±0.158	0.621±0.023	1.024±0.0457	0.406±0.013
Wenchang	30	3.000±0.000	2.518±0.653	0.589±0.107	0.969±0.1787	0.336±0.019
Silikes	30	3.000±0.000	2.682±0.345	0.624±0.048	1.036±0.071	0.409±0.026
Red jungle	33	2.500±0.707	1.845±0.915	0.382±0.307	0.654±0.494	0.239±0.155

Na, Observed number of alleles; Ne, Effective number of alleles; He, gene diversity; I, Shannon's Information index; PIC, Polymorphism information contents

Table 3: Genetic variation statistics of lipoprotein lipase loci

Locus	Sample size	Na	Ne	He	I	PIC
LPL9	239	3.000	1.983	0.496	0.838	0.288
LPL10	243	3.000	2.827	0.646	1.068	0.388
Mean	241	3.000	2.405	0.571	0.953	0.338
St. Dev		0.000	0.597	0.107	0.162	0.050

Na, Ne, He, I and PIC were as defined in Table 2

Table 4: Lipoprotein lipase gene diversity in subdivided populations

Locus	Sample size	H_T	H_S	G_{ST}	Nm
LPL9	239	0.496	0.459	0.074	6.238
LPL10	243	0.658	0.610	0.072	6.404
Mean	241	0.577	0.535	0.073	6.332
St. Dev		0.013	0.011		

H_T, Total genetic diversity; H_S, Genetic diversity within population; G_{ST}, coefficient of genetic differentiation; Nm, gene flow

Table 5: Nei's original measures of lipoprotein lipase genetic identity and distance

Pop ID	Rugao	Anka	Wenchang	Silkies	Red jungle fowl
Rugao	1.0000	0.8024	0.9216	0.9306	0.8245
Anka	0.2202	1.0000	0.9507	0.9538	0.8729
Wenchang	0.0816	0.0505	1.0000	0.9876	0.9075
Silkies	0.0720	0.0473	0.0125	1.0000	0.8832
Red jungle fowl	0.1930	0.1360	0.0970	0.1243	1.0000

Genetic identity above diagonal and genetic distance below diagonal

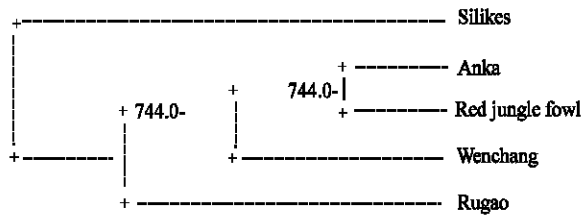

Fig. 2: Phylogeny tree of lipoprotein lipase in Red jungle and domestics chicken populations

Content (PIC) was an ideal index to measure the polymorphism of allele fragments. PIC>0.5, indicated that the locus of high polymorphism, 0.25<PIC<0.5, indicated the locus of medium-polymorphism and PIC<0.25, indicated the locus of low polymorphism. In the present study all populations observed medium polymorphism for lipoprotein lipase gene.

F-statistics and gene flow: The average total genetic diversity (H_T), genetic diversity within population (H_S) and coefficient of genetic differentiation (G_{ST}) across all loci of lipoprotein lipase gene were 0.577±0.013, 0.535±0.011 and 0.073, respectively (Table 4). LPL10 shows high estimates compared with LPL6. The G_{ST} estimates are used to predict gene flow which was a fundamental micro evolutionary force that can determine the potential for genetic differentiation among populations and for local adaptation and also influences the geographical spread of novel adaptations. In this study the average lipoprotein lipase gene flow was 6.332 (Table 4).

Genetic distance and phylogeny tree: The highest lipoprotein lipase genetic distance was 0.220 between Rugao and Anka populations, while the lower was 0.013 between Wenchang and Silikes populations (Table 5). The constructed lipoprotein lipase phylogeny tree shows

that Anka and Red jungle fowl are genetically related than the other breeds with 74.4% bootstrapping percentages and then both are related by 74.4% bootstrapping percentages with Wenchang followed by Rugao and Silikes breeds (Fig. 2).

DISCUSSION

Genetic diversity and distance: In this study Silkies shows highest lipoprotein lipase gene diversity. The low genetic diversity observed may be due to high rates of selection pressure among populations. Therefore, the ideal measure of gene diversity within and between breeds would be based on the genes that control variation in relevant quality, disease resistance, fitness and other traits. The mean number of alleles and observed and expected heterozygosity are the most commonly

calculated population genetic parameters for assessing within breed diversity (Hanotte and Jianlin, 2005).The effective number of allele and Shannon information index in lipoprotein lipase gene was high in Silikes. Polymorphism Information Content (PIC) is an ideal index to measure the polymorphism of allele fragment (Chen *et al.*, 2004). The reason behind medium polymorphism may be due to geographical distribution and selection intensity of population, as well as a functional gene polymorphism was expected to be very low.

G_{ST} is a genetic statistic describing differentiation of populations values of G_{ST} range from zero to one, with low values indicating that little variation is proportioned among populations, high values denote that a large amount of variation is found among populations. The most obvious explanation for this genetic subdivision would be the geographical barriers preventing genetic exchange among the five chicken populations.

The past four decades have seen a proliferation of studies describing the genetic structure of natural populations and the accumulating body of evidence suggests there is no single role of gene flow in differentiation and adaptation, but that its role varies depending on its strength relative to other evolutionary forces and on the geographical distribution of populations (Slatkin, 1985). Therefore, the relative strength of gene flow may be affected by a variety of ecological factors that vary intra and inter-specifically such as dispersal ability, dietary specialization and phonological asynchrony among populations, habitat persistence, population persistence and spatial structure of habitat within the landscape (Peterson and Denno, 1998).

A formula for the variance of gene identity was derived for the case of neutral mutations using diffusion approximations for the changes of gene frequencies in a subdivided population. The genetic distance of lipoprotein lipase gene was found high between Rugao which was very lean and Anka which was very fat. In addition, the lower distance was found between Wenchang and Silikes which were genetically similar. On the other hand, there is an intermediate range of gene flow in which population subdivision can increase the variance. The formula for obtaining the variance allows us to study such statistics as the coefficient of gene differentiation and the correlation of heterozygosity (Takahata, 1981). The phylogeny tree indicated that Anka and Red jungle fowl are genetically related than the other breeds. This result was in contrast to the phenotypic background in terms of body weight, while it may concern the physiological function of gene. The ability to identify associations between markers and traits of economic interest can be considerably improved if the genetic distance between the two founder lines is maximized. The geographical distribution of the respective breeds was as follow; Silkies, Red jungle fowl, Wenchang and Rugao were native chicken breeds in China and the Anka was exotic breed. The economic value of breed indicated that Silkies and Red Jungle Fowl were very lean, Wenchang and Rugao was dual purpose however and Anka was fat chicken breed. From this study we can conclude that lipoprotein lipase gene was highly diverse between chicken populations. In addition, approximately 100 naturally occurring mutations in the LPL gene have been described in humans (Merkel *et al.*, 2002).

REFERENCES

Avise, J.C., 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, New York.

Baker, C.M.A. and C. Manwell, 1980. Chemical classification of cattle. I. Breed group. Animal Blood Groups and Biochemical. Genetics, 11: 127-150.

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

Chen, G.H., X.S. Wu, D.Q. Wang, J. Qin and S. Wu *et al.*, 2004. Cluster analysis of 12 Chinese native chicken populations using micro satellite markers. Asian-Aust. J. Anim. Sci., 17: 1047-1052.

Hanotte, O. and H. Jianlin, 2005. Genetic characterization of livestock populations and its use in conservation decision- making the role of biotechnology. Villa Gualino, Turin, Italy, 5-7 March.

Hermier, D., M.J. Chapman and B. Leclercq, 1984. Plasma lipoprotein profile in fasted and refed chickens of two lines selected for high or low adiposity. J. Nutr., 114: 1112-1121.

Hermier, D., A. Quignard-Boulangé, I. Dugail, G. Guy, M.R. Salichon, L. Brigand, B. Ardouin and B. Leclercq, 1989. Evidence of enhanced storage capacity in adipose tissue of genetically lean and fat chickens. J. Nutr., 119: 1369-1375.

Hodges, J., 1984. Conservation of animal genetic resources. Livest. Prod. Sci., 11: 1-2.

McDonald, B.A., 1997. The population genetics of fungi: Tools Tech. Phytopathol., 87: 448-453.

Merkel, M., J. Heeren, W. Dudeck, F. Rinninger, H. Radner, J.L. Breslow, R. Zechner and H. Greten, 2002. Inactive Lipoprotein Lipase (LPL) alone increases selective cholesterol ester uptake *in vivo*, whereas in the presence of active LPL it also increases triglyceride hydrolysis and whole particle lipoprotein uptake. J. Biol. Chem., 277: 7405-7411.

Peterson, M.A. and R.F. Denno, 1998. The influence of dispersal and diet breath on patterns of genetic isolation by distance in polyphagous insects. Am. Naturalist., 152: 438-446.

Sambrook, J., E.F. Fristch and T. Manistais, 1989. Molecular cloning: A Laboratory Manual. Vol. 3. Cold Spring Harbor Laboratory Press. Cold Spring Harbor. USA.

Simon, D., 1984. Conservation of animal genetic resources. A review. Livest. Prod. Sci., 11: 23-36.

Slatkin, M., 1985. Rare alleles as indicators of gene flow. Evolution, 39: 53-65.

Takahata, N., 1981. Genetic variability and rate of gene substitution in a finite population under mutation and fluctuating selection. Genetics, 98: 427-440.

Zhang, X., F.C. Leung, D.K.O. Chan, G. Yang and C. Wu, 2002. Genetic diversity of Chinese native chicken breeds based on protein polymorphism, randomly amplified polymorphic DNA and micro satellite polymorphism. Poult. Sci., 81: 1463-1472.