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RESEARCH ARTICLE

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Analysis of genetic distribution and population genetic structure of the *MyoD* gene in 10 pig breeds

Abstract Restriction fragment length polymorphism (RFLP) data was applied to analyze the distribution of the *MyoD* gene in 10 pig breeds and pig breed crosses. The population genetic information about genetic distribution, variation, and heterozygosity of the *MyoD* gene in different breed populations were analyzed. Based on the allele frequency, genetic distance and evolution distance among each breed populations were calculated and Unweighted Pair Group Method with Arithmetic mean (UPGMA) phylogenetic tree was gained based on the evolution distances between populations. The results indicated that the distribution of the *MyoD* genotype kept in Hardy-Weinberg equilibrium in most tested groups but not in Duroc (D) and Duroc × (Landrance × Yorkshire) (DLY) population. Generally, the genetic diversity of the *MyoD* gene was abundant and these tested breed populations had high genetic variations. The evolution of the *MyoD* gene was under natural selection pressure. On the phylogenetic tree, 10 pig breeds were divided into 4 clusters. The first cluster consisted of four breeds developed from Landrace. The second cluster was two indigenous Chinese pig breeds. The third cluster was three breeds developed from Duroc. The fourth cluster was a Tibetan pig breed. The constitution of the topology of the phylogenetic tree was consistent with the breeding history of each pig breed. From this experiment, we can conclude that some RFLP data obtained from functional gene can be used in the genetic deviation research between some closely related species or between different populations in certain species.

Keywords pig, *MyoD* gene, genetic diversity, phylogenesis

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1 Introduction

The *MyoD* gene is a member of the myogenic regulatory factors (MRFs) gene family, which is believed to play an important regulatory role in starting and maintaining the differentiation and development of skeletal muscle (Olson et al., 1991; Weintraub, 1993). Experiments have proven that the expression of the *MyoD* gene is associated with the differentiation of myoblasts. The overexpression of the *MyoD* gene will repress the proliferation of myoblast and thus speed the formation of mature muscle fiber cell from myoblasts (Megeney et al., 1996; Smith et al., 1994). In addition, the *MyoD* gene can also indirectly affect the terminal differentiation of muscle fiber cell through changing the activity of the *MyoG* gene (Weintraub, 1993). Postnatal *MyoD* expression is found only in satellite cells in muscles and the level of *MyoD* mRNA expression is related to the activity of the satellite cells (Megeney et al., 1996; Smith et al., 1994). Because the proliferation and differentiation of satellite cells will affect the muscle growth rate and production, the genetic diversity of the *MyoD* gene may probably affect these productive traits related with muscle (Te Pas et al., 2000; Knol et al., 1997; Cieslak et al., 2000; Jiang et al., 1997; Liu et al., 2003). In view of its important function, the genetic structure of the *MyoD* gene is relatively conservative.

In the research of the population genetic structure and evolution, genetic markers with high polymorphism information content, such as microsatellite and nucleotide sequence, are usually utilized (Nei and Kumar, 2000; Avise, 2000; Wang and Li, 2002). Because of its relatively small nucleotide replacement number, the RFLP marker is not ideal for researching the population genetic structure and phylogenesis. However, the RFLP marker is a quick and cheap method to detect the nucleotide replacement with the characteristic of codominance (Liu et al., 2005; Teng et al., 2005). Avise has proven that when the relationship between nucleotide sequences is very close, the RFLP marker can reflect the genetic structure of the population and therefore

can be used to research the genetic diversity and evolution relationship between different and closely related species or different populations in certain species (Avise, 2000).

Using the RFLP technique, Cieslak researched the genetic distribution of the *MyoD* gene in 7 different Polish pig breeds and proved the existence of significant difference on the composition of carcasses between different genotype individuals (Cieslak et al., 2000). The genetic resource of indigenous Chinese pig breeds is very abundant, but reports about the genetic variation and genetic structure of the *MyoD* gene in indigenous Chinese pig breeds are very limited (Zhu and Li, 2005). In our study, the PCR-RFLP technique was used to detect the *MyoD* genotype of individuals from 10 pig breeds. These 10 pig breeds were from four genetic types including indigenous Chinese breeds, developed breeds, hybrid breeds and foreign breeds, respectively. The genetic distribution and population genetic structure of the *MyoD* gene were analyzed and the evolution distances between the breeds were calculated based on the gene frequency data. The phylogenetic tree of these populations was obtained by calculating their evolution distances. Additionally, the possibility of utilizing RFLP data in researching of genetic relationship and evolution was discussed.

2 Materials and methods

2.1 Sampling

Tissue samples of 168 individuals from five indigenous Chinese breeds [Yanan pig (YN), Rongchang pig (RC), Tibetan pig (TB), Wuzhishan pig (WZS) and Dahe pig (DH)], two foreign breeds [Duroc (D) and Landrace (L)], one developed breed [Dahewu pig (DW)] and two hybrid breeds [Landrance × Yanan (LYa), Duroc × (Landrance × Yorkshire) (DLY)] were collected and detected in this study.

2.2 Genotype detection

Based on the pig *MyoD* gene sequence (GenBank accession No. U12574), amplification primers were designed by the Primer 5.0 software. The length of the amplification product is 497 bp which encompasses the complete intron 1 and part exon 2 of the *MyoD* gene. The amplification product was digested by *Ded* I. In the 497-bp fragment, a polymorphic *Ded* I restriction site existed. Allele *MyoD**C is characterized by the presence of 497-bp fragment (in the absence of *Ded* I restriction site) while allele *MyoD**A possesses the *Ded* I restriction site and the 497-bp band is cleaved to 268-bp and 229-bp fragments.

2.3 Data analyses

2.3.1 The genetic distribution analysis of the *MyoD* gene in different pig breeds

The frequency of each allele and genotype in every pig breed population was calculated. Two different methods (χ^2-test and Likelihood ratio test) were performed to test if the distribution of the *MyoD* genotypes follows the Hardy-Weinberg equilibrium in each tested breeds.

2.3.2 The population genetic structure analysis of the *MyoD* gene in different pig breeds

The population genetic structure analysis of the *MyoD* gene in different pig breeds was performed by the PopGene 16 software (Population Genetic Analysis, Version1.31), which was developed by Francis C. Yeh (Department of Renewable Resource, University of Alberta). The calculating method of each parameter was reported by Nei and Kumar (2000) and Avise (2000). The analysis involved the following indexes:

The genetic variation statistics for all loci of the *MyoD* gene in different pig breeds were mainly concerned about the observed number of alleles, the effective number of alleles and the Shannon's Information index.

The heterozygosity statistics for all loci of the *MyoD* gene in different pig breeds were mainly concerned about the polymorphism information content (*PIC*), homozygosity (*Hom*), heterozygosity (*Het*) and Wright's (1978) fixation index (F_{is}).

The Ewens-Watterson Test for Neutrality was performed to test if the *MyoD* gene follows the Natural Selection model in the tested breeds and all the statistics were calculated using 1000 simulated samples. Besides the above, *F*-statistics and Gene Flow for tested loci of the *MyoD* gene in different pig breeds were also performed.

The genetic relationship analysis between different pig breeds: the Nei's Original Measures of Genetic Identity and Genetic distance (D_A) between breeds were calculated. Based on D_A, the UPGMA method was utilized to calculate the evolution distance and the phylogenetic tree of these populations was obtained.

3 Results

3.1 Genetic distribution of the PCR-RFLP polymorphism of the *MyoD* gene in different pig populations

The frequencies of the *MyoD* alleles and genotypes and their distributions among different pig populations are listed in Table 1. Table 1 shows the *Ded* I polymorphism of the *MyoD* gene is abundant. In most of tested pig

Table 1 The distribution of the genotypes and the allele of the *MyoD* gene in different pig breeds

breed type	breed	*n*	genotype attribution			allele frequency		Chi-square test		Likelihood ratio test	
			AA	AC	CC	A	C	χ^2	P	G^2	P
indigenous	YN	30	0	5	25	8.33	91.67	0.195 3	0.6586	0.3642	0.5462
Chinese breed	RC	7	0	4	3	28.57	71.43	0.800 0	0.3711	1.2403	0.2654
	WZS	15	1	8	6	33.33	66.67	0.419 2	0.5174	0.4404	0.5069
	TB	6	2	3	1	58.33	41.67	0.023 8	0.8774	0.0237	0.8778
	DH	10	0	1	9	5.00	95.00	0.000 0	1.0000	0.0000	1.0000
developed breed	DW	10	2	6	2	50.00	50.00	0.217 8	0.6407	0.2197	0.6392
hybrid breed	LYa	31	3	11	17	27.42	72.58	0.492 2	0.4829	0.4736	0.4913
	DLY	39	3	25	11	39.74	60.26	4.139 4*	0.0419	4.3629*	0.0367
foreign breed	D	10	0	9	1	45.00	55.00	5.890 9*	0.0152	7.7120**	0.0055
	L	10	0	4	6	20.00	80.00	0.450 0	0.5023	0.7593	0.3836
total		168	11	76	81	29.17	70.83	1.421 0	0.2332	1.4661	0.2260

Note: Allele frequencies are given by $n \times 100$. The values of χ^2 and G^2 are from the test of the distribution of different genotypes for Hardy-Weinberg equilibrium in different pig breeds. * and ** represent means different significantly at $P < 0.05$ and $P < 0.01$, respectively. The meanings of breed symbols are annotated in 2.1.

populations, the C allele can be found with high frequency and mainly exists in the form of heterozygotes.

Two different methods (χ^2-test and Likelihood ratio test) were performed to test if the distribution of the *MyoD* genotypes follows the Hardy-Weinberg equilibrium in the tested populations. The results indicated that, except for Duroc and DLY, the distribution of the genotypes were kept in Hardy-Weinberg equilibrium in all of the other tested populations ($P > 0.05$). That means that when considering the *Ded* I locus, no special artificial selection was performed on it at the beginning of the breeding program for these tested breeds. All of these populations were following the random mate model. In the DLY population, both the χ^2-test and Likelihood ratio test proved the distribution of the *MyoD* genotypes were significantly biased from the Hardy-Weinberg equilibrium ($P < 0.05$). However in the Duroc population, the Likelihood ratio test proved the distribution of the *MyoD* genotypes were significantly biased from the Hardy-Weinberg equilibrium at the level of 0.01. The χ^2-test proved the bias was at the level of 0.05. The confidence probability of the Likelihood ratio test is stricter than that of the χ^2-test.

3.2 Genetic variation analysis of the *MyoD* gene in different pig populations

The heterozygosity is a measure of the frequency of the heterozygotes in the tested site of the populations. It is the best parameter to measure the population genetic variation. The lower the heterozygosity is, the higher the identity of the population is. In Table 2, the average heterozygosity of all the tested populations is relatively high. That means the genetic diversity of each population is abundant and the selection potential is relatively high. Among these tested breeds, the average observed

Table 2 Heterozygosity analysis of the *MyoD* gene loci in different pig breeds

breed type	breed	sample size	observed value		Levene's expected value		Nei's expected heterozygosity (*H*)	Nei's effective number of allele	Wright's fixation index (F_{is})
			homozygosity (*Hom*)	heterozygosity (*Het*)	homozygosity (*Hom*)	heterozygosity (*Het*)			
native breed	YN	60	0.8333	0.1667	0.8446	0.1554	0.1528	1.1803	−0.0909
	RC	14	0.4286	0.5714	0.5604	0.4396	0.4082	1.6897	−0.4000
	WZS	30	0.4667	0.5333	0.5402	0.4598	0.4444	1.8000	−0.2000
	TB	12	0.5000	0.5000	0.4697	0.5303	0.4861	1.9459	−0.0286
	DH	20	0.9000	0.1000	0.9000	0.1000	0.0950	1.1050	−0.0526
developed breed	DW	20	0.4000	0.6000	0.4737	0.5263	0.5000	2.0000	−0.2000
hybrid breed	LYa	62	0.6452	0.3548	0.5955	0.4045	0.3980	1.6612	0.1085
	DLY	78	0.3590	0.6410	0.5148	0.4852	0.4790	1.9192	−0.3384
foreign breed	D	20	0.1000	0.9000	0.4789	0.5211	0.4950	1.9802	−0.8182
	L	20	0.6000	0.4000	0.6632	0.3368	0.3200	1.4706	−0.2500
total		336	0.5476	0.4524	0.5856	0.4144	0.4132	1.7041	−0.0948

population heterozygosity was 0.4524. The Duroc population had the highest heterozygosity (0.9000), while the heterozygosity of the Dahe pig (0.1000) and the Yanan pig (0.1667) were among the lowest. The trend of homozygosity was opposite to heterozygosity. In the tested breeds, the average observed population homozygosity was 0.5476. Two different methods (Levene's method and Nei's method) were utilized to calculate the expected population heterozygosity under the random mate model. With the Levene's method, the average expected heterozygosity was 0.4144. The Tibetan pig (0.5303), the Dahewu pig (0.5263) and the Duroc (0.5211) populations had the highest expected heterozygosity, while the Dahe pig (0.1000) and the Yanan pig (0.1554) populations had the lowest expected heterozygosity. The trend of expected heterozygosity had no significant difference with that of the observed heterozygosity. With the Nei's method, the average expected heterozygosity was 0.4132. The Dahewu pig (0.5000), Duroc (0.4950) and Tibetan pig (0.4861) populations have the highest expected heterozygosity. Besides the above two calculations, the fixation index (F_{is}) was calculated to judge the bias that exists between the observed heterozygosity and Nei's expected heterozygosity. Generally speaking, the fixation index will be a negative value if the observed heterozygosity is larger than the expected heterozygosity under the random mate model. However, when there exists an inbreeding effect in the population, the observed heterozygosity will be smaller, but the fixation index will be a positive value. Among the tested populations, there existed an inbreeding effect in the Landrance × Yanan (LYa) population.

The observed number of alleles in the tested site was 2 in all of the populations. When calculating the effective number, the Dahewu pig, Duroc and Tibetan pig populations had the highest effective number of alleles, higher than 1.9, while the Dahe pig and Yanan pig populations had the lowest one, smaller than 1.2. The result was consistent with the result of Nei's expected heterozygosity.

3.3 The neutrality selection analysis of the *MyoD* gene in different pig populations

According to the neutrality theory of molecular evolution, natural selection is the main selection pressure for a natural population and the random genetic drift is the determinative factor to decide whether a genetic variation can exist or not. The observed heterozygosity of the tested site was calculated first and then the Ewens-Watterson Test for Neutrality was performed to test if the *MyoD* gene follows the Natural Selection model in the tested breeds. All the statistics were calculated using 1000 simulated samples. In Table 3, there are significant differences between the observed and expected values ($P < 0.05$), which indicates that the *MyoD* gene did not follow the neutrality selection model and in the history of evolution, all of the tested breeds had experienced the pressure of natural selection.

Shannon's information index is a measure of the genetic deviation degree of a population, which is determined by the incorporated effects of selection, variation and random genetic drift in the breeding process of a random mate population. Among all of the tested breeds, the average information index was 0.6036. The Dahewu pig (0.6931), Duroc (0.6881), Tibetan (0.6792) and DLY (0.6720) populations had the highest information index, while the information index of the Dahe pig (0.1985) and the Yanan pig (0.2868) were among the lowest. The high information index means that the genetic variation in the tested breeds was relatively high. The result was consistent with the result of average heterozygosity calculated based on the gene frequency.

3.4 *F*-statistics and gene flow for tested loci of the *MyoD* gene in different pig breeds

Fixation index is a series of parameters which are used to measure the genetic deviation degree of a population. Based on the assumption that different subpopulations originated from a common ancestor, the F_{is} and F_{it}

Table 3 Ewens-Watterson test for neutrality of *MyoD* gene in different pig breeds

breed type	breed	sample size	Shannon's information index *I*	observed *F*	expected value*	
					F	*SE*
native breed	YN	60	0.2868	0.8472	0.7897	0.0270
	RC	14	0.5983	0.5918	0.7149	0.0186
	WZS	30	0.635	0.5556	0.7608	0.0236
	TB	12	0.6792	0.5139	0.6854	0.0174
	DH	20	0.1985	0.9050	0.7237	0.0227
developed breed	DW	20	0.6931	0.5000	0.7379	0.0218
hybrid breed	LYa	62	0.5874	0.6020	0.7906	0.0267
	DLY	78	0.6720	0.5210	0.8095	0.0262
foreign breed	D	20	0.6881	0.5050	0.7299	0.0232
	L	20	0.5004	0.6800	0.7304	0.0216
total		336	0.6036	0.6036	0.8535	0.0259

Note: * These values were calculated using 1000 simulated samples.

(defined by Wright) are used to estimate the correlation coefficient between two mated gametes in a subpopulation and population, respectively. The F_{st} is the correlation coefficient between two gametes gained randomly from two different subpopulations, which is used to measure the genetic difference between subpopulations. The Fixation index can be used to reflect the mate model of the population and the selection model related with the allele polymorphism. From Table 4, we know that if we take every breed as a subpopulation of the whole experimental population, strong genetic deviation had happened in the history of population evolution and highly genetic diversity had formed between subpopulations. At the same time, these subpopulations had a genetic impact on each other and the gene flow between subpopulations was 1.7414. However, because of the complexity of the formation of natural population and the lack of basic evolution parameters, further research was not performed.

Table 4 *F*-statistics and gene flow analysis of *MyoD* gene loci

sample size	Fixation index			gene flow (*Nm**)
	F_{is}	F_{it}	F_{st}	
336	−0.2617	−0.1033	0.1255	1.7414

Note: * Nm = Gene flow estimated from $F_{st} = 0.25(1–F_{st})/F_{st}$.

3.5 Analysis of the genetic identity and genetic distance between breeds and the construction of the phylogenetic tree of the tested populations

There are many methods introduced to measure the genetic distance between populations, but no method has been confirmed as the best one. In our study, the Nei's genetic distance (D_A) between tested breeds was calculated based on the gene frequency data and the result was listed in Table 5.

Based on the Genetic distance (D_A), the UPGMA method was applied to calculate the evolution distance and the phylogenetic tree of these populations were obtained (Fig. 1). In Fig. 1, the numbers marked on the branches are the evolution distances based on Nei's genetic distance.

4 Discussion

4.1 The *Ded* I polymorphism of the *MyoD* gene

In most of the tested pig populations, the *C* allele could be found with high frequency and mainly existed in the form of heterozygotes. Except for the Duroc and DLY, the distribution of the genotypes kept in Hardy-Weinberg equilibrium in all of the other tested populations. The result was consistent with the reports of Knoll et al. (1997) and Cieslak et al. (2000).

4.2 The genetic structure of the *MyoD* gene

In the history of population evolution, the tested site had experienced the pressure of natural selection and all of the tested populations had a relatively high heterozygosity. Our results were consistent with the conclusion that the genetic deviation is relatively high in the indigenous Chinese pig breeds reported in other researches (Liu et al., 2005; Teng et al., 2005). Because of the limited population size and the existing inbreeding effects in the population, the Dahe pig and Yanan pig populations had the lowest heterozygosity. The genetic deviation was relatively high in the Tibetan pig population which was due to the plateau environment.

4.3 The construction of the phylogenetic tree

At first, the tree constructed using the UPGMA method was used to measure the identity between populations and was named as the phenogram. With the improvement of the method, the UPGMA method was also used to construct the molecular phylogenetic tree. Nei has proven that when the phylogenetic tree is constructed on the base of gene frequency data, the UPGMA method is considered better than any other methods based on genetic distance. Generally speaking, if the number of nucleotide is small and the genetic replacement rate is not stable, topology error will be formed. The UPGMA is a good method to construct the phylogenetic tree, which can get both the right topology and the evolution distance (Nei and Kumar, 2000).

Table 5 Nei's genetic identity and genetic distance

breed	YN	RC	WZS	TB	DH	DW	LYa	DLY	D	L
YN	–	0.9583	0.9312	0.6525	0.9993	0.7682	0.9636	0.8812	0.8281	0.9881
RC	0.0426	–	0.9965	0.8419	0.9467	0.9191	0.9998	0.9796	0.9538	0.9908
WZS	0.0712	0.0035	–	0.8838	0.9167	0.9487	0.9948	0.9929	0.9754	0.9762
TB	0.4269	0.1721	0.1235	–	0.6232	0.9864	0.8313	0.9332	0.9651	0.7612
DH	0.0007	0.0548	0.0870	0.4729	–	0.7433	0.9528	0.8626	0.8062	0.9815
DW	0.2637	0.0843	0.0527	0.0137	0.2967	–	0.9114	0.9796	0.9950	0.8575
LYa	0.0371	0.0002	0.0053	0.1848	0.0484	0.0928	–	0.9755	0.9478	0.9933
DLY	0.1265	0.0207	0.0071	0.0691	0.1479	0.0206	0.0248	–	0.9947	0.9434
D	0.1886	0.0473	0.0249	0.0355	0.2155	0.0050	0.0536	0.0053	–	0.9044
L	0.0120	0.0092	0.0241	0.2728	0.0186	0.1537	0.0068	0.0583	0.1004	–

Note: Values above diagonal and below diagonal are Nei's genetic identity and genetic distance, respectively.

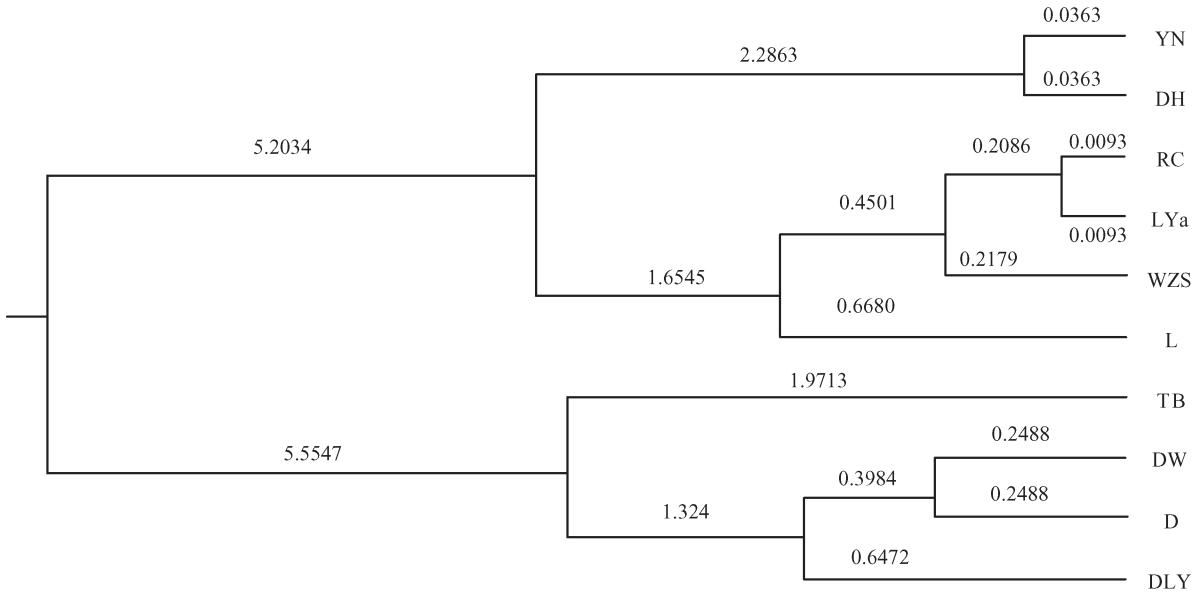

Fig. 1 A dendrogram based Nei's (1972) genetic distance (method = UPGMA)
Note: Numbers marked on the branches are the evolution distance based on Nei's genetic distance.

On the phylogenetic tree, all the tested breeds were divided into four clusters. The first cluster was formed by the Rongchang pig, the L × Yanan, the Wuzhishan pig and the Landrance. The second cluster included the Yanan pig and the Dahe pig. The third cluster included the Dahewu pig, Duroc and D × (L × Y). The Tibetan pig formed the fourth cluster of the phylogenetic tree.

Judging from the breeding history of each tested breed, in the first cluster, the Landrance is used in the breeding process of the new Rongchang pig and the L × Yanan pig is a hybrid pig between the Landrance and the Yanan pig. Both of these two breeds have genetic relations with the Landrance. The first cluster can be named as the Landrance-origined cluster. In the second cluster, both of the Yanan pig and the Dahe pig belong to primary breed and experience less selection, which can be defined as primary indigenous-breed cluster. In the third cluster, the Dahewu pig is a developed breed that has genetic relations with the Duroc, and the D × (L × Y) is a hybrid pig between the Duroc and the L × Y. All of these breeds have genetic relations with the Duroc. The third cluster can be defined as the Duroc-origined cluster. The Tibetan pig is a typical plateau pig breed which has a typical wild pig character and is different from any other pig breeds. The fourth cluster can be defined as plateau-Tibetan-pig cluster.

4.4 The possibility of utilizing RFLP data in genetic deviation research between species

Compared with other molecular markers, the RFLP marker has smallest nucleotide replacement. But Avise has proven that when the relationship is very close between populations. RFLP is a quick and cheap method to estimate the genetic structure of the population which can be used to research the genetic deviation of populations in a certain specie or closely related species (Avise, 2000). In the present research, the constitution of the topology of the phylogenetic tree is consistent with the breeding history of each pig breed. This means that the phylogenetic tree constructed on the base of the genetic distribution of the *MyoD* gene in each tested pig population is believable and can reflect the phylogenetic relationship between different pig breeds. From this study, we can conclude that some RFLP data from a functional gene can be used in the genetic deviation research between closely related species or between different populations in certain species. Certainly, increasing the detecting number of a gene locus can increase the certainty probability.

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