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Full Length Research Paper

Comparison of genetic detection efficiency of different markers under the same genetic background

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Chinese native sheep populations, Hu sheep, Tong sheep, Small-tailed Han sheep and Tan sheep were used to study the efficiency of genetic markers. The genetic markers used in this study include morphological and ecological indices, blood protein enzyme, microsatellite DNA and the combination of three markers. The results showed that the morphological and ecological indices are not accurate tools to determine the relationships among populations. In contrast, blood protein enzyme and microsatellite DNA were the best markers for genetic diversity among sheep populations. Therefore, selection of markers depends on the aim of research; for instance neutral markers are selected to study the origin of breeds, trait indices for economical or ecological classification, and multi-combined genetic markers for the relations among populations.

Key words: Genetic markers, genetic differentiation, genetic detection efficiency.

INTRODUCTION

Genetic characterization and differentiation of populations are often necessary for the conduct of valid case-control association studies (Stein et al., 2004; Kaufman et al., 2004; Luo et al., 2005), determining the role of ancestry in phenotypic differences (Parra et al., 2003; Brutsaert et al., 2005), assigning population groups for valid linkage analysis (Gelernter et al., 2005), examining the distribution of neutral genetic variation among popula-tions, and inferring migration histories (Romualdi et al., 2002; Fejerman et al., 2005). Two grouping methods are generally used in sheep breeding; one is based on distribution of morphology and ecological features, which have a great significance to guide regional animal production (Zheng, 1980). The other is based on molecular markers such as blood protein enzyme polymorphisms, microsatellite DNA fingerprinting, mainly revealing the genetic relationship among breeds and exploring the evolution and origination of breeds and it has great significance to reserve and develop livestock genetic resources (Chang, 1998; Sun et al., 2003; Sun, 2006). The efficiency of markers assisted selection (MAS) relative to purely phenotypic selection has been widely studied, mainly in the case of populations derived from the cross of two inbred lines through analytical approaches (Luo et al., 1997; Moreau et al., 1998) and simulations (Zhang and Smith, 1993; Whittaker et al., 1997; Hospital et al., 1997). All these theoretical studies concluded that in many situations, MAS could be more efficient than phenotypic selection. To study the genetic differentiation in sheep populations under different ecological types, we analyzed the detecting effect of genetic markers at morphological and ecological level, as well as blood protein enzymes and microsatellite DNAs. The aim was to provide valuable evidence for selecting efficient genetic markers for further study of genetic differentiation in sheep breeds and other species.

MATERIALS AND METHODS

Materials

Sheep population used including 63 Hu sheep (HU), 65 Tong sheep (TONG), 60 Small-tailed Han sheep (XWH) and 70 Tan sheep (TAN) were from Huzhou of Zhejiang province, Baishui of Shanxi province, Liangshan of Shandong province and Yanchi of Ningxia province in China, respectively. Samples were collected based on

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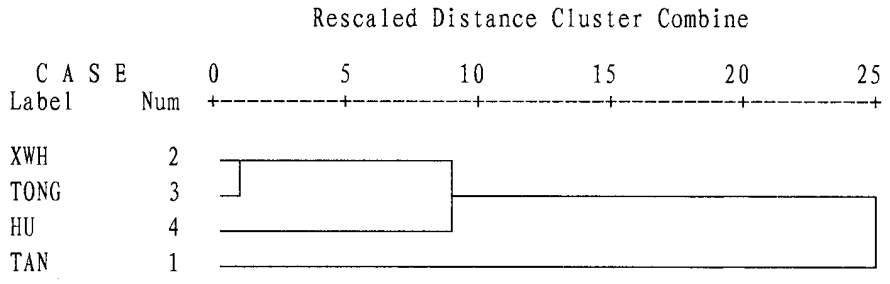

Figure 1. Clustering results of the principal component values calculated by R-type method based on morphological and ecological data of sheep populations.

random sampling methods in the typical colonies from the central area of habitat.

Morphological and ecological methods

The morphological and ecological parameters include 5 body size parameters (height, length, breast circumference, tail length and tail breadth); 7 morphological features include fleece color and tail type; and 7 ecological indices include elevation, average temperature, average of the lowest temperature, average of the highest temperature, range of annual temperature, range of annual rainfall and local environmental features.

Blood protein and enzyme methods

A 12 structural loci on constant chromosomes including albumin (Alb) transferrin (Tf) alkaline phosphatase (Alp), leucine aminopeptidase (Lap), arylesterase (Ary-Es) hemoglobin-β(Hb-β), X – protein (X-p) carbonic anhydrase (CA) catalase (Cat), esterase D (Es-D), lysine (Ly) and posassium (Ke) were detected as previously described by Tsunoda et al. (1995, 1998,1999).

Microsatellite DNA methods

The genomic DNA was extracted by common method described by Sun (2006). Seven microsatellite markers (OarFCB11, OarFCB48, OarFCB128, OarFCB304, MAF33, MAF70 and OarAE101) on different chromosomes recommended by FAO and ISAG were adopted to analyze the genetic polymorphism within sheep populations.

Statistical analysis

The data were analyzed using principal component analysis of SAS software (Yuan and Zhou, 2002). The principal component values of each population were used to calculate the Euclid's genetic distance under R type systematic clustering. R type systematic clustering was conducted based on the data of 17 quantitative morphological and ecological indices, 28 alleles of 12 structural loci of blood protein enzyme, 193 alleles of 7 microsatellite DNA markers, and combined genetic analysis based on 17 quantitative morphological and ecological indices, 12 structural loci and 7 microsatellite markers.

Relativity among the inter-population Euclid's genetic distance at different levels of markers was analyzed using SPSS software. The Euclid's genetic distances of the pairwise populations were first calculated at different levels of markers, and then the coefficients of the paired Euclid's genetic distances were calculated, with one Euclid's genetic distance as the independent variable, the other as dependent variant. All the computation was completed by SAS6.12 and SPSS14.0 software.

RESULTS

Morphological and ecological markers

The principal component values of sheep populations were calculated by R-type method. Three principal component values of each population were used to compute the inter-population Euclid's genetic distances of the four populations clustered by linkage between-groups. From Figure 1, the four populations were divided into two types; one was Tan sheep in the interlaced zone of agrarian and pasturing areas, which were characterized by high elevation, and with a low precipitation and a low annual mean temperature. The others Hu sheep, Tong sheep, Small-tailed and Han sheep were in the agrarian areas, which were characterized by low elevation, high precipitation and a high annual mean temperature.

Based on the 17 quantitative morphological and ecological indices, systematic clustering was conducted (Figure 2). The 17 morphological and ecological features of the four populations were divided into three types; one group is the combination of morphological and ecological indices except for the elevation (X_{12}) and rainfall (X_{17}). The elevation (X_{12}) and rainfall (X_{17}) consist of the other two groups, respectively. Therefore, elevation and rainfall are important characters that may decide the distribution of the sheep populations.

Blood protein enzyme

Three principal component values of each population were used to calculate the inter-population Euclid's genetic distances of the four populations (Figure 3). Sheep populations were divided into two groups, but the inter-population genetic divergence were different from Figure 1. Hu sheep and Tong sheep formed a distinct cluster first and then Tan sheep and Small-tailed Han

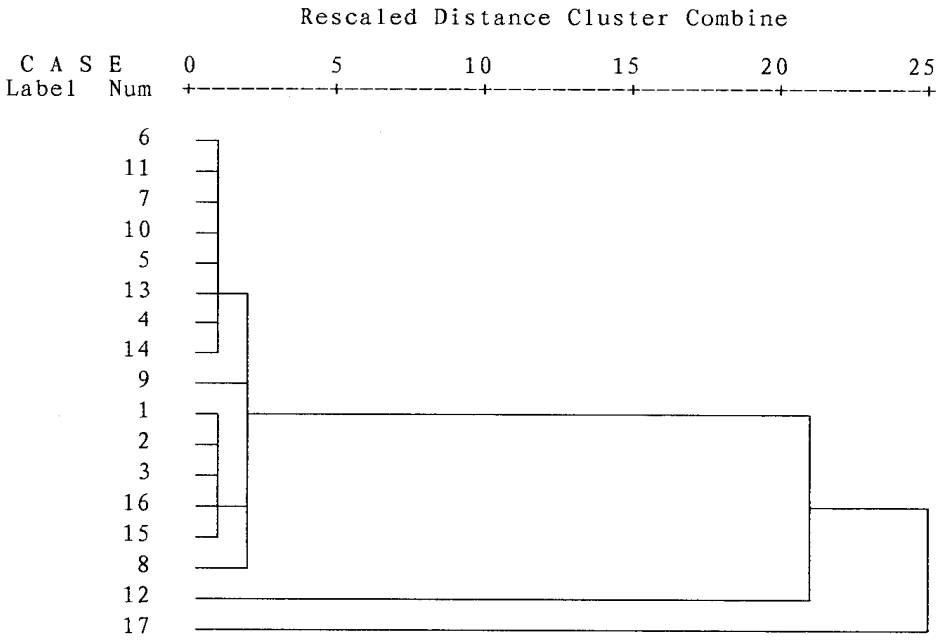

Figure 2. Clustering results of 17 morphology and ecology indices of sheep populations. X_1, height at withers; X_2, body length; X_3, breast circumference; X_4, tail length; X_5, tail breadth; X_6, horned; X_7, polled; X_8, self-color; X_9, head and legs with colored; X_{10}, spotted; X_{11}, dark brown color; X_{12}, elevation; X_{13}, average temperature; X_{14}, average of the lowest temperature; X_{15}, average of the highest temperature; X_{16}, annual temperature; X_{17}, annual rainfall.

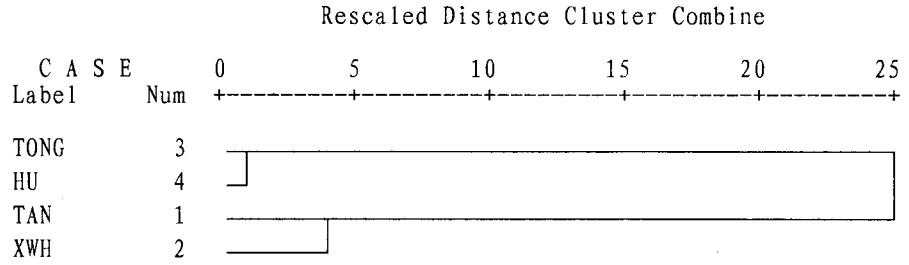

Figure 3. Clustering results of the principal component values calculated by R-type method based on blood protein and enzyme data of sheep populations.

sheep gathered as another cluster.

Microsatellite DNA

Sheep populations were divided into two cluster groups, the inter-population genetic divergence was different from Figure 1, but is similar to Figure 3. At this level, Tan sheep and Small-tailed Han sheep had a high level of genetic divergence (Figure 4).

Combined genetic markers

The result of all combined genetic markers; morphological and ecological, blood protein enzyme and microsatellite DNA is presented in Figure 5. The four populations were divided into two clusters, but the inter-population genetic divergences were different from the above-mentioned results. However, at the blood protein enzyme level, Hu sheep and Tong sheep clustered first followed by Small-tailed Han sheep and Tan sheep, which was identical with the morphological and ecological result.

The inter-population Euclid's genetic distances and correlation coefficients matrix of the four populations were estimated based on the morphological and ecological indices, structural loci, microsatellite DNA marker and combined genetic markers (Tables 1 and 2). Based on microsatellite DNA, the inter-population Euclid's genetic distances and correlation coefficient of morphological and ecological indices and blood protein enzyme were

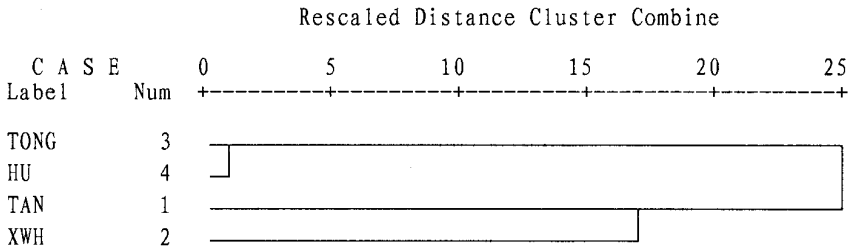

Figure 4. Clustering results of the principal component values calculated by R-type method based on microsatellite DNA data of sheep populations.

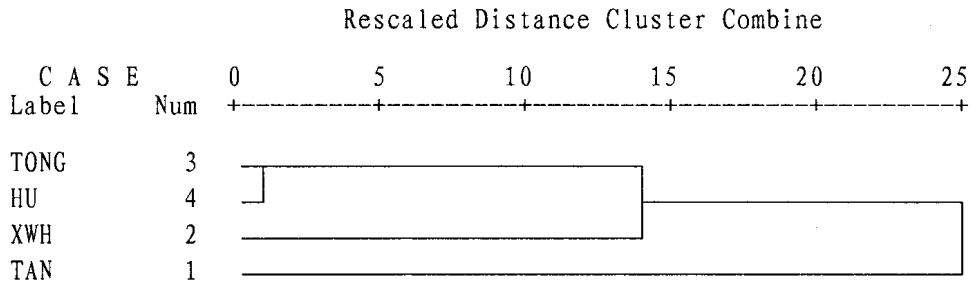

Figure 5. Clustering results of the principal component values calculated by R-type method based on multi-combined markers data of sheep populations.

Table 1. Genetic distance of different markers among sheep populations.

Markers and population	Morphological and ecological indices	Blood protein enzymes markers	Microsatellite DNA markers	Combined genetic markers
TAN-XWH	5.935	6.702	18.884	21.945
TAN-TONG	6.046	7.259	21.371	23.878
TAN-HU	7.243	7.475	21.066	23.692
XWH-TONG	4.258	7.340	18.838	20.839
XWH-HU	5.170	7.836	19.230	20.652
HU-TONG	4.786	6.576	16.668	17.949

Table 2. Correlation coefficient matrix of different markers in sheep populations.

Genetic markers	Morphological and ecological indices	Blood protein enzymes markers	Microsatellite DNA markers	Combined genetic markers
Morphological and ecological indices	1.000	0.141	0.711	0.762
Blood protein enzymes markers	0.141	1.000	0.573	0.399
Microsatellite DNA markers	0.711	0.573	1.00	0. 967**
Combined genetic markers	0.762	0.399	0. 967**	1.000

**Pearson correlation is significant at the 0.01 level (2-tailed).

r = 0.711 (P = 0.113) and r = 0.573 (P = 0.234), respectively. Based on combined genetic markers, the correlation coefficients of the inter-population Euclid's genetic distances of the morphological and ecological, blood protein enzyme and microsatellite DNA were r = 0.762 (P = 0.078), r = 0.399 (P = 0.433) and r = 0.967 (P = 0.002), respectively. The findings indicated that the results based on the microsatellite DNA were close to that based on combined genetic markers. The distribution of sheep was affected by ecological features, and the detec-

tion efficiency of blood protein enzyme for sheep populations was lower than that of morphological and ecological indices, indicating that the difference in breeds was not only related with their origin, but also related with the ecological environment.

DISCUSSION

Phenotypic selection, which does not require marker evaluation, allows breeders to increase the population size and thus the intensity of selection compared with the one used for MAS (Moreau et al., 2000). In this study, we showed that the morphological and ecological features were less important in exploring the origin of breeds compared to molecular genetic markers of neutral loci such as blood protein enzymes and the microsatellite DNA markers. Their detection accuracy and efficiency of the relationships among sheep populations were hence affected, but their affect in genetic divergences of breeds should not be neglected (Sun et al., 2004).

As neutral, structural loci, the microsatellite DNA markers are not affected by selection in breed origination, and the population divergences based on these neutral loci could reflect better the origin of populations. Structural loci are located in the DNA coding region with a variation occupying 10% of the total DNA variation. They are good representatives of genomes, but a low polymorphism limits their application (Tsunoda et al., 1995, 1998). Microsatellite DNA was generally distributed in the non coding region and had rich polymorphisms for a small selective pressure compared to the coding regions. They are more suitable to analyze closely related species or breeds determined by origin, or relationships randomly fixed by neutral genes than other low polymorphic markers. Comparing the blood protein polymorphisms with the microsatellite DNA polymorphisms for the same breed, we found that the polymorphisms revealed by microsatellite DNA markers were higher than the blood protein polymorphisms, which make the classification among individuals more accurate. More-over, the inter-population genetic distances estimated by microsatellite DNA markers were bigger than that of blood protein markers. However, the phylogeny trees of both had the same trend, and this indicates the efficiency of micro-satellite DNA marker (Sun et al., 2004). Micro-satellite markers are useful for genetic studies because they are co-dominant, multi-allelic, widely distributed across the genome, polymerase chain reaction (PCR)-based, and transferable between different genotypes (Grisi et al., 2007). Information generated by these markers allows comparisons and information exchange between different studies, especially for comparative genetic mapping (Grattapaglia, 2000).

Our result showed that the combined genetic analysis was more efficient than single marker analysis.

Statistically, genetic information of each level has correlation with another, and the principal component analysis induces some complicated variables into only a few independent variables, that are to cluster systematically based on genetic markers with statistical correlations among common genetic background (Chang, 1995). Singh and Bellmann (1974) indicated that the genotypically constructed selection indices were more efficient than the phenotypically constructed selection index. It was, however, observed that the genotypically constructed indices were more sensitive to linkage and the reduction in their performance was relatively greater if characters included in the index had varying heritability coefficients (Singh and Bellmann, 1974).

Conclusion

Morphological and ecological data are not accurate tools to determine the relationships among populations, but their weight in genetic divergences of breeds should not be neglected. Selection of genetics marker depends on the aim of research. For instance, if the aim was to trace back the origin of some breeds, neutral markers should be used; if the aim was to classify economically or ecologically, the related trait indices should be considered; and if the aim was to analyze the current relations among populations, the multi-level combined genetic markers is a better choice. The highlight of the principal component analysis provided a useful method to induce some complicated variables (indices or traits, and in genetic resource, markers) into only a few independent variables.

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