

Estimation of genetic relation of mithun (*Bos frontalis*) with other livestock species through RAPD-PAGE

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This study was undertaken to determine genetic differences and similarities between 4 strains of mithun based on RAPD-PAGE markers. Cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), sheep (*Avis aries*), goat (*Capra hircus*) and mithun (*Bos frontalis*) were included for the present study. Individuals (6) from each species were selected to collect the blood samples. Blood (15 ml) from representative 6 individuals of each species was collected from juglar vein in 50 ml centrifuge tube containing EDTA as anticoagulant. The genomic DNA was isolated from each sample using whole blood DNA isolation kit. After isolation of the DNA from each sample, the DNA pool representing each of the five livestock species was made by mixing equal amounts of genomic DNAs from individual sample for RAPD study. DNA pooled samples were further diluted to the concentration of 25–30 ng/μl with deionized water for PCR amplification.

The PCR amplifications were carried out in 25 μl volume containing 75 ng of genomic DNA, 25 PM of random primer, 200 μm of each dNTPs, 2.0 mM MgCl₂, 1.5 U of Taq DNA polymerase and 1×PCR buffer (10mM Tris-HCl, pH 8.3, 50 mM KCl). Amplifications were performed on DNA thermal cycler. The amplification profile consists of 4 min initial denaturation at 95°C, followed by 40 cycles of 1 min at 94°C, 1 min at 40°C and 2 min at 72°C. After the final cycle, samples were further incubated at 72°C for 5 min. The samples were stored at 4°C. All aliquots of 15 μl were used for electrophoresis. The amplicons were resolved on 1.4% agarose gel on a constant voltage of 60 V and visualized by illumination with ultraviolet light after staining with ethidium bromide. A mixture of lambda DNA digested with Hind III

and EcoR I was used as DNA molecular size marker. The samples were resolved on 6% native polyacrylamide gel. The gels were run at constant current of 11 Amp for 10–12 h. The gels were removed from the assembly. After electrophoresis the gel was washed in 10% ethanol for 5 minute and followed by rinsing with 1% nitric acid for 3 min to oxidize DNA. Then gel was rinsed for a few seconds in distilled water. Subsequently the gel was impregnated with 0.006 M Ag NO₃ for 20 min to stain DNA. Gel was again rinsed with distilled water to remove unstained Ag NO₃ and the gel was again placed in developer solution (0.28 M sodium carbonate, 0.019% formaldehyde) till the bands of desired intensity and contrast achieved. Thereafter, the reaction was stopped by transferring the gel into solution containing 10% glacial acetic acid and kept for 2 min. The gel was viewed over the X-ray viewer and photographed. Molecular sizes of various fragments were estimated by using standard molecular size markers (8 × 174 Hal III digests, Hind III/Eco RI digest).

The genetic similarities as well as genetic distances were estimated using 2 different measures i.e. based on band sharing proportion as well as based on band frequency. The genetic similarities within and between species were calculated in the terms of proportion of common bands between the individuals within the species as well as between the species. The band sharing frequency between 2 animals was calculated as an expression of similarity of RAPD fingerprints of animals from either same or different genetic groups (Jeffrey and Morton 1987, Wtton *et al.* 1987, Dunnington *et al.* 1990).

The within species similarity (WS_i) were calculated as the average of B_{ab} across all the possible comparisons between individuals within ith species.

$$D'_{ij} = -\ln [BS'_{ij}/\sqrt{(WS_i \cdot WS_j)}]$$

The characteristics of the amplification profiles of all the primers are presented in Table 1. The total number of bands amplified ranged from 12 to 23. R-17 random primer amplified least number of bands while B-14 random primers

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Table 1. The number of polymorphic bands, total number of bands and their size ranges from different random primers on 6% native PAGE

Code No.	Total loci amplified	Polymorphic loci	Size ranges in bp	
			Minimum	Maximum
B1	13	13	391	1826
B2	14	14	353	1689
B3	18	18	179	1826
B7	17	17	255	1241
B9	16	16	296	1060
B10	13	12	396	827
B11	15	15	252	1292
B13	15	15	399	1318
B14	23	23	316	1149
B16	15	15	302	159
R-2	18	18	291	1564
R-10	19	19	228	4420
R-13	16	16	338	4318
R-14	16	15	380	3352
R-17	12	12	333	3019
R-18	20	20	317	2020
Total	279	277	179	4420

amplified maximum number of bands. Number of amplified bands of B-1, B-2, B-10 primers were close to the number of amplified bands of R-17 primer while number of amplified bands of R-18, R-16, R-10, R-2 and B-7 primers were close to the bands of B-14 primers that showed one less polymorphic loci than total number of loci amplified by them. Out of the total 279 loci amplified, 277 were polymorphic. The bands amplified from these primers also varied in their size ranges. The size difference between amplified band ranged between 431 bp (by B-10) and 4192 bp (R-10). The minimum and maximum size of polymorphic loci ranged from 179 to 4420 bp. Joshi *et al.* (1998) studied different species for RAPD profile and comparison. Performance of RAPD profile from goats, sheep, buffaloes, cattle and dogs indicated that amplified bands were unique for each species in terms of the number and position of the bands. In present study, all the random primers detected a large amount of

polymorphism as all the primers generated species-specific RAPD profile. All species differed for the RAPD profiles generated from these random primers. The characteristics of amplification profiles in various species generated by the random primers are given in Table 2. Goat showed minimum and maximum number of bands among 5 species. Cattle and buffalo as well as sheep and mithun expressed similar number of minimum bands, however, sheep and cattle showed similar number of maximum bands. In other side, the average number of bands amplified by primers were lowest in goat followed by buffalo, cattle, sheep and mithun. Sheep and mithun expressed equal average number of bands. The amplified products size was lowest in sheep followed by goat, cattle, mithun and buffalo. Kantanen *et al.* (1995) reported greater number of polymorphic RAPD markers in sheep than cattle, however, sheep populations were more homogenous than cattle populations. The maximum size of bands in bp was in cattle, followed by goat, buffalo, sheep and mithun. Cattle expressed highest range of minimum and maximum size of bands in bp followed by goat, buffalo and sheep. Lowest range between minimum and maximum size of bands in bp was in mithun. The average numbers of bands of these 5 livestock species are in comparison with published reports for other mammalian species i.e. 1 to 12 in sheep (Cushwa *et al.* 1992), 3–12 in cattle (Gwakisa *et al.* 1994), and 5–16 in dogs (Rothuizen and Van 1994).

The between species genetic similarity and genetic distances based on band sharing (BS) (with pooled over all the primers) among the 5 livestock species are presented in Table 3. The genetic similarity estimates, pooled over the primers ranged from 0.295 to 0.452. While mithun and goat followed by buffalo and sheep showed the minimum genetic similarity. Maximum genetic similarity was observed between goat and sheep, followed by mithun and buffalo. Results revealed that small ruminants and large ruminants had lesser genetic similarity as it was expected, however, species of small ruminants and large ruminants had more genetic similarity among themselves. Among large ruminants, buffalo expressed least genetic similarity with cattle and highest genetic similarity with mithun. Although buffalo and cattle are famous milch type species but had less

Table 2. Average number of bands per primer and size ranges of bands amplified in various livestock species from the polymorphic random primers on 6% native PAGE

species	No. of bands		Average No. of bands	Size range	
	Minimum	Maximum		Minimum	Maximum
Goat	1	15	5.80	207	4318
Sheep	4	10	7.00	179	3019
Cattle	2	10	6.24	228	4420
Buffalo	2	13	6.00	291	3352
Mithun	4	11	7.00	261	2092

Table 3. Genetic similarities (BS) (above diagonal) and genetic distances (below diagonal) based on band shering among the five livestock species pooled over all the random primers on 6% native PAGE

Species/ species	Goat	Sheep	Cattle	Buffalo	Mithun
Goat	–	0.452	0.312	0.318	0.295
Sheep	0.795	–	0.339	0.300	0.339
Cattle	1.164	1.081	–	0.346	0.393
Buffalo	1.144	1.204	1.061	–	0.418
Mithun	1.221	1.082	0.934	0.872	–

genetic similarity. Bhusan and Rajkhowa (2003) reported that physical appearances and rearing practices of mithun were much closer to buffalo than cattle and with other livestock species. The similarity of mithun and buffalo might be expressed at DNA level in the present study. Genetic similarity between large and small ruminants, cattle and mithun expressed same genetic similarity with sheep and these were highest while mithun expressed least genetic similarity with sheep and these were highest while mithun expressed least genetic similarity with goat. The livestock species expressed low to moderate genetic similarity with each other and at this level the sheep and goats were more genetically similar to each other in comparison to other livestock. There were no more reports available in the literature regarding genetic similarity between different studies although genetic similarities between the 5 species of poultry were in the range of 0.326 to 0.395 (Sharma *et al.* 2001), which are consistent with present observations.

The genetic distances, based on band sharing (DS) among the livestock species with RAPD markers pooled over the primers ranged from 0.795 to 1.227 while goat and sheep followed by mithun and buffalo showed the minimum genetic distance. Maximum genetic distance was observed between mithun and goat followed by buffalo and sheep. Between large ruminants and small ruminants, highest genetic distance was observed between mithun and goat and least genetic distance was found between cattle and sheep. Among large ruminants cattle showed maximum genetic distance with buffalo followed by genetic distance between mithun and cattle and genetic distance between mithun and buffalo. On the other hand mithun expressed higher genetic distance with cattle than buffalo. These results confirmed the result of genetic similarity between these five species because where genetic similarities were higher there genetic distances were lower and vice versa. The perusal of each result revealed that the livestock species at low to moderate genetic distances with each other and at this level the mithun and goats were more genetically distanced to each other in comparison to other livestock pairs. There were no more studies for genetic distance between various species however genetic distances between the 5 species of poultry were in the range of 0.998 to 1.236 (Sharma *et al.* 2001), which are consistent with the present study.

SUMMARY

Six individuals (included both sex) in each of cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), sheep (*Ovis aries*) and goat (*Capra hircus*) were utilized in the present investigation.

The genetic similarity estimates, pooled over the primers ranged from 0.295 to 0.452. Mithun and goat (BS=0.295) followed by buffalo and sheep (BS=0.300) showed the minimum genetic similarity. Maximum genetic similarity was observed between goat and sheep (BS=0.452), followed by mithun and buffalo (BS=0.418). The genetic distances, based on band sharing (DS) among the livestock species with RAPD markers pooled over the primers ranged from 0.795 to 1.227. Goat and sheep (DS=0.795) followed by mithun and buffalo (DS=0.877) showed the minimum genetic distance. Maximum genetic distance was observed between mithun and goat (DS=1.221) followed by buffalo and sheep (DS=0.204).

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