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Genetic Differentiation and Phylogeny Relationships of Functional ApoVLDL-II Gene in Red Jungle Fowl and Domestic Chicken Populations

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Abstract: A total of 243 individuals from Red Jungle Fowl (*Gallus gallus spadiceus*), Rugao, Anka, Wenchang and Silikes chicken populations were used for polymorphism analysis in functional apoVLDL-II gene by Restriction fragment length polymorphism and single strand conformation polymorphism markers. The results show that Anka population has highest gene diversity and Shannon information index, while Red jungle fowl shows highest effective number of allele. In addition, the higher coefficient of genetic differentiation (G_{ST}) across all loci in apoVLDL-II was indicating that high variation is proportioned among populations. As expected total gene diversity (H_T) has upper estimate compared with within population genetic diversity (H_S) across all loci. The mean G_{ST} value across all loci was (0.194) indicating about 19.4% of total genetic variation could be explained by breeds differences, while the remaining 80.6% was accounted for differences among individuals. The average apoVLDL-II gene flow across all loci in five chicken populations was 1.189. The estimates of genetic identity and distance confirm that these genes are significantly different between genetically fat and lean population, because fat type breed Anka shows highest distance with the other Silikes and Rugao whish are genetically lean. In addition, Wenchang and Red jungle fowl were found more closely and genetically related than the other breeds with 49.4% bootstrapping percentages, then they were related to Silikes by 100% bootstrapping percentages followed by Rugao and finally all of them are related with exotic fat breed Anka.

Key words: Genetic differentiation, phylogenetic relationship, apoVLDL-II, Red jungle fowl, domestic chicken

INTRODUCTION

Chickens are belonging to the genus *Gallus* spp. which include four species of Jungle fowl; *Gallus gallus* (Red jungle fowl), *Gallus sonneratti* (Grey jungle fowl), *Gallus lafayetti* (Ceylon jungle fowl) and *Gallus varius* (Green jungle fowl). All reports indicate Red jungle fowl as ancestor of domestic fowls (West and Zhou, 1989). Because the genetic distance between Red jungle fowl and domestic fowl was very low (Mohd-Azmi *et al*., 2000). Defining the genetic structure of populations is a logical first step in studies of chicken population genetics because the genetic structure of a population reflects its evolutionary history and its potential to evolve. For evolution to occur by natural selection there must be variation in fitness among individuals. Genetic variability in a species occurs both among individuals within populations as well as among populations (Wright, 1978). Variation within populations is lost through genetic drift (Allendorf *et al*., 1987), while variation among populations is lost when previously restricted gene flow between populations is increased for some reason (e.g., stocking, removal of natural barriers such as waterfalls). Campton (1987) indicated the lost of differentiation between populations is a result of the homogenization of two previously distinct entities. Beyond this loss of genetic variation, mixing two groups can result in out breeding depression, which is the loss of fitness in offspring that results from the mating of two individuals that are too distantly related (Templeton, 1987). This loss in fitness is caused by the disruption of the process that produced advantageous local adaptations through natural selection. Analysis of the genetic diversity of a function gene is an important component for the success of population conservation. Most chicken growth and fitness traits are known controlled by multiple genes (Deeb and Lamont, 2002), which have been identified as a candidate used to improve animal traits through Markers Assisted Selection (MAS) on genotype (Dekkers, 2004). ApoVLDL-II gene is a major transporter of triglycerides and attempts to reduce excessive fatness in bird have involved the control of VLDL metabolism. Selection for low plasma VLDL concentration for 10 generations in chicken has reduced the rate of VLDL secretion by at least 50% whereas

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Table 1: Primers sequences, location, PCR product and annealing temperature of chicken apoVLDL-II gene

Primers name	Sequences (5-3 flanking region)	Direction	Location	PCR product (bp)	Annealing temperature (°C)
VLDL6	CCTCTATGACATGGT TGCCT	Sense	1549-2041	492	58
VLDL6	ATGGGTTTGACCCTGCTA TG	Antisense			
VLDL-9	CACCTTTCTAAATGCACAGT	Sense	2490-2759	289	53.4
VLDL-9	GCAATGATCTTCTGAATGAC	Antisense			
VLDL-10	ATTGACTAGCGTGAGATTCC	Sense	2788-3071	303	57
VLDL-10	ATGATGGTGCAGTTCTTCTT	Antisense			
VLDL-17	ACTGCCTATTCCTGCCTTCT	Sense	4199-4479	280	56
VLDL-17	CACCGACTTTTCTTCCAACT	Antisense			

selection for high VLDL concentration has increased the rate of VLDL secretion over 2-fold (Griffin *et al*., 1989). According to Griffin *et al*. (1989) we consider the genetic polymorphism of apoVLDL-II among five chicken populations which ranged from very lean Red jungle fowl to very fat Anka, to estimate the functional apoVLDL-II gene diversity and to determine the genetic distance and develop a divergence dendrogram for chicken populations that may be suitable for conservation purpose.

MATERIALS AND METHODS

Animal populations: This study was conducted in College of Animal Science and Technology, Yangzhou University, China. Approximately 243 individual were used for this study, blood samples were collected from Rugao (89), Anka (59) and Wenchang (30) chickens in Jiangsu Poultry Institute, Yangzhou, China in September 2005. In addition, DNA of Silkies (32) and Red jungle fowl (33) were taken from the lab of Animal Genetic Resource of our College.

Primers and DNA extraction: Genomic DNA was isolated from the whole blood using saturated salt method (Sambrook *et al*., 1989). Primers were designed by Primers 5.0 and Oligo 6.0 based on the published sequences in Genebank, accession number (J00810), one pair was designed by (Li *et al*., 2005) (Table 1).

PCR-SSCP and PCR-RFLP genotype: Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was used for polymorphism analysis in VLDL6 locus by *SfcI* restriction enzyme (Sangon, China) (Li *et al*., 2005). While polymerase chain reaction single strand conformation polymorphism technique (PCR-SSCP) as described by Orita *et al*. (1989) was developed for the polymorphism analysis for other loci.

Genetic diversity analysis: The effective allele number was estimated as a reciprocal of homozygosity, genetic diversity and Shannon index estimates were performed using the Popgene version 1.31. Correlation coefficient among the heterozygosity and Shannon index were estimated using Pearson bivariate correlation coefficient of SPSS 11.5. Polymorphism Information Content (PIC) was estimated according to the following formula (Botstein *et al*., 1980):

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

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

The Popgene program was farther used to calculate total genetic diversity (H_T), genetic diversity within population (H_S), coefficient of gene differentiation (G_{ST}).

$$G_{ST} \frac{H_T -- H_S}{H_T}$$

Moreover, gene flow was estimated from G_{ST} or G_{CT} as

$$Nm = \frac{0.5(1 - G_{ST})}{G_{ST}}$$

Phylogeny analysis: Genetic diversity among Red Jungle Fowl and domestic chicken populations was quantified using Nei *et al*. (1983) anaqular genetic distance (DA). The Neighbor Joining (NJ) method implemented in the phylip version 3.63 was used to construct the phylogeny tree. Reliability of tree Topology was examined by bootstrap re-sampling.

RESULTS

Genetic diversity: The genotypes of apoVLDL-II gene polymorphism in various loci were presented in (Fig. 1). In addition, apoVLDL-II genetic variation statistics in various populations and loci were presented in (Table 2 and 3). ApoVLDL-II gene was found high diverse in Anka breed and lower in Silkies breed, whereas VLDL17 locus showed highest diversity with an average 0.363"0.162 of the gene. The effective number of allele estimate the reciprocal of homozygosity, it was ranged from 1.571±0.697 to 1.204±0.135 in Red jungle fowl and Wenchang breeds, respectively, while among loci was ranged from 1.999-1.207 in VLDL17 and VLDL9, respectively. Shannon's information index a measure of

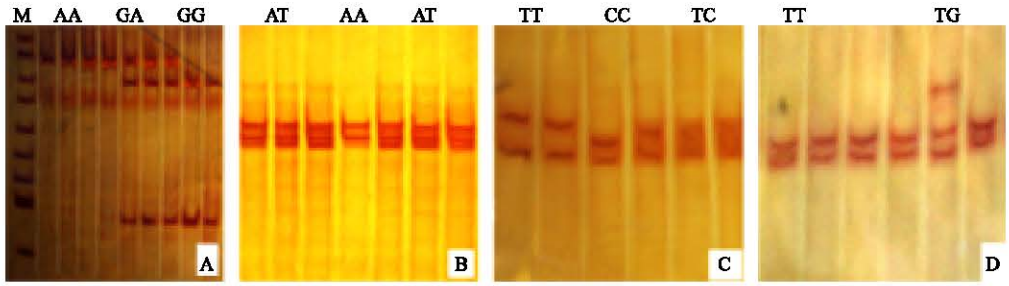

Fig. 1: Analysis of apoVLDL-II gene polymorphisms (A) PCR-RFLP of VLDL6 locus (B) PCR-SSCP of VLDL9 locus (C) PCR-SSCP of VLDL10 locus (D) PCR-SSCP of VLDL17 locus

Table 2: Genetic variation statistics of apoVLDL-II gene in various populations

Population	Sample size	Na	Ne	He	I	PIC
Rugao	89	2.250±0.500	1.543±0.539	0.288±0.246	0.466±0.357	0.307±0.088
Anka	59	2.250±0.500	1.538±0.350	0.324±0.154	0.516±0.213	0.312±0.071
Wenchang	30	2.000±0.000	1.204±0.135	0.162±0.091	0.292 ±0.129	0.177±0.089
Silkies	32	2.000±0.817	1.221±0.221	0.160±0.153	0.290±0.262	0.273±0.063
Red Jungle	33	2.000±0.817	1.571±0.697	0.270±0.288	0.432±0.449	0.271±0.093

Na, observed number of alleles; Ne, effective number of alleles; He, gene diversity; I, Shannon's Information index and PIC, polymorphism information contents

Table 3: Genetic variation statistics of apoVLDL-II gene in various loci

Locus	Sample size	Na	Ne	He	I	PIC
VLDL6	243	3.000	1.399	0.286	0.508	0.174
VLDL9	243	2.000	1.207	0.171	0.313	0.419
VLDL10	243	3.000	1.974	0.493	0.794	0.191
VLDL17	243	2.000	1.999	0.499	0.693	0.287
Mean	243	2.500	1.645	0.363	0.577	0.268
St. Dev		0.577	0.403	0.162	0.212	0.056

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

gene diversity in apoVLDL-II gene was ranged from 0.516±0.213-0.290±0.262 for Anka and Silikes, respectively. Correlation between heterozygosity and Shannon index was highly significance 0.973. The mean Polymorphism Information Content (PIC) was obtained using the gene frequencies data, all populations shows medium Polymorphism Information Content (PIC), except Wenchang breed has observed low polymorphism (PIC).

F-Statistics and gene flow: In order to evaluate the genetic diversity within and between chicken populations, genetic diversity (H_T and H_S) and genetic subdivision (G_{ST}) for each locus across all populations were estimated. The average total genetic diversity (H_T), genetic diversity within population (H_S) and coefficient of genetic differentiation (G_{ST}) across all loci were 0.342±0.019, 0.241±0.007 and 0.296, respectively (Table 4). As expected total gene diversity (H_T) has upper estimated compared with within population genetic diversity (H_S) across all loci. In this study the mean G_{ST} value across all loci was (0.194) indicating around 19.4% of total genetic variation could be explained by breeds differences, while the remaining 80.6% was accounted for differences among individuals. Frequently, estimates of G_{ST} are used to predict other genetic phenomena, such as gene flow

Table 4: Nei's analysis of apoVLDL-II gene diversity in subdivided populations

Locus	Sample size	H_T	H_S	G_{ST}	Nm
VLDL6	243	0.3480	0.3003	0.1372	3.1454
VLDL9	243	0.1490	0.1227	0.1765	2.3321
VLDL10	243	0.3924	0.2811	0.2837	1.2627
VLDL17	243	0.4773	0.2580	0.4594	0.5884
Mean	243	0.3417	0.2405	0.2960	1.1890
St. Dev		0.0194	0.0065		

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 apoVLDL-II genetic identity and distance

Pop ID	Rugao	Anka	Wenchang	Silkies	Red jungle fowl
Rugao	1.000	0.905	0.788	0.797	0.761
Anka	0.100	1.000	0.735	0.699	0.753
Wenchang	0.238	0.308	1.000	0.994	0.952
Silikes	0.226	0.357	0.006	1.000	0.943
Red jungle fowl	0.273	0.284	0.049	0.058	1.000

Genetic identity above diagonal and genetic distance below diagonal

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 our recent study the average apoVLDL-II gene flow across all loci in five chicken populations was 1.189 Table 4.

Genetic distance and phylogeny relationship: The reliable measures of differences between populations are genetic distance, which can be estimated from the differences in gene frequencies as a number of marker loci. The identity of apoVLDL-II gene was found higher between Silikes and Wenchang breeds 0.994 and lowers between Silikes and Anka 0.699, in contrast the higher genetic distance was 0.357 between Anka and Silikes and lower was 0.006 between Wenchang and Silikes Table 5. The relationships

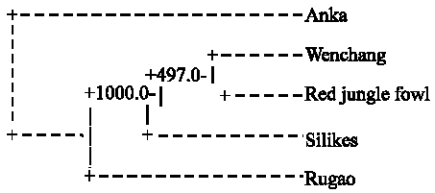

Fig. 2: Polygenetic relationships of apoVLDL-II in Red jungle and domestics chicken populations

between populations can be constructing using phylogeny tree. In this study the dendrogram inferred for the modern cultivars and breeding lines is given in (Fig. 2), it was obtained after neighbor joining cluster analysis in PHYLIP. In apoVLDL-II gene Wenchang and Red jungle fowl are more closely and genetically related than the other breeds with 49.4% bootstrapping percentages, then they were related to Silikes by 100% bootstrapping percentages followed by Rugao and finally all of them are related with exotic fat breed Anka.

DISCUSSION

Genetic diversity: Analysis of the patterns of molecular genetic variation within a species is usually motivated by the desire to identify genetic relationships among populations. This information can be used to determine phylogenetic associations and ultimately the underlying evolutionary history of the species (Chenyambuga *et al*., 2004). In this study Anka chicken breed shows highest apoVLDL-II gene diversity. Our results are lower than those estimated for Chinese chicken populations using microsatellite marker (Zhang *et al*., 2002; Shen, 2004). 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 higher Shannon information index and Polymorphism Information Content (PIC) in apoVLDL-II gene were recorded in Anka, while the lower was recorded in Silkies and Wenchang, respectively. It is confirms that apoVLDL-II gene was highly diverse between chicken pupolations. The Polymorphism Information Content (PIC) was an ideal index to measure the polymorphism of allele fragment (Chen *et al*., 2004). The reason behind medium polymorphism in this study may be due to geographical distribution and selection intensity of population, as well as a functional gene polymorphism was expected to be very low and RFLP is relatively low level of polymorphism (Liu and Cordes, 2004).

F-Statistics and gene flow: The higher coefficient of genetic differentiation (G_{ST}) across all loci in apoVLDL-II indicating that high variation is proportioned among populations. In this study total apoVLDL-II gene diversity (H_T) has upper estimated compared with within population genetic diversity (H_S) across all loci. The mean G_{ST} value across all loci was (0.194) indicating around 19.4% of total genetic variation could be explained by breeds' differences, while the remaining 80.6% was accounted for differences among individuals. The most obvious explanation for this genetic subdivision would be the geographical barriers preventing genetic exchange among the five chicken populations. Weir and Basten (1990) indicated that the simplest parameters for assessing the distribution of diversity between breeds using genetic markers are the genetic differentiation or fixation indices (e.g., FST, GST, θ). Gene flow between or among populations can still be detected as a genetic signature in allele frequency variation for many generations after the cessation of migration between two populations (Slatkin and Barton, 1989). Levels of gene flow are expected to be proportional to the geographic distance between discrete populations (Kimur and Weiss, 1964). In this study the average gene flow across all loci in five chicken populations was found to be 1.189. It has been demonstrated that diffusive gene flow will prevent substantial genetic differentiation due to genetic drift if gene flow is greater than unity (Slatkin, 1985).

Genetic distance and Phylogeny relationship: Molecular estimates of the evolutionary distance between divergent chicken breeds have profound implications for the prediction of heterosis. Generally, the degree of heterosis will increase as the genetic distance between two populations becomes larger. This is a direct result of the mathematical formulation of all measures of genetic distance (Nei, 1987). A formula for the variance of gene identity (homozygosity) was derived for the case of neutral mutations using diffusion approximations for the changes of gene frequencies in a subdivided population. In this study apoVLDL-II genetic distance was high between Anka and Silikes and low between Wenchang and Silikes, These estimates confirm that these genes are significantly different between genetically fat and lean population, because Anka which was fat type breed shows highest distance with the other Silikes and Rugao whish are genetically lean. It is shown that when gene flow is extremely small, the variance of gene identity for

the entire population at equilibrium is smaller than that of the panmictic population with the same mean gene identity. On the other hand, although a large amount of gene flow makes a subdivided population equivalent to a panmictic population, there is an intermediate range of gene flow in which population subdivision can increase the variance (Takahata, 1981).

The genetic structure of the five chicken populations can also be investigated through phylogenetic analysis. In apoVLDL-II gene Wenchang and Red jungle fowl are more closely and genetically related than the other breeds, then they were related to Silkies followed by Rugao and finally all of them are related with exotic Anka breed. Low bootstrap values and varying trees were constructed from distance matrices with individual animals. The lower degree of clustering observed in Wenchang and Red jungle fowl is presumably due to higher allelic heterogeneity as a consequence of their evolutionary history. 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. Generally when constructing phylogeny tree, it is difficult to judge succinctly, which is the best one with regard to the genetic relationship of the examined populations. Phylogeny relationship was support the history of geographical location and the economic value of the populations (Pandey *et al*., 2002). Finally from the genetic diversity, differentiation, distance and phylogeny trees we can conclude that apoVLDL-II gene was significantly different between fat and lean chickens which support the selection study for low plasma VLDL concentration for 10 generations carried out by (Griffin *et al*., 1989).

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