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RESEARCH ARTICLE

Exploration of the Exogenous Male Yak Introduction Breeding Model and its Effects on Tibetan Small-Sized Family Farms

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ABSTRACT

A total of three family farms including Village No. 9 in Nima township, Nerong Naqu County (NQA); Village No. 11 in Nima township, Nerong Naqu County (NQB); and Yare township, Gegi County, Ali District (GJ) from three ecology yak populations (EYP) were selected for this study to identify the most optimized mode of exogenous male adult yak introduction (EMI) within EYP for solving the inbreeding problem caused by the small-scale yak husbandry system. Exogenous adult male yaks from the same EYP with different proportions (100% to NQA, 50% to NQB, and 0% to GJ) were introduced, and 10 microsatellites were used to detect the genetic diversity of these populations before (in 2017) and after (in 2019) the introduction of exogenous adult male yaks (EMI). Results showed that the divergence between the observed and the expected heterozygosity of the NQA and NQB populations was reduced in 2019, while the number of markers significantly deviating from Hardy–Weinberg equilibrium ($P<0.05$) and F_{IS} (inbreeding coefficient) within populations decreased compared with that in 2017. In contrast, the F_{IS} of GJ population without EMI continued to increase (from 0.011 to 0.033) over the years 2017 to 2019. Moreover, genetic differences between the populations (Pairwise Fixation index, F_{ST}) showed that EMI increased the genetic divergence between populations. Overall, this study shows that the introduction of exogenous male adult yaks not only effectively reduces the degree of population deviation from equilibrium but also decreases the inbreeding level within the population within a few generations. This study also provides a valuable management model for stable yak production on small sized family farms.

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INTRODUCTION

Molecular markers can effectively reveal the genetic resources of livestock and poultry at the DNA level. Microsatellite markers are available in large numbers and show co-dominant inheritance and high polymorphism. These markers have been used in many human and livestock genetic studies, such as parent–child relationship identification, species classification, population or individual genetic variation, genetic structure analysis and genetic diversity exploration (Fan *et al.*, 2008; Zhou *et al.*, 2008). These markers are also effective tools commonly used in research on topics such as marker-assisted selection, quantitative trait locus mapping and population clustering analysis (Sewalem *et al.*, 2002).

Most of the complete genetic linkage maps of cattle, sheep and pigs were constructed on the basis of microsatellite markers. For example, Barendse *et al.* (1997) developed genetic linkage maps in cattle by using 746 molecular markers, including 601 microsatellite markers. Subsequently, a more accurate genetic linkage map for cattle was generated using 3960 markers comprising 3802 polymorphic microsatellites, with the marker distance reduced to 1.4 cM (Ihara *et al.*, 2004). Moreover, genetic maps of pigs (Rohrer *et al.*, 1994), sheep (Crawford *et al.*, 1995) and goats (Vaiman *et al.*, 1996) have been developed. To date, many studies on the genetic diversity of livestock based on microsatellites, including those of E *et al.* (2019), Habimana *et al.* (2020) and Ba *et al.* (2020) have been reported.

As one of the main yak husbandry areas in China, Tibet has 4.57 million heads of yaks, which account for 30% of the total yak population in this country. Since 1950s, Chinese scholars have conducted detailed research on yak production performance. Particularly since 1990s, the genetic diversity of yak breeds has been comprehensively and systematically investigated at the molecular level by using different genetic markers. For example, Liao *et al.* (2008) revealed the rich genetic diversity and low genetic differentiation levels of yak breeds from five ecotype regions in China with 16 microsatellites. Li *et al.* (2013) used eight microsatellite markers and showed that the genetic diversity of yaks in eastern Tibet was higher than that in western Tibet and that eastern Tibet was a possible cradle of yak diversity. Luo *et al.* (2017) used 15 microsatellite loci to study the genetic diversity of the Maiwa yaks and found that the breed had rich genetic diversity but no genetic differentiation. Recent studies (Pei *et al.*, 2018; Zhu *et al.*, 2019) investigated the genetic diversity of several ecological groups of Tibetan yaks using microsatellite DNA, which provided helpful information on the conservation and utilization of local ecotype population resources for Tibetan yaks.

The appropriate management of high-quality yak resources considering conservation and utilization in core yak husbandry areas, especially due to the existing livestock husbandry system, needs to be ensured. The inbreeding problem within the yak population is becoming increasingly serious under the small-scale family farming model. In the present study, the population structure and genetic diversity level of populations before and after the introduction of exogenous male yaks were investigated with microsatellites to evaluate the effect of such introductions and identify the most optimized mode for exogenous adult male yak introduction.

MATERIALS AND METHODS

Experimental animals: For this study, sampling before exogenous adult male yaks introduction (EMI) was done in October 2017, when 129 healthy yaks (1–3 years old) were selected from three Tibetan yak groups including Village No. 9 in Nima township, Nerong Naqu County (NQA; n = 47), Village No. 11 in Nima township, Nerong Naqu County (NQB; n = 59) and Yare township, Gegi County, Ali District (GJ; n = 23). One milliliter of venous blood was collected from each individual and stored at -80°C for further analysis.

Subsequently, in December 2017, exogenous adult male yaks equal to 0% (control), 50%, or 100% of the original male population size were introduced into the GJ, NQB, and NQA populations, respectively, to participate in intragroup mating for that year. The exogenous male yaks belonged to other family farms located within their own ecological population, and there had been no blood relationship between the yaks on the farms for the past 5 years.

For sampling after EMI, healthy newborn and juvenile yaks (1-3 years old) were identified at the 3 sites in October 2019. Then 32 animals were randomly selected from each ecology population with EMI, about 1 ml venous blood was collected from each animal and stored at −80°C.

Genomic DNA extraction: The genomic DNA from all blood samples collected from yaks before and after EMI was extracted, using the standard phenol-chloroform protocol, as described earlier (Sambrook and Russell, 2001). This extracted genomic DNA was stored at -20°C.

Ten microsatellite markers of the genetic diversity estimation system for bovines, recommended by the Food and Agriculture Organization (FAO) of the United Nations and the International Society of Animal Genetics (ISAG), were used in the present study to estimate the diversity of yak populations (Zhu *et al.*, 2019). Information regarding these 10 microsatellite markers and their primers is given in Table 1.

PCR analysis: A 20 μL PCR system was used for PCR analysis. In the analysis, the final concentrations of each component were as follows: dNTPs, 0.2 mmol/L; Mg^{2+}, 1.5 mmol/L; mixed upstream and downstream primers, 0.5 mmol/L; Taq enzyme, 5 U/2 L; and DNA template, 1 μL (approximately 60 ng). The PCR procedure was as follows: pre-denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30s, annealing at 50°C–65°C for 30s, and extension at 72°C for 30s; extension for 7 min at 72°C; and storage at 4°C. The PCR products were genotyped using an ABI 3130 xl automatic genetic analyzer (AB, USA).

Statistical analysis: Microsatellite Toolkit software (Attard *et al.*, 2010) was used to calculate the mean number of alleles (N_A), polymorphism information content (PIC), observed heterozygosity (H_O) and expected heterozygosity (H_E). The genetic differentiation index (F_{ST}) among populations was calculated using Arlequin 3.5 software (Excoffier and Lischer, 2010). Hardy–Weinberg equilibrium (HWE) was assessed by GENEPOP 3.4 software (Raymond and Rousset, 1995), while FSTAT 2.9 software (Goudet, 1995) was used to calculate the inbreeding coefficient (F_{IS}).

RESULTS

Results of the present study showed that the H_E and PIC of ILSTS008 in 2017 were the lowest (H_E = 0.5949, PIC = 0.4936), and the H_O of AGLA293 was the lowest (H_O = 0.2571). However, MGTG7 had the highest levels of H_E (0.8691) and PIC (0.8260). Among the three populations in 2019, ILSTS008 had the lowest H_E (0.5676), H_O (0.5060), and PIC (0.4737). By contrast, TGLA73 had the highest H_E (0.8433), H_O (0.8730), and PIC (0.8085), as shown in Table 2.

The results for genetic diversity at the population level are shown in Table 3. The H_E of the three populations in 2017 ranged from 0.7291±0.0345 (NQB) to 0.7695±0.0286 (NQA), H_O ranged from 0.6094±0.0212 (NQB) to 0.7302±0.0332 (GJ), and N_A ranged from 5.90±1.73 (GJ) to 8.00±2.91 (NQA). In addition, the PIC of the three populations was larger than 0.6 (0.6692 to 0.7154), which indicated that the markers were highly polymorphic within the three yak populations.

Table 1: Primer sequence, fragment size, and PCR annealing temperature (Tm) for 10 microsatellite markers used in the study

Marker name	Sequences (5'-3')	Size (bp)	Tm (°C)
ILSTS008	F: GAATCATGGATTTCTGGGG R: TAGCAGTGAGTGAGGTTGGC	175-187	58
BM1824	F: GAGCAAGGTGTTTTTCCAATC R: CATTCTCCAACTGCTTCCTTG	180-194	57
ETH225	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	144-162	64
SPS115	F: AAAGTGACACAACAGCTTCTCCAG R: AACGAGTGTCCTAGTTTGGCTGTG	234-254	64
ETH152	F: TACTCGTAGGGCAGGCTGCCTG R: GAGACCTCAGGGTTGGTGATCAG	194-212	56
MGTG7	F: TTCATTGCAGCAACTATTTACAATAG R: TAAGTTCCCTGTATCATTTTTGAA	278-312	55
TGLA53	F: GCTTTCAGAAATAGTTTGCATTCA R: ATCTTCACATGATATTACAGCAGA	143~191	55
TGLA73	F: GAGAATCACCTAGAGAGAGGCA R: CTTTCTCTTTAAATTCTATATGGT	111~143	55
AGLA293	F: GAAACTCAACCCAAGACAACTCAAG R: ATGACTTTATTCTCCACCTAGCAGA	210~240	55
TGLA122	F: CCCTCCTCCAGGTAAATCAGC R: AATCACATGGCAAATAAGTACATAC	143-175	54

F = Forward; R = Reverse.

Table 2: Comparative genetic diversity analysis of 10 microsatellite loci in the overall yak group between 2017 and 2019

Marker	2017			2019		
	H_E	H_O	*PIC*	H_E	H_O	*PIC*
SPS115	0.8054	0.7223	0.7599	0.7054	0.6882	0.6495
ETH152	0.8172	0.7274	0.7603	0.7969	0.8289	0.7540
TGLA122	0.7265	0.7150	0.6739	0.7411	0.5958	0.6914
ETH225	0.7371	0.6934	0.6797	0.7123	0.7153	0.6504
MGTG7	0.8691	0.6014	0.8260	0.8127	0.8515	0.7730
TGLA73	0.8210	0.7957	0.7856	0.8433	0.8730	0.8085
AGLA293	0.6463	0.2571	0.5687	0.6163	0.5426	0.5484
TGLA53	0.7357	0.7371	0.6912	0.7463	0.7903	0.6933
BM1824	0.7012	0.7385	0.6367	0.6318	0.6300	0.5704
ILSTS008	0.5949	0.6723	0.4936	0.5676	0.5060	0.4737

The H_E of the populations in 2019 ranged from 0.7060±0.0373 (NQB) to 0.7251±0.0311 (NQA), the H_O ranged from 0.6871±0.0260 in GJ to 0.7175 ± 0.0254 in NQB, and the N_A ranged from 5.90±1.10 (GJ) to 7.00±2.40 (NQA). The PIC of the three populations was larger than 0.6 (0.6613 to 0.6876). Specifically, compared with the results from 2017, NQA and NQB showed remarkable increases in H_O in 2019, while no such change was observed in the GJ population (Table 3).

The inbreeding coefficient (F_{IS}) values of NQA and NQB were 0.148 and 0.166 in 2017, respectively. However, after the introduction of the male adult yaks, the F_{IS} values of the two populations dropped to 0.048 and −0.017. Interestingly, the F_{IS} of the GJ population without the introduction of male yaks increased from 0.011 to 0.033 after two years (Table 3). These results suggest that the introduction of exogenous male adult yaks can quickly reduce F_{IS} to a certain extent.

A comparison of the results of the HWE test for the three populations before and after the introduction of male yaks is shown in Table 4. In 2017, three markers (ETH152, MGTG7, and AGLA293) showed significant deviation from HWE (P<0.05) in NQA, five markers (SPS115, ETH152, ETH225, MGTG7, and AGLA293) deviated from HWE in NQB, while only one marker (AGLA293) significantly deviated from HWE (P<0.05) in GJ. However, in 2019, only one marker (AGLA293) significantly deviated from HWE (P<0.05) in NQA and NQB each. The GJ population had two markers (AGLA293 and TGLA122) that showed significant deviation from HWE (P<0.05). The number of markers deviating from HWE generally decreased in 2019 compared with 2017 in NQA and NQB. However, GJ showed the opposite trend.

Estimation of the genetic differences among the populations showed that the F_{ST} values of the three populations ranged from 0.0148 to 0.0362 in 2017 and from 0.0235 to 0.0378 in 2019. The genetic differentiation index among the populations revealed a significant genetic difference (P<0.05) among the populations (Table 5). Moreover, the genetic divergence of yaks was higher after the introduction of male yaks (in 2019) than that before the introduction of male yaks (in 2017). It indicates that the genetic differences among the populations generally increased, which would be conducive to yak conservation and the further development and utilization of local yak resources.

DISCUSSION

Microsatellites are commonly used as molecular genetic markers, with a wide and uniform distribution in the genome, rich polymorphism information content, and co-dominant inheritance; thus, they are considered to be the best marker system for evaluating the genetic diversity of livestock and poultry (Fathi *et al.*, 2018; Gvozdanović *et al.*, 2019). The results of the present study showed that the three populations were still highly polymorphic after the introduction of the exogenous adult male yaks based on PIC and N_A because the difference among the three populations was not notable (Table 3). The difference between H_O and H_E before the introduction of exogenous adult male yaks in 2017 was more than 0.1 on the average in NQA and NQB populations. However, this difference decreased approximately by 0.01–0.02 after the introduction of exogenous adult male yaks in 2019. The introduction of exogenous male yaks reduced the divergence between H_O and H_E by 5 to 10 times in NQA and NQB.

Theoretically, large disparities between the expected heterozygosity (H_E) and the actual heterozygosity (observed heterozygosity, H_O) and high degrees of deviation from HWE lead to the substantial risks of inbreeding and bottleneck effects (Montarry *et al.*, 2015; Selvam *et al.*, 2017; Furlan-Murari *et al.*, 2019).

Table 3: Genetic diversity assessment of three different farm-scale yak populations before and after the introduction of exogenous adult male yaks

Sampling year	Population	H_E±SD	H_O±SD	No. of alleles	*PIC*	F_{IS}
2017	NQA	0.7695±0.0286	0.6585±0.0291	8.00±2.91	0.7154	0.148
	NQB	0.7291±0.0345	0.6094±0.0212	7.10±2.96	0.6781	0.166
	GJ	0.7378±0.0233	0.7302±0.0332	5.90±1.73	0.6692	0.011
2019	NQA	0.7251±0.0311	0.7018±0.0262	7.00±2.40	0.6876	0.048
	NQB	0.7060±0.0373	0.7175±0.0254	6.30±1.77	0.6659	-0.017
	GJ	0.7209±0.0221	0.6871±0.0260	5.90±1.10	0.6613	0.033

Table 4: Hardy–Weinberg analysis of 10 microsatellite loci within different farm-scale yak populations before and after the introduction of exogenous adult male yaks

Marker	2017			2019		
	NQA	NQB	GJ	NQA	NQB	GJ
SPS115	*P* =0.144	*P* =0.000*	*P* =0.793	*P* =0.793	*P* =0.754	*P* =0.368
ETH152	*P* =0.031*	*P* =0.000*	*P* =0.722	*P* =0.722	*P* =0.877	*P* =0.667
TGLA122	*P* =0.702	*P* =0.290	*P* =0.600	*P* =0.600	*P* =0.084	*P* =0.044*
ETH225	*P* =0.364	*P* =0.023*	*P* =1.000	*P* =1.000	*P* =0.712	*P* =0.061
MGTG7	*P* =0.018*	*P* =0.000*	*P* =0.273	*P* =0.273	*P* =0.362	*P* =0.762
TGLA73	*P* =0.119	*P* =0.206	*P* =0.946	*P* =0.946	*P* =0.335	*P* =0.834
AGLA293	*P* =0.000*	*P* =0.000*	*P* =0.000*	*P* =0.000*	*P* =0.003*	P =0.000*
TGLA53	*P* =0.152	*P* =0.389	*P* =0.585	*P* =0.585	*P* =0.167	*P* =0.246
BM1824	*P* =0.949	*P* =0.396	*P* =0.165	*P* =0.165	*P* =0.858	*P* =0.583
ILSTS008	*P* =0.059	*P* =0.117	*P* =0.285	*P* =0.285	*P* =0.072	*P* =0.106

*Represents significant lack of heterozygosity (P<0.05).

Table 5: Genetic differentiation index (F_{ST}) among three different farm-scale yak populations before and after the introduction of exogenous adult male yaks

Population code		2019		
		NQA	NQB	GJ
2017	NQA	0.0000	0.0235*	0.0284*
	NQB	0.0148*	0.0000	0.0378*
	GJ	0.0362*	0.0152*	0.0000

Note: The F_{ST} values of the three groups in 2017 are in the lower left, and those in 2019 are in the upper right corner.: *Represents a significant genetic divergence between populations of the years of 2017 and 2019 (P<0.05).

This prediction is consistent with the dynamic results observed for the higher number of markers deviating from HWE in the NQA and NQB populations in 2017.

In addition, the control population (GJ) used in this study showed very small difference between H_E and H_O in 2017 (the difference was approximately 0.0076). However, the difference between H_E and H_O in the GJ population in 2019 increased by approximately 0.0338. Moreover, the number of markers that deviated from HWE decreased after the introduction of exogenous male yaks decreased from 3 (in 2017) to 1 (in 2019) in NQA and from 5 (in 2017) to 1 (in 2019) in NQB. These results show that the introduction of exogenous adult male yaks helps in maintaining, and even recovering, natural population status. Yaks in China are generally free to mate within the population, and manual intervention is minimal. Therefore, maintaining the natural balance of the population is extremely critical.

This study also revealed that NQA and NQB had high inbreeding risks in 2017, with F_{IS} values of 0.148 and 0.166, respectively. However, the F_{IS} values of the two populations decreased to 0.048 and −0.017 after the introduction of exogenous adult male yaks. This indicates that the young generation within population was almost completely free from inbreeding risk in comparison with that before EMI. On the contrary, the GJ population (control group) that did not receive exogenous male yaks, showed an increase in F_{IS} from 0.011 in 2017 to 0.033 in 2019. These results employ that the risk of rapid increase in inbreeding coefficient is more likely to occur in family farming due to the limited number of breeding bulls. Therefore, the introduction of exogenous adult male yaks can rapidly reduce F_{IS} within few generations, which can help curb the risk of inbreeding.

Notably, the F_{IS} of the original population can be reduced on the basis of the introduced yak proportion (50% and 100% of the original male yak population). However, considering the economic cost, such effects can be achieved in a short period by introducing exogenous yaks at a frequency of 50% of the number of male yaks in the original herd. The test results revealed that the F_{IS} of NQB significantly decreased to −0.017 (Table 3), which also had a negative value, after the introduction of yaks at a frequency of 50%. The excess heterozygotes, indicated by negative F_{IS} were found in the population possibly because the exogenous males decreased the mating opportunities for the original yaks via their size and other advantages (Balloux, 2004; Carlson *et al.*, 2017). This phenomenon may lead to a risk of bottleneck effects in the population within a short period and inbreeding after many generations (Saccheri *et al.*, 1999; Seyedabadi *et al.*, 2017). However, the excess of heterozygotes can help improve the adaptability of individuals and populations to the ecological environment, especially the resistance to pathogens (Apanius *et al.*, 1997; Chowell *et al.*, 2019).

It is well known that F_{ST} represents genetic divergence between populations (Holsinger and Weir, 2009; Chen *et al.*, 2019). In this study, it was found that EMI can generally increase the genetic divergence between different ecological populations, which is conducive to maintaining and enriching the genetic diversity within the population. It also helps in maintaining the unique genetic material within population and phylogenetic genetic structure of inter-population.

Unfortunately, for the present study, 3 local ecological yak populations were used which are not recognized yak breeds. However, it is well known that there are a large number of regional yak ecological groups on the Qinghai-Tibet Plateau due to the natural selection of geographical barriers and natural climate conditions. So, the genetic structure of ecological groups is independent due to their long-term stable habitat and limited range of pasture. Moreover, yaks on the Qinghai-

Tibet Plateau in China mainly have two ancestral genetic backgrounds, including the Kunlun Mountain branch and the Qilian Mountain branch, with the existing domestic yak population basically has these two kinds of ancestral blood. Moreover, the purpose of this study was to use a family farm yak in the same ecological group to mix with male yak from different family farms in the same ecological group for evaluation of the changing level of genetic diversity, so as to find a solution to the ancestor effect in yak family farming.

Conclusions: The current study indicates that the EMI can effectively reduce the possibility of population equilibrium deviations and the risk of inbreeding. It reduced the inbreeding level in the population within a few generations. The optimal mode for the EMI considered the production needs and the actual features of the population to determine the number and proportion of exogenous male yaks to be introduced.

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Authors contribution: Guang-Xin E, Yan-Bin Zhu, Basang Wang-Dui conceived and designed the experiments. Pingcuo Zhan-Dui, Luosang Dun-Zhu, Dawa Yang-La performed the lab work. Guang-Xin E, Yan-Bin Zhu, Basang Wang-Dui analyzed the data and wrote the paper.

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