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Estimation of inbreeding in cattle using RAPD markers

Tarun K Bhattacharya*, Pushpendra Kumar, Jamuna D Joshi and Satish Kumar

Blood Biochemical Polymorphism Laboratory, Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P. - 243 122, India

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Inbreeding indicates the degree of homozygosity at a locus within a population. Normally inbreeding is estimated in terms of a coefficient calculated from the pedigree of an individual. If no history is available, however, there is no way to estimate the inbreeding coefficient. Sometimes, data on individuals are missing, and that too can prevent the estimation of the inbreeding coefficient, which is essential for formulation of a breeding programme at the farm level and for breed development.

Molecular biological approaches may solve some of these problems. RAPD (random amplified polymorphic DNA) markers use oligonucleotides to prime arbitrary amplification from genomic DNA. RAPD markers are multilocus ones which may have the power to estimate band sharing among individuals within a breed. This sharing of bands may show the genome with levels of homozygosity between animals. This homozygosity is used to calculate the inbreeding coefficient. Band sharing in cattle has been estimated (Cho & Han, 1994; Gwakisa et al. 1994; Antoniou & Skidmore, 1995; Kantanen et al. 1995; Teale et al. 1995). Band sharing has also been used to calculate inbreeding coefficient in poultry (Kuhnlein et al. 1990; Grunder et al. 1994). In this study we report the inbreeding coefficients in cattle using band sharing between individuals within a population.

Materials and Methods

Isolation of genomic DNA

The study was carried out on 20 randomly selected animals of three Indian cattle breeds namely Red Sindhi, Hariana and Tharparkar maintained at three farms: Central Cattle Breeding Farm, Chiplima, Orissa, India; Shree gaushala, Jind, Hariana, India; Central Cattle Breeding Farm, Lakhimpur-Kheri, U.P., India. All three breeds were maintained at all three farms, but the herd sizes of each breed were different. Blood samples were collected in 50-ml sterile polypropylene vials containing 0·5 ml 0·5 M-EDTA. Genomic DNA was isolated from blood following the protocol of Sambrook et al. (1989). Samples were adjusted to a concentration of 20 μg/ml before the PCR reaction.

*For correspondence; e-mail: tarun@ivri.up.nic.in

Amplification and analysis of DNA

Genomic DNA was amplified by nine published primers (Table 1). The PCR reaction was carried out in a final volume of 50 μl containing 60–90 ng DNA; 100 μM of each dNTP; 40 mg primer; 1U Taq polymerase enzyme; 2 mM-$MgCl_2$; 10X PCR assay buffer (500 mM-KCl; 100 mM-Tris-HCl, pH 8·8). The programme followed for amplification was 40 cycles of denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min and extension at 72 °C for 1 min followed by final extension at 72 °C for 5 min. PCR products were electrophoresed at 150 V in 1·6% EEO agarose containing 1X TBE and ethidium bromide (0·5 μg/ml). λ DNA digested with EcoRI and HindIII was used as a molecular weight marker. Bands were visualized under a u.v.-transilluminator and photographed.

Only distinct and prominent bands were scored from the gel. The whole study was replicated twice. The resultant bands were used to estimate band sharing within a population and hence the inbreeding coefficients. Band sharing or similarity coefficients were calculated as an expression of the similarity of RAPD fingerprints of animals within a breed (Jeffreys & Morton, 1987; Welton et al. 1987; Dunnington et al. 1990). Mathematically it could be expressed as BS=2Cab/(Na+Nb); where BS is band sharing, Cab is the number of common bands shared by individuals a and b, Na and Nb are the total number of bands for individuals a and b. The inbreeding coefficient of each breed was calculated using the formulae:

$$U=1 \Big/ N\sum_{i=1}^{N} Vi$$

and Fx=(U−0·417)/0·566 (Kuhnlein et al. 1990), where U is the linear relationship between band frequency and variability, N is the number of different bands scored, Vi is the frequency of band i and Fx is the inbreeding coefficient.

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Table 1. RAPD primers producing polymorphic bands

Primer	Sequence (5′ to 3′)	GC%†	Reference
A1	GCACTGAGTA	50	Kantanen et al. 1995
A2	ACGTCGAGCA	60	Kantanen et al. 1995
A5	GAATCGGCCA	60	Castellanos et al. 1996
P1	ACAACGCCTC	60	Appa Rao et al. 1996
P2	GGGAACGTGT	60	Appa Rao et al. 1996
P3	CTGGGCAACT	60	Appa Rao et al. 1996
P4	CCGTGACTCA	60	Appa Rao et al. 1996
T3	GCTGCTCGAGT	64	Kantanen et al. 1995
T4	AACCGCGGTCT	64	Kantanen et al. 1995

†Guanine plus cytosine content of primer (%)

Results

The number of bands that could be scored in the three indigenous breeds of cattle across the nine primers ranged from 2–18 having a molecular size of 0·15–3·0 kb. When A1 primer was used for the study, most of the bands were monomorphic. The other primers, A2, T3, T4, A5, P1, P2, P3 and P4, yielded respectively 7, 2, 7, 7, 5, 4, 4 and 2 bands during agarose gel electrophoresis.

The overall band sharing frequency across the primer was 0·75 ± 0·04 in Hariana cattle, 0·76 ± 0·02 in Tharparkar and 0·86 ± 0·04 in Red Sindhi. On the basis of the primer-wise contribution, A1 primer revealed the highest value of band sharing in all three breeds, whilst the lowest estimate was found in A2 primer (Table 2).

The different number of shared fingerprints were taken into account for inbreeding studies. The highest inbreeding coefficient was found in A1 primer whereas the lowest magnitude was from P2 primer in Hariana and Tharparkar populations. The primers T4 and P1 derived, respectively, the highest and lowest estimates of inbreeding coefficient in Red Sindhi. The overall inbreeding coefficient was highest in the Hariana breed, followed by Tharparkar and Red Sindhi (Table 2).

Table 2. Average band sharing frequencies (R) and average inbreeding coefficient (Fx) in three Indian Zebu cattle populations

	Hariana		Tharparkar		Red Sindhi	
Primer	R	Fx	R	Fx	R	Fx
A1	0·95 ± 0·05	9·22	0·83 ± 0·03	8·11	0·90 ± 0·01	7·46
A2	0·74 ± 0·03	6·50	0·76 ± 0·02	6·34	0·68 ± 0·03	5·71
A5	0·75 ± 0·02	6·39	0·77 ± 0·02	7·46	0·86 ± 0·01	7·86
T3	0·79 ± 0·02	8·27	0·73 ± 0·04	6·88	0·76 ± 0·03	6·54
T4	0·73 ± 0·03	6·81	0·69 ± 0·03	6·16	0·79 ± 0·02	8·50
P1	0·75 ± 0·02	6·65	0·73 ± 0·02	6·61	1·00 ± 0·06	5·34
P2	0·60 ± 0·03	5·28	0·66 ± 0·04	5·96	1·00 ± 0·04	5·39
P3	0·62 ± 0·04	6·05	0·80 ± 0·02	6·94	0·86 ± 0·03	6·14
P4	0·83 ± 0·02	7·85	0·84 ± 0·02	8·06	0·86 ± 0·02	7·56
Overall	0·75 ± 0·04	7·00	0·76 ± 0·02	6·95	0·86 ± 0·04	6·72

Discussion

All the primers used in the study showed polymorphic bands, which is the basic requirement for analysing variability within and between populations (Gwakisa et al. 1994). The breed-specific bands were observed for primers T4, A5 and P4. Such breed/population-specific primers were reported earlier (Cho & Han, 1994; Lee et al. 1994; Chung et al. 1995; Carpio et al. 1996; Yang et al. 1996). The major advantage of RAPD analysis over other ways of examining polymorphism, such as RFLP or microsatellite analysis is in the ease with which informative markers are found without prior sequence information.

The inbreeding coefficient was highest in Hariana, although the band sharing frequency was highest in Red Sindhi. This may be due to the Hariana population being small. Animals are mostly bred within high and low-yielding groups within the population. On the other hand, the Red Sindhi animals on the farms were large in number and random mating was followed, which created a more even balance of genetic material in the herd and which might be the reason for the highest similarity coefficient with a low inbreeding measure. This method is very useful, however, when formulating a breeding plan in a herd for which there is no information on inbreeding coefficient or relatedness amongst animals.

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