



Genetic identity of Malnad gidda, Punganur and Vechur – Indian breeds of dwarf cattle

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Malnad gidda, Punganur and Vechur are important dwarf breeds of zebu cattle, native of Southern part of India. Malnad gidda, the zebu breed of cattle (Figs 1 and 2) is well adapted to the climatic conditions of Western Ghats of Karnataka. These animals are known for disease resistance, adaptability to the hot and humid conditions of the heavy rainfall region of Western Ghats. Punganur breed of dwarf cattle (Figs 3, 4) originating from Punganur area in Chittoor, Andhra Pradesh, India. This endangered breed of cattle is now taken up for conservation at Livestock Research Station, Palamaner, Andhra Pradesh (Narendra Nath 1993 and Reddy *et al.* 2004). Vechur, yet another dwarf breed of zebu cattle, has its origin from Vechur, Kottayam, Kerala, India. These animals are short in size and with light red, black, fawn and white colour. Cows are extremely small in size, weighing about 125 kg with a height of not more than 90 cm (Figs 5 and 6). They are capable of producing 2–3 kg milk / day (Susamma 1996).

The home tracts of Malnad gidda and Vechur are contiguous. Their number is reducing at a faster rate due to large scale crossbreeding programme. Recently, the programme for the conservation of indigenous germplasm has been initiated, which requires a large-scale intervention from R&D institutions for effective conservation and propagation of valuable native germplasm. Any programme aiming at *ex-situ* conservation warrants genetic characterization of breeds, precise identification of superior germplasm and its effective use in the genetic improvement. To characterize these breeds, adequate knowledge about genetic variation within and between populations is important. Random amplified polymorphic DNA (RAPD) fingerprinting, used for characterization of different breeds of cattle and buffaloes (Bhattacharya *et al.* 2003, Saifi *et al.* 2004 and Suprabha and Anilkumar 2006), help in identification of informative markers without prior knowledge of sequence information. The study therefore, aimed at evaluating the genetic identity among Malnad gidda,

Punganur and Vechur breeds of cattle using RAPD-PCR.

DNA was isolated from 10 ml of venous blood collected from 40 Malnad gidda, 20 Punganur and 10 Vechur cattle using the high salt method (Montgomery and Sise 1990) with minor modifications. The samples were adjusted to a concentration of 20 µg/ml before PCR amplification. Ten short oligonucleotide primers chosen arbitrarily and having GC content of 60–90 % were used in this investigation. DNA samples were amplified using thermal cycler. The reaction mixture of 25 µl in each PCR tube consisted of 2.5 µl taq DNA polymerase buffer, 2.0 µl of 10 mM dNTP mix, 1.7 µl (25 mM) magnesium chloride, 2 µl (40 ng) of primer, 2 µl (50 ng) of genomic DNA diluted in TE buffer, 14.3 µl ultra pure water and 0.50 µl of Taq DNA polymerase (3U/ µl). The thermal cycles consisted of initial denaturation at 94°C for 2 min, 40 cycles of denaturation at 94°C for 45 sec, primer annealing at 55°C for 1 min, and primer extension at 72°C for 45 sec, followed by final extension for 5 min at 72°C. The PCR products were subjected to electrophoresis at 100V in 2.5 % Agarose gel in 1x TBE buffer containing 0.5 mg/ml of ethidium bromide along with DNA molecular size marker. The amplified products were read with the help of 100 to 1000 bp molecular size marker. Only distinct and prominent bands representing RAPD fingerprints were scored using *Quantity one* band analysis software and data were subjected to statistical analysis.

Statistical analysis: Band frequency (BF), which gives an estimation of number of bands shared by different breeds, was estimated as the ratio of number of animals (n) carrying a particular band to the total number of animals (N) screened per breed (BF= n/N).

Band sharing frequency (BSF): Within as well as between breeds band-sharing frequency (BSF) which is an indicator of closeness between breeds was calculated as per Smith *et al.* (1996).

$$BSF = 2 B_{ab} / (B_a + B_b)$$

where, B_{ab} is the number of bands shared by individuals, a and b and B_a and B_b are the total number of bands for individual a and b.

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Figs 1–6. 1. Malnad Gidda Bull; 2. Malnad Gidda cow with calf; 3. Punganur Bull; 4. Punganur Cow; 5. Vechur Bull 6. Vechur Cow

Genetic distance (D_{xy}): Between population band sharing frequency (BSF) values were used to estimate the genetic distance as described by Smith *et al.* (1996).

$$D_{xy} = -\ln((B_{xy}) / \sqrt{(B_x \cdot B_y)})$$

where, B_x and B_y are within breed BSF of breeds x and y , B_{xy} is BSF between breeds x and y and D_{xy} is the genetic distance between breeds x and y .

RAPD fingerprints: All 10 primers used in this study exhibited satisfactory amplification and polymorphic bands ranging from 0.053 to 1.47 Kb. The average number of scorable bands across the primers were in the range of 6 to 14 in these 3 breeds. This is in agreement with 2–18 bands reported in baboons and humans (Reidy *et al.* 1992), 5–6 in dogs (Rothuizen and Van 1994), 4 to 13 in cattle (Suprabha and Anil Kumar 2006) and 8 to 14 in buffaloes (Das *et al.* 2007). In the present investigation, the primer no. 6 (P6) produced 11 bands in Malnad gidda, 10 bands in Punganur and 13 bands in Vechur cattle. With this primer most of the bands were found to be monomorphic, except for 2, which were polymorphic with a size of approximately 0.573 and 0.824 kb. In this study, primers, P3, P4, P5, and P10 revealed 5 polymorphic bands each, while, in each primer pair of P1 and P2 as well as in P7 and P9, the number of polymorphic bands was found as 4 and 6 respectively.

The within breed band sharing frequencies ranged from 0.76 to 0.95 in Malnad gidda, 0.65 to 0.91 in Punganur and 0.75 to 0.92 in Vechur breeds of cattle. The average within breed BSF for all the primers screened was 0.83 ± 0.01 in Malnad gidda, 0.81 ± 0.02 in Punganur and 0.84 ± 0.01 in Vechur cattle. Present findings may be considered as baseline information on molecular characterization of Malnad gidda cattle in comparison with other dwarf breeds of zebu cattle.

A few reports on other breeds of cattle also demonstrated breed specific RAPD fingerprints with different primers (Gwakisa *et al.* 1994, Shivakumar 1997, Ahn *et al.* 1999). The between breed BSF values determine the genetic relation between breeds or populations. The average band sharing frequency between Malnad gidda and Punganur was observed as 0.73 ± 0.02 , whereas, the BSF values between Malnad gidda and Vechur, and Punganur and Vechur were 0.74 ± 0.02 and 0.76 ± 0.02 respectively. The present result was similar as reported in Rathi and Tharparkar breeds of cattle (Sharma *et al.* 2004) and in Gir and Kankrej breeds of cattle (Joshi *et al.* 2007).

The mean average percent difference (MAPD), being an indirect measure of similarity indices value, depicts the breed difference or dissimilarity between breeds. The MAPD values within Malnad gidda, Punganur and Vechur breeds were 17.33 ± 1.92 , 18.39 ± 2.65 and 15.84 ± 2.21 respectively. The MAPD values between Malnad gidda and Punganur, Malnad gidda and Vechur, and Punganur and Vechur were 26.36 ± 2.23 , 25.94 ± 2.08 and 23.57 ± 2.75 , respectively. The estimates of genetic distance values between Malnad gidda and Punganur, Malnad gidda and Vechur, and Punganur and Vechur were 0.113, 0.123 and 0.084, respectively. Bhattacharya *et al.* (2003) reported similar trend of results in Haryana, Tharparkar and Red Sindhi breeds of cattle. In another study, it was reported that Malnad gidda breed was different from each of the other South Indian medium/ large sized cattle breeds viz., Hallikar, Khillari, Krishna Valley, Ongole and Deoni (Ramesha *et al.* 2002).

Our results revealed that the inter-breed similarity indices were quite high among these breeds. The values in terms of MAPD and genetic distance revealed surprisingly that Vechur

Table 1. Band sharing frequency (BSF)

Primer	Within breeds			Between breeds		
	MG	PU	VE	MG×PU	MG×VE	PU×VE
P1	0.955±0.02	0.810±0.08	0.845±0.03	0.732±0.02	0.612±0.03	0.627±0.03
P2	0.773±0.04	0.916±0.04	0.92±0.02	0.836±0.04	0.826±0.02	0.89±0.01
P3	0.84±0.03	0.65±0.01	0.77±0.05	0.58±0.04	0.72±0.01	0.67±0.03
P4	0.88±0.04	0.84±0.02	0.90±0.01	0.816±0.02	0.86±0.02	0.74±0.05
P5	0.793±0.05	0.946±0.01	0.90±0.01	0.73±0.01	0.748±0.03	0.921±0.008
P6	0.874±0.07	0.769±0.04	0.799±0.04	0.754±0.03	0.767±0.01	0.724±0.03
P7	0.811±0.04	0.723±0.05	0.832±0.05	0.750±0.02	0.780±0.02	0.739±0.04
P8	0.84±0.02	0.797±0.09	0.749±0.04	0.677±0.02	0.66±0.02	0.762±0.02
P9	0.779±0.07	0.769±0.05	0.879±0.04	0.751±0.01	0.766±0.01	0.780±0.03
P10	0.761±0.05	0.869±0.03	0.88±0.03	0.70±0.05	0.725±0.04	0.762±0.03
Overall	0.830±0.01	0.809±0.02	0.847±0.01	0.732±0.02	0.741±0.02	0.762±0.02

Table 2. Average percent difference (APD)

Primer	Within breeds			Between breeds		
	MG	PU	VE	MG×PU	MG×VE	PU×VE
P1	20.51±5.58	5.55±1.39	9.97±1.14	26.77±1.47	25.30±.45	7.87±0.89
P2	22.51±4.36	8.78±4.44	8.11±2.31	16.17±4.17	17.19±2.77	11.11±0.98
P3	17.0±2.51	35.33±1.20	23.0±5.29	41.44±4.05	27.44±.53	32.66±3.68
P4	11.7±4.76	16.11±2.62	9.61±1.13	18.26±2.05	19.88±2.83	25.27±2.27
P5	4.4±2.21	19.33±7.71	15.66±3.84	29.2±4.85	39.52±3.24	36.33±4.26
P6	12.56±1.73	23.9±2.33	20.33±4.9	24.45±3.45	23.33±1.33	29.11±2.60
P7	18.85±4.67	20.42±1.93	16.76±5.9	24.92±2.30	22.0±2.98	23.84±4.53
P8	19.96±1.29	18.38±2.97	28.66±3.33	32.3±2.05	33.61±2.54	23.73±2.10
P9	21.99±7.5	23.06±5.93	12.06±0.43	24.94±1.50	23.32±1.95	22.03±3.78
P10	23.88±9.71	13.06±3.84	11.2±0.03	25.14±2.73	27.86±4.78	23.78±3.00
MAPD	17.33±1.92	18.39±2.65	15.84±2.21	26.36±2.23	25.94±2.08	23.57±2.75

breed is closer to Punganur than Malnad gidda breed of cattle. It is in contrary to the expectation since the habitats of Vechur and Malnad gidda are contiguous, while the habitat of Punganur breed is Andhra Pradesh which is almost 300–500 km away from the habitats of other 2 breeds. Because of low values of both MAPD and genetic distance, it can be concluded that these dwarf cattle breeds might have originated from a common source in the distant past. Probably, due to the process of evolution through implication of natural selection after migration from a common place and remained confined to a particular geoclimatic region, the major genes controlling some of the qualitative characters could have been fixed in these dwarf breeds.

SUMMARY

Malnad gidda, Punganur and Vechur, native breeds of Karnataka, Andhra Pradesh and Kerala, respectively, are small in size with low productivity profile. These dwarf cattle do not require any special management care in terms of feeding, herd management and health care. In addition to this, these breeds are known for disease resistance and adaptability to the hot,

humid and high rainfall areas with low input situations. The results, in terms of inter-breed similarity indices, MAPD and genetic distance estimates, revealed that there are some differences among these dwarf breeds. However, low estimates of both MAPD as well as genetic distance indicated that these dwarf cattle breeds might have originated from a common source in the distant past. Probably due to the process of evolution and through implication of natural selection after migration from a common source and remained confined to a particular geoclimatic region for years together, the major genes controlling qualitative characters might have been fixed in these dwarf breeds thereby creating some inter-breed differences.

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