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RESEARCH ARTICLE

Assessment of genetic diversity among cross breed hybrids of Yak and cattle using geo-phenotypic and molecular marker

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Abstract

Three weeks field survey was conducted to validate the indigenous practices of Bhotia tribe about the hybridization between local cow with Yak (♂) in high altitude which is their summer settlement. For this study. One male Yak, three Cows with its offspring of two Jhupu (♂, sterile hybrid), seven Jumo (♀ fertile hybrid), two Talbuni (♀, fertile hybrid), three Talbu (♂, sterile hybrid), and four Bulls (fertile hybrid) were taken. Geo-phenotypic data showed that Yak can survive at high altitude (above 3500 m asl) only while the cross breed animals Jhupu, Jhumo, Talbuni and Talbu can survive at both the altitudes (2000-4000 m asl). The distinguishing characters of some hybrids like Talbuni and Jhumo are golden brown body color and small thick sickle shape horn respectively. A RAPD profiling was used to validate the parent and hybrids relationships. When the RAPD data were used to construct dendrogram, Talbuni,Talbu, Jhumo and local bull were in one group. Dendrogram data suggested that parent and hybrids used in the study were following standard hybridization pattern but their actual parent were different. In correlation studies Jumo showed negative correlation with Talbu, Bull and Yak. These results are well supported by traditional practices and also suggest that introgression of yak gene in the local should be tightly controlled.

INTRODUCTION

The Himalayas is known for its rich cultural diversity linked with equally rich biological diversity along with complex socio-ecological systems. The socio-economic conditions of the ethnic groups are marginalizing based on indigenous practices. The local and indigenous traditional knowledge is a key resource for empowering communities against marginalization, poverty and impoverishment to lead agro-pastoral life and develop their indigenous knowledge with availability of raw material in their surroundings; hence development of any society largely based on their social cultural and traditional knowledge system (Sahani, 2012).

Today, domestication of yak is distributed in Central Asia to the Altai and Hangai mountains of Mongolia and Russia, and from the Pamir Plateau and Tian-shan Mountains in the west to the Qi-lian and Min-shan mountains in the east and well adoptive in cold and high altitude environment. The hybridization of yak with cattle has been documented in ancient historical records (Cai, 1989; Zhang, 2000). Yak hybridization is widely practiced even till today in pastoral and agro-pastoral areas across the entire geographical distribution range of the species. It was observed that yak-cattle F1 hybrids are superior to both parental types in many aspects like better beef, greater size, produce higher milk yields and have better ability to withstand a warmer climate at lower altitudes than yak (Phillips et al., 1946a; b; White et al., 1946; Joshi, 1982; Zhang, 2000; Wiener et al., 2003). Traditionally, local cattle bulls / cows are used to interbreed naturally with yak cows at higher altitudes, while reciprocal interbreeding is more common at lower altitudes. Their F1 hybrid males are sterile, while females remain fertile. It was also noted that after four generations of backcross of hybrid cows to parental bulls, hybrid males becomes fertile and the offspring

are indistinguishable from pure yak or pure cattle in body conformation and appearance. Therefore, some animals which resemble yak probably carry genes that have been introgressed from cattle several generations earlier (Phillips et al., 1946a; b). To elaborate this concept, the development of DNA-based nuclear and mitochondrial (mt) markers allows for better identification of morphologically similar species and hybrids (Qi et al., 2009).

In Central Himalaya one ethnic community called 'Bhotia' inhabited in Harsil, Niti, Mana, Malhari in Garhwal region and Darma, Byans, Johar and Choudhas valley of Kumaon region. Generally they have two homes, one in higher altitude for summer(April-September) where they cultivated different crops like Ogal (*Fagopyrum esculentum*), Phaphar (*Fagopyrum tataricum*), Palthi (*Hordeum vulgare*), Rajma (*Phacilus vulgaris*), Potato, and some other vegetables like peas, cabbage and radish with collection of medicinal herbs like Gandrayan (*Angelica gluca*), *Silajeet*, Kutki(*Picrorihiza kurooa*) and *Keedajadi* (*Cordyceps*) locally known as *Yarsa Gambu* etc, for petty business and another home in low altitude for winter(October-March) settlement, where they cultivated wheat and other Rabi crops. In both the settlements they are accompanied with their large number of domestic animals like sheep, cattle and ponies. But they leave yak in the summer settlement as because it could not survive in low altitude for its physiology. These animals are the secondary sources of income in Bhotia economy. These animals help in adopting the Bhotia society in the extreme in-hospitable condition in higher altitudes (high mountains, steep slopes, deep valleys, rough terrains and in-hospitable weather condition and limited agriculture), through various production processes, by which the community derived their secondary income (Farooquee 1998). Their socio-economic is self sufficient and sustainable in this agro-pastoral mode of economic with minor trade of medicinal herbs in lower altitude. In view of the above traditional practices, a systematic attempt has been made to validate the hybridization in cattle by following objectives (1) Geo-phenotypic analysis of Yak, local Cow, Bulls and their hybrids (2) Assessment of genetic diversity between parents and crossbreed cattle relationships using RAPD primers.

Materials and Methods

Animals (hybridization pattern and geo-phenotypic analysis)

Three weeks field tour was conducted in high altitude to explore the indigenous traditional knowledge and practices of hybridization between yak and local cattle. Generally Yak is costly for village peoples, so only one Yak was purchased (in the study area) by 3/4 villages which was used only for hybridization purpose and number of local cattle were more than hybrids. Therefore in this study, one male Yak, three local Cows, four local Bulls, two Talbuni, three Talbu, seven Jumo and two Jhupu were taken. Yak (♂) was crossed with local Cow (♀) produced (F1 hybrid) Jhupu (♂, sterile) and Jumo (♀). When this Jumo (♀) was crossed with Yak (♂) produced Talbuni (♀) and Talbu (♂, sterile; Fig 1). All the studied animals were also evaluated geo-phenotypically using altitude, body weight (kg), body color, hairs in body, horn pattern and height (feet).

DNA isolation and PCR from Blood samples

Fresh blood of yak, cow and their hybrids (4 ml) were collected in vacutainer containing liquid EDTA for DNA isolation. Five ml of 1X SSC buffer was added to the blood samples and gently mixed for several times. Tubes were centrifuged for 10 min at 3000 rpm in room temperature (RT) and then 5 ml of supernatant was discarded. These steps were repeated several times till the pellet is totally clear. Supernatant was discarded and 1 ml of 0.2M Sodium acetate, 50 µl SDS (20%) and 25 µl proteinase K were added and kept for digestion in water bath at 56^{o}C for overnight. Digested samples were transferred in 2ml tube in equal volume and 125µl phenol and 125µl choloroform/isoamyle alcohol (24:1) was added. Solution was properly mixed and centrifuged (10,000 rpm at RT). Supernatant was transferred and equal volume of chilled absolute alcohol was added for precipitation. Centrifuged at 12000 rpm for 10 min and supernatant was discarded and 200µl TE buffer was added. Tubes were vortexed and incubated at 56^{o}C till pellet dissolves (overnight). Then 80µl, 1.2 M Sodium acetate was added and mixed thoroughly by adding 500µl chilled absolute alcohol to precipitate DNA and centrifuged at 12000 rpm at RT for 10 min. Pellet was washed with 70% alcohol. Then 200µl of TE buffer was added and incubated till pellet was dissolved at 56^{o}Cfor 24 hours. This concentrated DNA was used for further molecular analysis.

Polymerase chain reactions (PCR) were carried out in 25 µl volume. A reaction tube contained 25 ng of DNA, 0.2 units of *Taq* DNA polymerase, 10 mM of each dNTPs, 1.5 mM $MgCl_2$ and 5 pmol of decanucleotide primers (Operon, USA). The amplification was carried out in the DNA Engine thermal cycler (Biometra, USA) using 5 min in 94^{o}C, 1 min in 94^{o}C, 2 min in 35^{o}C and 2 min in 72^{o}C and final extension 5 min in 72^{o}C temperatures for 35 cycles. The DNA profile was analyzed following Nei and Li (1979) and the dendrogram of the genetic relatedness among the parents and hybrids was produced by means of UPGMA (unweight pair group method with arithmetic average) cluster analysis.

Data interpretation

Statistical analysis was carried out using Statistical version 8.0.

Results and Discussion

In the present investigation Yak (body wt: 550-600kg, thick and small horns) was survived at the high altitude of more than 3500 m amsl with extreme cold, while local cow and bull were in low survive in lower altitude. Interestingly, the cross breed animals from Yak (above 3500 m amsl) and low land cow were survived in both the altitude (2000-4000 m amsl) and they were more useful by the Bhotia in both the settlements. The male offspring from Yak and cow cross breed was known as Jupu (body wt: 400-500 kg), which was sterile in nature, and was survive in wide range of altitude (2000-4000 m amsl) and can carry loads like Yak. They also produce hairs which have high value and used as sacred broom in temple in different part of country with high demand. On the other hand the female offspring from the cross breed are known as Jhumo (app. body wt: 350-400 kg). Jhumo has long tail with hairs and thick and sickle shaped horns. They also produced more and thicker milk than low land cow. If Jhumo has been crossed with low land bull their female offspring was called Talbuni which is golden brown, containing few hairs in body and small horns and male sterile offspring called Talbu which was similar to Talbuni except black in color (Table 1). If Jhumo has been crossed with yak their hybrids called Goru ((♂, sterile like Yak) and Garmo (♀, bearing characters of cow as well as yak and produce enough milk; Fig 1). By cross breeding, the communities getting the cattle population, which can be survive in both the settlements and solve the purpose of their different mode of utility in both the settlement.

The common factors such as the geographical locations where yak pastoralist is practiced like, yak-cattle hybridization is not common in the pastoral areas at high altitude where cattle cannot survived well, while it is widespread in areas of agro-pastoral zone at relatively low altitudes (Wiener et al. 2003). This has been consistent with our observations that the surrounding hybrid animals displayed a significantly higher frequency of cattle introgression. In particular, the highest frequency of cattle introgression was detected in Jhumo (76%) and Jupu (68%) populations, which are located in the altitude of 2800-3048 m asl. In the case of the Jhumo (female), hybridization between yak and local cow has been widely carried out to improve its milk production and other used, while Talbuni and Talbu are the descendants of a small population that survived in both the settlements (Farooquee, 1998) .

To confirm parent and hybrids relationship molecular profiling was carried out using RAPD markers. It was found in molecular analysis that Jhumo 4 did not have any similarity with Jupu 1 and 4 local Cow and Talbu1. Similarly, Jhumo 2 and local cow also did not showed any similarity with Talbu 2. When these RAPD data were used for correlation study, the negative relationship was observed in Jhumo with Jupu, Bull and Yak. Maximum positive relationship was observed with Talbuni with Talbu (0.97). Minimum positive relationship was observed with Cow and Yak (Table 2). Phylogenetic tree generated through similarity matrix comprise two major clusters, Cluster A comprises of cow 4 and Yak and rest of the parents and their hybrids were in another cluster (B). Cluster (B) was again divided in two sub clusters (C and D) interestingly Bull 5, Talbuni 5 and 2, Talbu 2, Jhumo1, Jhumo 4 and Jhumo 6 were in one subcluster (D). And other hybrids were in other subclusters (C; Fig 2). The average data of all the studied animals of individual parents and offsprings were taken to construct phylogenetic tree; showed Cow, Yak and Jupu were individual clusters whereas Bull, Jhumo, Talbuni and Talbu were in cluster (Fig 3). This dendrogram was well supported by PCA analysis (Fig 4).

Since a long time, the local community in worldwide has long history of interaction with their natural environment as a treasure of knowledge, know-how practice and representations for their various needs. Yaks are the master of adaptation and survival in difficult conditions created by environment of the rugged unkempt and deep gorge with mountain terrains of the Himalayas. Hybridization practices Yak with local cattle were very much in used from more than 3000 years ago (Cai, 1989; Zhang, 1989; 2000). Cattle bulls are commonly used to hybridize with yak cows at relatively high altitudes, while reciprocal crossing is practiced at low altitudes of their distribution range (Phillips et al., 1946a; b; Cai, 1980; Joshi, 1982; Zhang, 1989; Adachi and Kawamoto, 1992; Davaa, 1996; Tshering et al., 1996). Hybrid males are sterile and their fertility does not resume until the fourth backcrossing generation (Deakin et al., 1935; Cai, 1989; Tumennasan et al., 1997; Zhang, 2000; Hisabumi et al., 2002), and therefore male-mediated cattle introgression in yak is not possible (Jianlin et al., 2002) and thus the cattle genes are only introduced into yak genome by hybridization of female F1 hybrids to yak. To validate the existing traditional knowledge male Yak has been used to introgression of gene in to local cow and these practiced was again validated by molecular markers which were also validated with our results. In another report a contemporary population of 1076 yak was developed from a small population of survivors, and hybridization between yak and local cattle which may have occurred in the process of population recovery and expansion, with a closed breeding programme and being responsible for high frequency of individuals carrying cattle-specific mtDNA sequences and/or autosomal microsatellite alleles in the population (Qi et al., 2009).

The classical example in this respect was from Sikkim Himalaya which is located in the north-eastern part of India. During the Cultural Revolution in the Tibetan (Autonomous Region of China) many Tibetan people were

moved towards Sikkim for seeking asylum. Only 10 families among them were given a plot at Khangchendzonga (Kanchenjunga) Biosphere Reserve for settlement (Chettri, 2009). They brought few dzo (male yak) from Holung of Nepal for carrying goods, training equipment and also for their livelihood. After some time dzo (male Yak) and dzomo (female) became very useful animals for them, due to their adaptability to the harsh physiographic features. Therefore in that place Yaks (dzo) are called 'nor' which means 'jewel' or 'wealth' for Tibetans. Yak/cow hybrids are larger and stronger than cattle or yaks showing heterosis (Singh et al., 2003). The female hybrids are fertile and produced more milk but the males are sterile, strongly supported with our field survey and study. This hybridization is practiced all over the Himalayas and trans-Himalayan areas and normally the dzos are used as draught animals (Chettri, 2008; Tambe and Rawat, 2009). Genetically, these hybridization patterns should follow Mendelian inheritance. where half the hybrids (Jhumo) resemble cows, presumably having received the 'cow' alleles in duplicate; while the other half (Jupu) resemble yaks, having presumably received one 'cow' and one (dominant) 'yak' allele. In addition, it was also reported that the pulmonary arterial pressure and resistance necessary for high altitude environment were significantly higher in indigenous Himalayan cattle than in the yaks (Anand et al., 1986). However, the pulmonary arterial resistance, an adaptive mutation in the arterial wall that enhances the capacity of animals to withstand a low oxygen supply, was slightly higher in the hybrids and yak than in domestic cattle. This is the reason why people prefer to cross a yak with a cow rather than a domestic bull with a Talbuni or other female hybrid. The indigenous knowledge practiced by high altitude pastoralist communities has a strong rationale base. The people of this area started using Jupu as pack animals. The rationale for using Jupu is that as a hybrid of a yak and cow, it can withstand with the high temperatures (30-32^{o}C) which yaks normally cannot stand. In addition, a Jupu and other male hybrids can carry more weight than a domestic cow or bull and can walk through any terrain with a load up to 5,000 meters, withstanding -15^{o}C without difficulties. Thus, this hybrid has become the most promising source of income for high altitude people. Similarly, these hybrids are more productive in terms of milk and can survive at a wide range of altitudinal zones during seasonal migration compared to yaks, which are normally known for less milk and being limited to alpine zones. In a report (1990), due to heavy and continuous snowfall almost 200 yaks and dri died in the high altitude area (near Dzongri) but people of those places were survived due to the presence of quite a number of hybrids like dzo, dzomo, ortoom and usanguzee to compensate for the loss (Chettri, 2009).

Table.1 Observed quantitative and qualitative characters of Yak, local cattle and their hybrids

S. No	**Traits**	**Yak (♂)**	**Cow (♀)**	**Local bull (♂)**	**Jupu (Sterile ♂)**	**Jhumo (♀)**	**Talbuni (♀)**	**Talbu (Sterile ♂)**
1	Altitude (m asl)	Above 3500	Below 2000	Below 2000	2000-4000	2000-4000	2000-4000	2000-4000
2	Body wt (approx in kg)	550-600	250-300	350-400	400-500	350-400	250-300	250-300
3	Body color	Black	Black/ white/ brown	Black/ white/ brown	Black/ white/ brown	Black/ white/ brown	Golden brown	Black
4	Hairs in body	Hairs started from juvenile vein to tail	No hairs	No hairs	Hairs started from juvenile vein to tail	Long hairs in tail	Less hairs	Less hairs
5	Horns	Small and thick	Thin and long	Thin and long	Big and long	small, thick, sickle shape	small	small
6	Height (feet)	5.5-6	4	4.5-5	5.5-6.5	5	4	4

Table 2: Correlation coefficient among cattle and hybrids

	JHUMO	**JUPU**	**BULL**	**COW**	**TALBUNI**	**TALBU**	**YAK**

JHUMO	1						
JUPU	-0.066	1					
BULL	-0.231	0.759	1				
COW	0.326	0.223	0.615	1			
TALBUNI	0.198	0.709	0.862	0.846	1		
TALBU	0.25	0.536	0.798	0.933	0.972	1	
YAK	-0.109	0.711	0.559	0.089	0.47	0.409	1

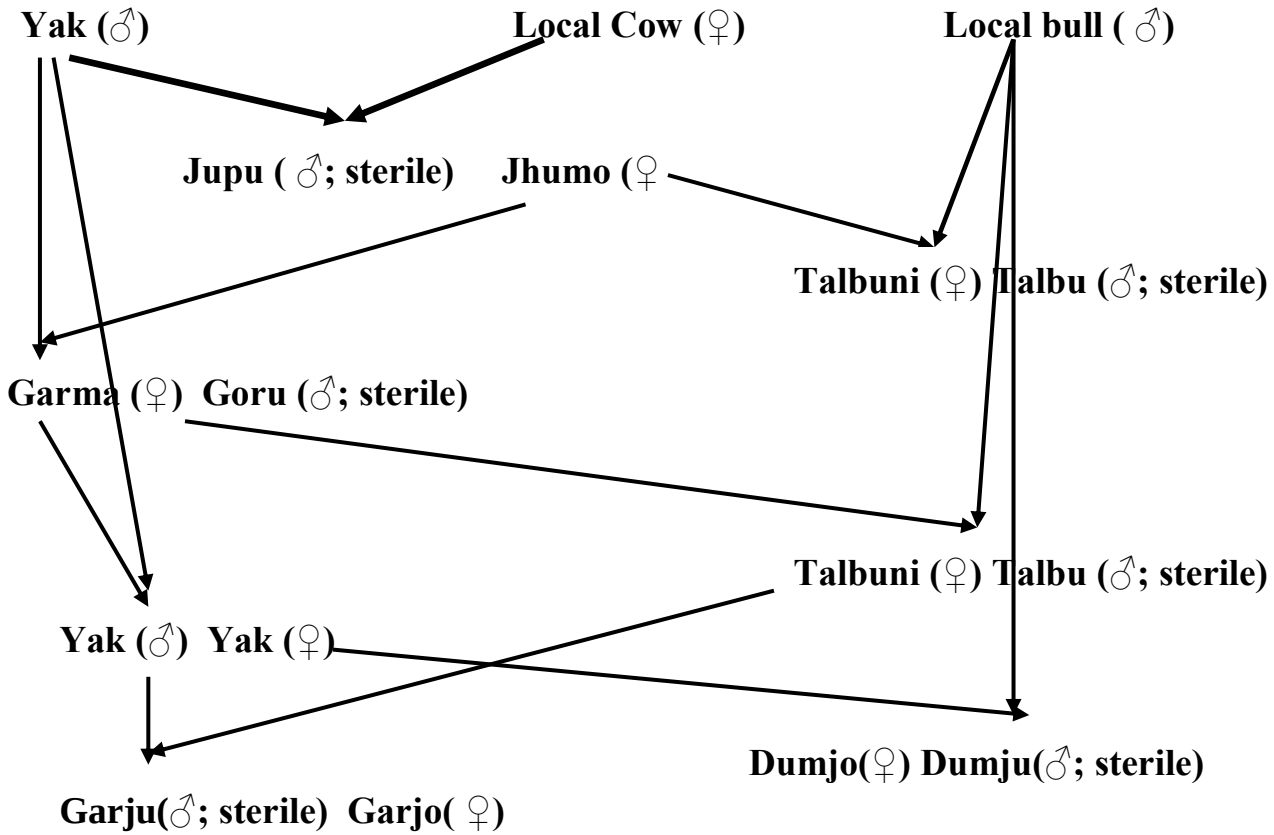

Fig 1. Hybridization pattern of yak, local cow, bull and hybrids in high altitude of Indian Central Himalaya

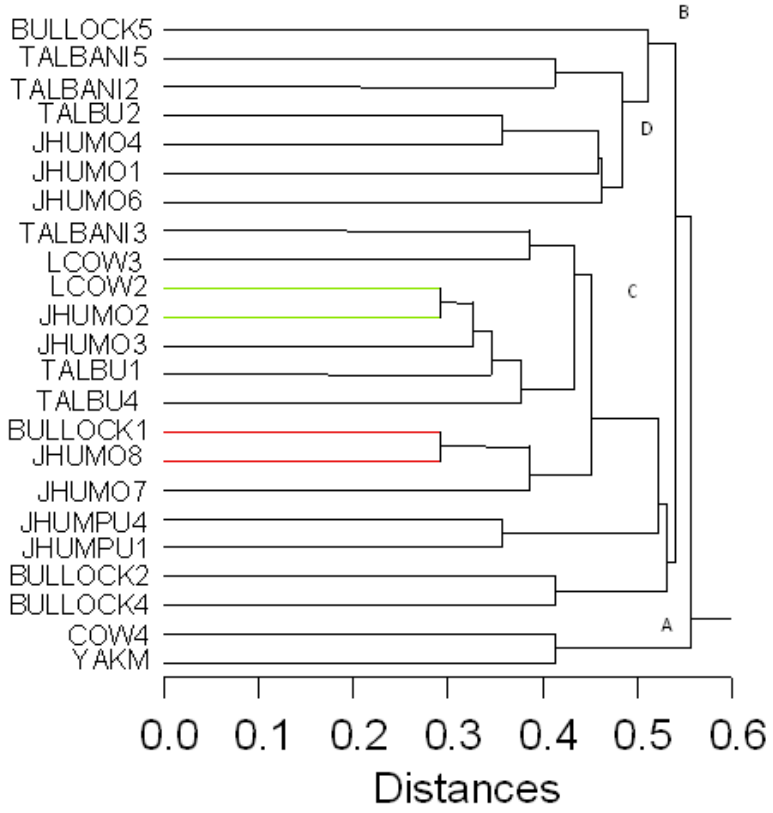

Fig 2. Genetic relationship among the Yak, local cow, bull and hybrids animals using RAPD data
Note: one male Yak, three local Cows, four local Bulls, two Talbuni (♀, hybrid), three Talbu (♂, sterile hybrid), seven Jhumo (♀, hybrid) and two Jupu (♂, sterile hybrid)

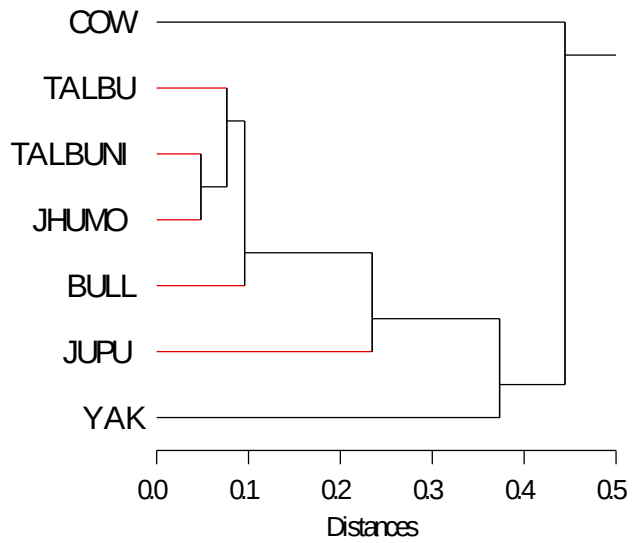

Fig 3. Phylogenetic relationships among the Yak, local cattle and hybrids

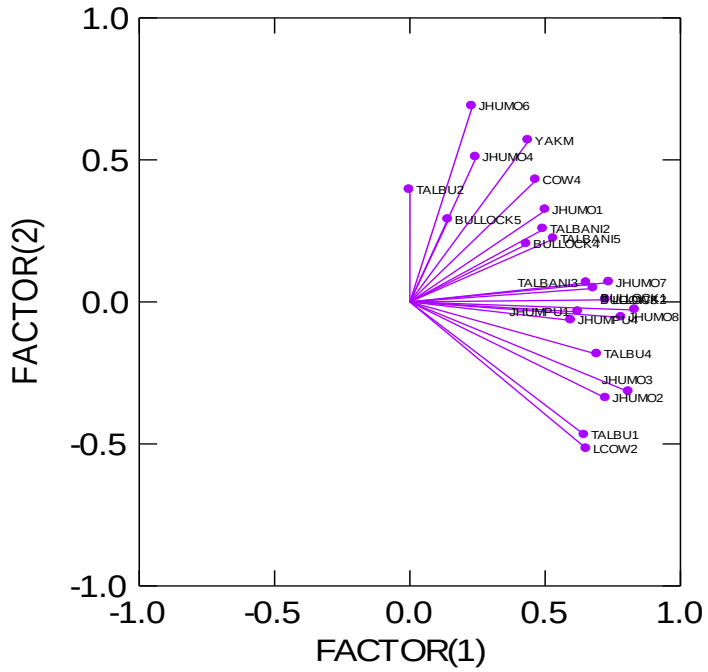

Fig. 4. Principle component analysis of Yak, local cow, bull and their hybrids
Note: one male Yak, three local Cows, four local Bulls, two Talbuni (♀, hybrid), three Talbu (♂, sterile hybrid), seven Jhumo (♀, hybrid) and two Jupu (♂, sterile hybrid)

Conclusion

In the present study a wide range of polymorphism was observed in different parents and hybrids. It is quite possible for such results that selected animals were following standard hybridization pattern but they belong to different parents of their origin. Although the level of cattle admixture showed a clear geographical structure based on our results, the level of cattle admixture is correlated with the altitude across geographical regions as well as within geographical region. Although yak-cattle hybridization is primarily driven to produce F1 hybrids, our results showed that the subsequent gene flow between yak and cattle took place and has affected contemporary genetic make-up of domestic yak. Such hybridization practices in cattle introgression is an ongoing process and might have been relatively more important in recent times. Similarly on the other hand to protect yak genetic integrity, the hybridization between yak and cattle should be tightly controlled.

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Conflict of Interest

All authors state that they have no conflict of interest.

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