

## Genetic differentiation of Indian camel (*Camelus dromedarius*) breeds using random oligonucleotide primers

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### Summary

The camel population in India is facing a severe decline which demands that immediate steps are taken to ensure its conservation. Characterisation is an integral part of the conservation program. The Polymerase Chain Reaction-Randomly Amplified Polymorphic DNA profile of unrelated camels of the Bikaneri (29), Jaisalmeri (30) and Kachchhi (18) breeds were analyzed. Reproducible polymorphic bands with varying frequencies among the three breeds of camel were obtained with five oligonucleotide primers. A total of 75 bands were amplified, of which 27 (36%) were polymorphic. The probability of obtaining identical fingerprints was observed to be the lowest in primer GC-10 (5.7%) followed by OP-08 (8.7%), GT-10 (11.3%), G-2 (15.5%) and G-1 (80%). Breed informative bands were amplified. The maximum genetic variability was observed in the Bikaneri ( $0.80 \pm 0.05$ ) followed by the Kachchhi ( $0.84 \pm 0.06$ ) and the Jaisalmeri ( $0.87 \pm 0.05$ ) breeds. The inter-breed genetic distance estimates indicated a closer relationship in the Bikaneri-Kachchhi camels, (0.075), followed by the Jaisalmeri-Kachchhi (0.106) and Bikaneri-Jaisalmeri (0.132) breeds. A similar genetic relationship was observed when the degree of population subdivision was measured between the Bikaneri-Kachchhi (0.529), Jaisalmeri-Kachchhi (0.558) and Bikaneri-Jaisalmeri (0.566) breeds.

### Resumen

La población de camélidos en la India se enfrenta con un declive importante que requiere iniciar con una rápida intervención en vistas de su conservación. Un parte integral del programa de conservación está representado por la caracterización. Se ha analizado el perfil de ADN polimórfico amplificado casualmente de la cadena de reacción de polimerasa en camélidos sin relación tales las razas Bikaneri (29), Jaisalmeri (30), y Kachchhi (18). Las bandas polifórmicas reproducibles con frecuencias variantes entre las tres razas se obtuvieron con cinco oligonucleótidos primarios. Un total de 75 bandas fueron amplificadas, de las cuales 27 (el 36%) resultaron polimórficas. La probabilidad de obtener huellas idénticas fue inferior en el primer GC-10 (5,7%), seguido por OP-08 (8,7%), GT-10 (11,3%), G-2 (15,5%) y G-1 (80%). Las bandas de información de raza fueron amplificadas. El máximo de variabilidad genética se observó en la raza Bikaneri ( $0,80 \pm 0,05$ ) seguida por la raza Kachchhi ( $0,85 \pm 0,06$ ) y la Jaisalmeri ( $0,87 \pm 0,05$ ). La distancia genética estimada entre razas indica una relación estrecha entre las razas Bikaneri y Kachchhi (0,075), seguida por Jaisalmeri-Kachchhi (0,106) y Bikaneri-Jaisalmeri (0,132). Se observó una relación genética similar cuando el grado de subdivisión de la población fue medido entre Bikaneri-Kachchhi (0,529), Jaisalmeri-Kachchhi (0,558) y Bikaneri-Jaisalmeri (0,566).

**Keywords:** Characterisation, RAPD, Camelus

*dromedaries, Statistical analysis, Genetic distance, Similarities.*

## Introduction

Conservation of livestock species is a matter of global concern. The characterisation of livestock breeds at the phenotypic and molecular genetic level has become essential to establish the current status of the different livestock species and breeds available in different agro-climatic zones of the country and the world. India had the third highest camel population in the world until 1999, but due to a severe decline in the camel population since then it has slipped to sixth position (FAOSTAT data, 2005). The population of camels in India numbers 632 000. Rajasthan state has the highest population of 498 000 followed by Gujarat with 53 000 and Madhya Pradesh with 8 000. There has been 29.65% decline in the overall population in the last five years (Livestock Census, 2003). This is an alarming situation requiring immediate attention to ensure conservation of the breeds. India has four main breeds of camel, these being the Bikaneri, Jaisalmeri, Kachchhi and Mewari. The Bikaneri breed is well known for the draught potential whereas the Jaisalmeri breed is known for race potential and long distance travel. The camels of the Bikaneri and Jaisalmeri breeds are adapted to the climatic conditions of the *Thar* desert where the temperature gets very high during the summer and very low in winter. The Mewari breed is known for the production of milk and is adapted to the hilly terrains of the *Mewar* area in Rajasthan. The Kachchhi breed has a medium level of milk production and draught capabilities, and is adapted to the marshy land of the Ran of Kachchh in Gujarat state (Rathore, 1986).

The phenotypic characteristics of different camel breeds have been already described (Rathore, 1986). Significant differences exist

between the breeds in the production of hair (Sahani *et al.*, 1996), milk (Sahani *et al.*, 1998), draught potential and speed and stride (Rai *et al.*, 1992) etc. However, no significant breed differences could be detected by cytogenetic (Sahai and Vijh, 1993) and biochemical studies (Tandon *et al.*, 1997a, b; Tandon, 1998).

PCR-RAPD (Polymerase Chain Reaction, Randomly Amplified Polymorphic DNA) has the potential to distinguish between strains of almost any organism without prior knowledge of its DNA sequence utilizing short random primers of arbitrary sequences (Welsh & McClelland, 1990; Williams *et al.*, 1990). It has been used to distinguish strains of mouse (Welsh *et al.*, 1991), breeds of cattle (Kemp & Teale, 1992; Chung *et al.*, 1995) and sheep (Kantanen *et al.*, 1995), lines and breeds of chicken (Plotsky *et al.*, 1995), meat of different species (Min *et al.*, 1996) and for the detection of genetic variation in camel (Shereif and Alhadrami, 1996; Mishra *et al.*, 1998). PCR-RAPD was therefore used in the present investigation to study the genetic variation within Indian camel breeds and to estimate the genetic distances between them.

## Materials and Methods

### Camel (*Camelus dromedarius*)

Blood samples were collected from the Bikaneri (Figure 1), Jaisalmeri (Figure 2) and Kachchhi (Figure 3) camels maintained at the National Research Centre for Camels (NRCC) in Bikaner, India. The Centre maintains ~270 camels, Bikaneri (~120 camels), Jaisalmeri (~100 camels) and Kachchhi (~50 camels). Pedigree records are available since the year 1960. Almost every fourth year breeding males were procured from the breeding tracts of the respective breeds to avoid inbreeding in the centre's herd. The DNA was isolated using the method utilized by Davis *et al.*, (1986).

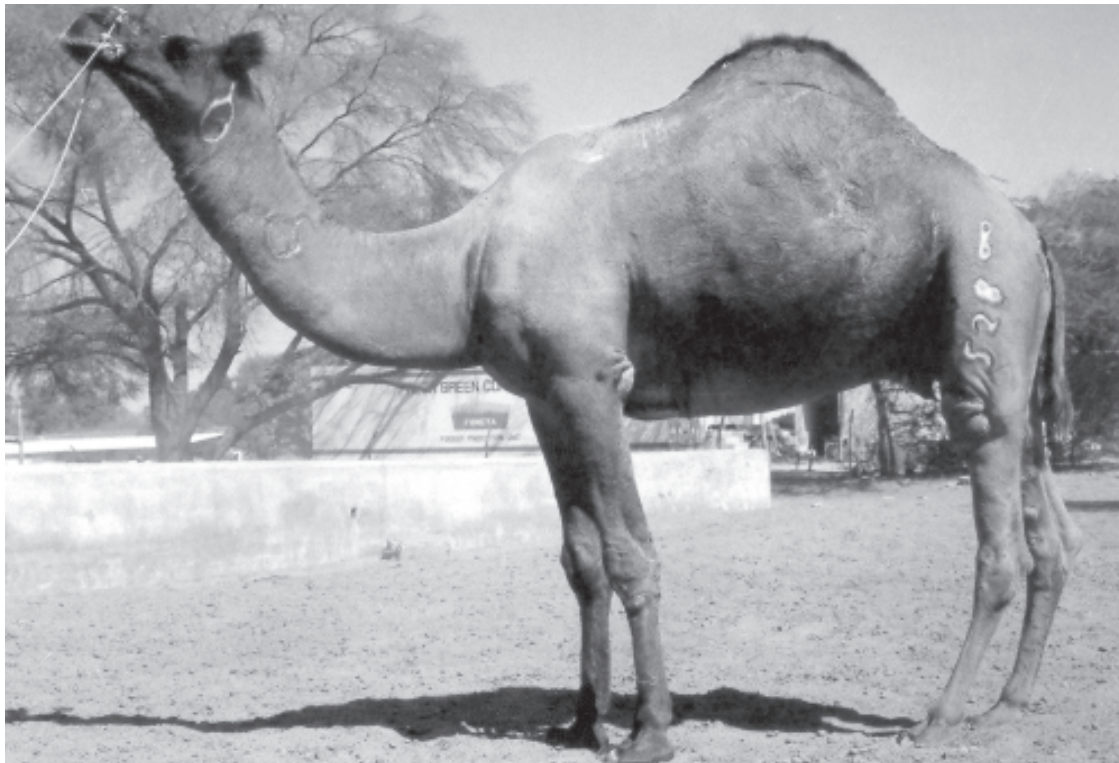


Figure 1. Adult Bikaneri male camel.

### PCR-Random Amplification of Polymorphic DNA

The PCR amplification reactions were carried out in 50  $\mu$ l reaction volume in 0.5 ml thin walled PCR tubes. The composition of PCR reaction was template DNA ~100 ng, primer ~5.0 pmol, each dNTP- 2.5 mM, *Taq* DNA polymerase- 2.5 U, *Taq* polymerase buffer 10X- 5.0 ml (10 mM pH 9.0 Tris HCl, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl and 0.01% gelatin). The PCR cycling conditions comprised initial denaturising at 94°C for 5 minutes, followed by 40 cycles of 94°C for one minute, 36°C for 45 seconds and 72°C for one minute. Final extension was carried out for five minutes at 72°C.

A total of six arbitrary short oligonucleotide primers reported to be informative in camel (Shereif and Alhadrami, 1996; Mishra, 1998) were used. The primer sequences were G-1 (5' GTG ACG TAG G 3'), G-2 (5' TCG CGA GCT G 3'), GC-10 (5'GCC GTC CGA G 3'), GT-10

(5' GTG ATC GCA G 3'), OP-08 (5' GTC CAC ACG G 3') and C-7 (5' GCG AGC GTC CC 3').

### Statistical analysis

Standard statistical analysis was used for analysing the PCR -RAPD data to obtain the RAPD characteristics with reference to the average number of bands ( $\epsilon$ ) and standard error (Nei and Li, 1979; Wetton, 1987; Lynch, 1990), the mean population frequency of a band (q) (Jeffreys and Morton, 1987), the mean probability for a band to be present in the heterozygous state (h) (Georges *et al.*, 1988), the probability that all bands of animal x are also present in animal y (but later may have additional bands) (Pg) (Morsch and Leibenguth, 1994) and the probability of two camels exhibiting identical fingerprints (no additional bands) (Pi) (Morsch and Leibenguth, 1994). The within breed similarity as band frequency ( $W^f$ ) and

Table 1. PCR-RAPD profile and frequency distribution of RAPD alleles in the polymorphic loci among different camel breeds.

| Primer | Number of bands |                 | Polymorphic bands and their frequencies |          |            |          |
|--------|-----------------|-----------------|---|----------|------------|----------|
|        | Total           | Size range (kb) | Size (kb)                               | Bikaneri | Jaisalmeri | Kachchhi |
| GT-10  | 17              | 0.40-3.10       | 1.51                                    | 0.14     | 0.37       | 0.24     |
|        |                 |                 | 1.02                                    | 0.64     | 0.83       | 0.35     |
|        |                 |                 | 0.93                                    | 0.64     | 0.97       | 0.94     |
|        |                 |                 | 0.87                                    | 0.64     | 0.40       | 0.94     |
|        |                 |                 | 0.67                                    | 0.00     | 0.43       | 0.29     |
|        |                 |                 | 0.50                                    | 0.04     | 0.93       | 0.18     |
|        |                 |                 | 0.41                                    | 0.75     | 0.97       | 0.94     |
| GC-10  | 15              | 0.60-2.90       | 1.45                                    | 0.21     | 0.04       | 0.22     |
|        |                 |                 | 1.32                                    | 0.10     | 0.43       | 0.06     |
|        |                 |                 | 1.24                                    | 0.83     | 0.61       | 0.61     |
|        |                 |                 | 1.00                                    | 0.21     | 0.04       | 0.22     |
|        |                 |                 | 0.95                                    | 0.72     | 0.82       | 0.94     |
|        |                 |                 | 0.83                                    | 0.03     | 0.32       | 0.22     |
|        |                 |                 | 0.77                                    | 0.17     | 0.18       | 0.11     |
| G-2    | 18              | 0.30-3.30       | 0.71                                    | 0.24     | 0.54       | 0.17     |
|        |                 |                 | 2.19                                    | 0.75     | 0.67       | 0.81     |
|        |                 |                 | 2.00                                    | 0.42     | 0.17       | 0.75     |
|        |                 |                 | 1.07                                    | 0.54     | 0.50       | 1.00     |
|        |                 |                 | 0.48                                    | 0.04     | 0.71       | 0.19     |
| OP-08  | 16              | 0.60-3.50       | 0.36                                    | 0.25     | 0.71       | 0.19     |
|        |                 |                 | 2.51                                    | 0.86     | 0.83       | 0.67     |
|        |                 |                 | 1.17                                    | 0.80     | 1.00       | 0.83     |
|        |                 |                 | 1.08                                    | 0.07     | 0.92       | 0.25     |
|        |                 |                 | 1.00                                    | 0.47     | 0.50       | 0.67     |
| G-1    | 9               | 0.57-2.00       | 0.82                                    | 0.47     | 0.42       | 0.67     |
|        |                 |                 | 0.80                                    | 0.67     | 0.67       | 0.83     |
| C-7    | 3               | 0.80-2.00       | 0.57                                    | 0.33     | 0.00       | 0.00     |
|        |                 |                 | 0.00                                    | -        | -          | -        |

band sharing rate ( $W^s$ ), between breed similarity as band frequency ( $B^f$ ) and band sharing rate ( $B^s$ ) (Kuhnlein *et al.*,1989), the genetic distances based on band frequency ( $D^f$ ) and band sharing rate ( $D^s$ ) (Kuhnlein *et al.*,1989), the population subdivision index  $S_{ij}$  (Lynch,1990) and the modified Wright's index  $F_{ST}$  of population subdivision (Lynch,1991) were calculated to estimate the genetic variation in the populations and genetic distances between them.

## Results and Discussion

### RAPD Polymorphism

The PCR-RAPD profile of camel genomic DNA with the primers used along with the number and size range of the bands scored are presented in table 1. The primers, GT-10, GC-10, G-2, OP-08 and G-1 amplified a total of 75 bands, of which 27 (36%) were polymorphic. The proportion of the



Figure 2. Adult Jaisalmeri male camel.

polymorphic bands was the highest with the primer GC-10 (53.33%) followed by GT-10 (41.18%), OP-08 (37.5%), G-2 (27.78%) and G-1 (11.11%). Since the RAPD technique is known to be sensitive to the amplification parameters (Williams *et al.*, 1993), the amplifications were repeated for a total of 54 samples spread over all primer-breed combinations.

Highly reproducible RAPD patterns were obtained in all combinations with the camel genome under precise conditions. The reproducibility of RAPD patterns in camel populations/breeds has also been reported by Shereif and Alhadrami (1996) and Mishra *et al.* (1998).

A varying number of scorable bands (3-18) were amplified in the three breeds of camel. The average number of bands and the degree of polymorphism differed significantly with the primer as well as with the breed (Table 1). Since RAPD markers have been shown to follow Mendelian inheritance and are dominant-recessive (Williams *et al.*, 1990; Kemp and Teale, 1992;

Rothuizen and Wolferen, 1994, Elo *et al.*, 1997; Liu *et al.*, 1998), the presence of a band in an individual indicates the presence of at least one dominant allele, while absence indicates homozygosity for recessive alleles (Wei *et al.*, 1997).

The analysis of RAPD patterns for breed differentiation was carried out considering only the clearly resolved bands. The primers GT-10 and OP-08 amplified a total of 17 and 16 bands, respectively. Shereif and Alhadrami (1996) used the above two primers and reported amplification of 4-16 DNA bands in the size range of 0.3~2 kb in association with five other decamer primers. The present results are quite consistent with that of Shereif and Alhadrami (1996) except for the size range of 0.3~3.5 kb. This could be either due to the differences in resolution under the 5% vertical non-denaturing polyacrylamide gel electrophoresis used in the previous study and the 1% horizontal agarose gel electrophoresis used in the present study or due to differences in the breeds. PCR-RAPD

Table 2. Data from PCR-RAPD of camel breeds from five oligonucleotide primers.

| Primer | Breed      | n  | $\epsilon \pm SE$ | N     | q    | h    | $P_g$ | $P_i$ |
|--------|------------|----|-------------------|-------|------|------|-------|-------|
| GT-10  | Bikaneri   | 28 | 12.86 $\pm$ 0.28  | 13.98 | 0.72 | 0.44 | 0.34  | 0.108 |
|        | Jaisalmeri | 30 | 14.90 $\pm$ 0.17  | 16.02 | 0.74 | 0.41 | 0.34  | 0.107 |
|        | Kachchhi   | 17 | 13.88 $\pm$ 0.26  | 14.92 | 0.74 | 0.41 | 0.37  | 0.125 |
| GC-10  | Bikaneri   | 29 | 9.52 $\pm$ 0.25   | 10.82 | 0.65 | 0.52 | 0.30  | 0.077 |
|        | Jaisalmeri | 28 | 9.96 $\pm$ 0.42   | 11.58 | 0.63 | 0.54 | 0.22  | 0.041 |
|        | Kachchhi   | 18 | 9.83 $\pm$ 0.32   | 11.30 | 0.64 | 0.53 | 0.25  | 0.055 |
| G-2    | Bikaneri   | 24 | 14.96 $\pm$ 0.24  | 16.09 | 0.74 | 0.41 | 0.34  | 0.106 |
|        | Jaisalmeri | 24 | 15.75 $\pm$ 0.23  | 16.94 | 0.74 | 0.41 | 0.32  | 0.094 |
|        | Kachchhi   | 16 | 15.94 $\pm$ 0.06  | 16.60 | 0.80 | 0.33 | 0.52  | 0.265 |
| OP-08  | Bikaneri   | 15 | 13.33 $\pm$ 0.23  | 14.49 | 0.72 | 0.44 | 0.33  | 0.099 |
|        | Jaisalmeri | 12 | 14.33 $\pm$ 0.19  | 15.41 | 0.74 | 0.41 | 0.35  | 0.117 |
|        | Kachchhi   | 12 | 14.08 $\pm$ 0.26  | 15.64 | 0.68 | 0.48 | 0.23  | 0.045 |
| G-1    | Bikaneri   | 12 | 8.33 $\pm$ 0.14   | 8.77  | 0.78 | 0.36 | 0.65  | 0.417 |
|        | Jaisalmeri | 12 | 9.00 $\pm$ 0.00   | 9.00  | 1.00 | 0.00 | 1.00  | 1.00  |
|        | Kachchhi   | 12 | 9.00 $\pm$ 0.00   | 9.00  | 1.00 | 0.00 | 1.00  | 1.00  |

n: Sample size, X: Average number of bands, SE: Standard error,  $\epsilon$ : Number of judged positions; q: Mean population frequency of a band; h: Mean probability for a band to be present in heterozygous state;  $P_g$ : Probability that all bands of animal x are also present in animal y;  $P_i$ : Probability of obtaining identical fingerprints.

of indigenous camel breeds amplified 5 to 15 bands with the same primers (Annual Report, NRCC, 1997-1998), which is similar to the number of bands amplified (3-18) in the present investigation.

### RAPD characteristics

The RAPD patterns of three camel breeds were characterised by distinguishable polymorphic bands in the size range of 0.36 to 2.51 kb (Table 1). The characteristics of RAPD patterns for the camel breeds from five oligonucleotide primers are presented in table 2. The mean population frequency of a band (q) was found to vary with the primer. The lowest value of q was observed for the three camel breeds in the primer GC-10 (0.64) followed by primer OP-08 (0.71), GT-10 (0.73), G-2 (0.76) and G-1 (0.93). Accordingly, the mean heterozygosity was the highest in the primer GC-10 followed by

rest of the primers in same sequence. The average band sharing rate ( $P_b$ ) was observed to be very high (0.92, 0.93 and 0.93) in the three camel breeds, which is in agreement with the findings of Shereif and Alhadrami, (1996). The higher values of the average band sharing rate (0.92-0.93) and the mean population frequency of a band (0.63-1.00) with low levels of heterozygosity (0.00 to 0.54) indicated reduced variability of the RAPD marker loci in the three breeds of camel. This might be due to the limited genetic variability in the species. The probability of two random camels exhibiting identical fingerprints ( $P_i$ ) was observed to be lowest ( $4.1 \times 10^{-2}$ ) in the Jaisalmeri breed with primer GC-10. Pooled over breeds, the primer GC-10 was found to give the lowest  $P_i$  value ( $5.7 \times 10^{-2}$ ). Hence, the above primer could be of great use in establishing individual identity and differentiating the camel breeds.

### Breed specific/Informative bands

All five primers generated RAPD patterns, which could be used to discriminate between the three breeds of camel (Table 1). The amplification of a DNA band having significantly less frequency ( $<0.10$ ) in one breed and a very high frequency ( $>0.90$ ) in other breed(s) could be of considerable use in distinguishing the camel breeds. The primer GT-10 amplified two bands (0.67 kb and 0.50 kb) with variable breed specificity. The 0.67 kb band was not scored in all camels belonging to the Bikaneri breed, whereas it was present with the frequency of 0.43 and 0.29 in the Jaisalmeri and Kachchhi breeds, respectively. The 0.50 kb band was observed with a frequency of only 0.0357 in the Bikaneri breed, whereas it was present with a very high frequency (0.93) in the Jaisalmeri but with a relatively low frequency (0.18) in Kachchhi breed indicating probable specificity for the Jaisalmeri breed.

The primer GC-10 revealed the highest polymorphism among the primers used,

which demonstrated four breed informative bands as the differences in the frequencies of these bands among the three breeds were not sufficient to designate them as breed specific. The 1.45 kb band had a frequency of only 0.04 in the Jaisalmeri breed as compared to 0.21 and 0.22 in the Bikaneri and Kachchhi breeds, respectively. The 1.32 kb band was observed in the Kachchhi breed with a very low frequency of 0.06, whereas this band was present with the frequencies of 0.43 in the Jaisalmeri and 0.10 in the Bikaneri. The 1.00 kb band had exactly the same frequency as that of the 1.45 kb fragment in the three breeds.

These frequencies were traced back to the camels in respective breeds and it was observed that in the Bikaneri and Jaisalmeri breeds the same camels exhibited the 1.45 kb and 1.00 kb fragments indicating the probable existence of a linkage between the two loci. However, in the Kachchhi breed, the camels exhibiting the 1.45 kb fragment were different from those exhibiting the 1.00 kb fragment, which was probably due

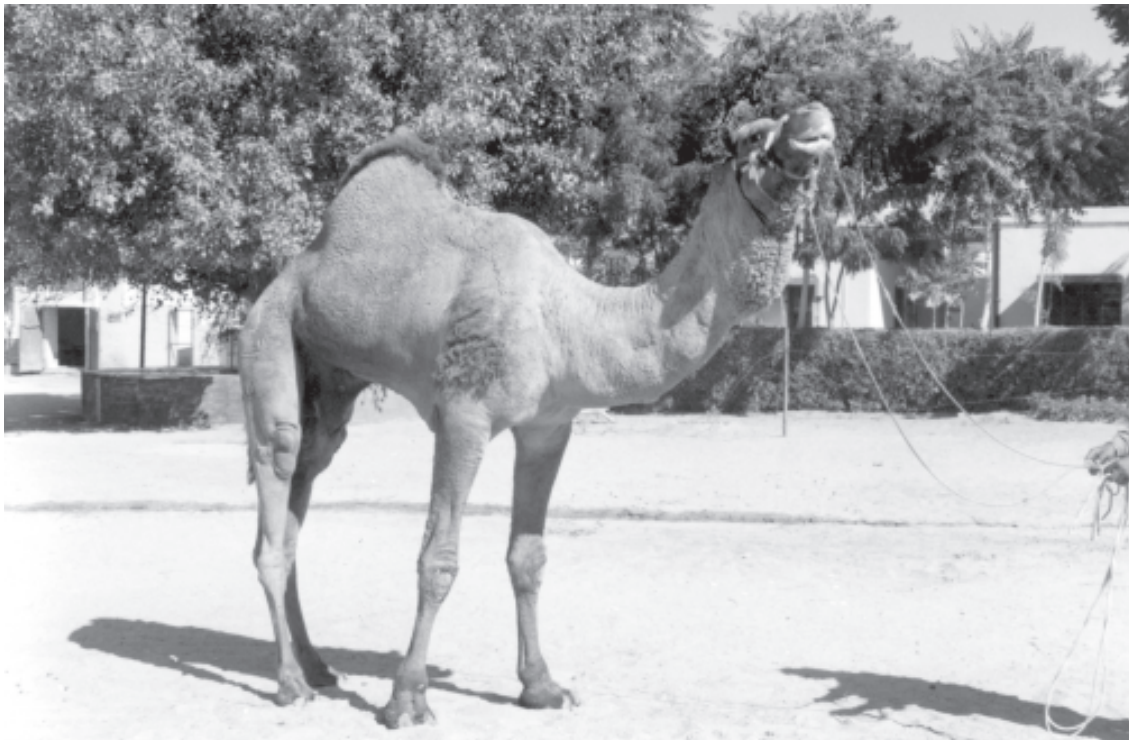


Figure 3. Adult Kachchhi male camel.

to segregation in this breed. The 0.83 kb fragment was observed with a frequency of only 0.03 in the Bikaneri, whereas it was observed with a frequency of 0.32 and 0.22 in the Jaisalmeri and Kachchhi breeds, respectively.

In primer G-2, the 0.48 kb band was present with a frequency of 0.71 in the Jaisalmeri breed as compared to 0.04 in the Bikaneri and 0.19 in the Kachchhi breed. In primer OP-08, a fragment of 1.08 kb had the frequency of 0.92 in Jaisalmeri, 0.07 in Bikaneri and 0.25 in Kachchhi camels. Primer G-1 amplified only one polymorphic band of 0.57 kb size, which was present only in the Bikaneri breed with a frequency of 0.33.

The frequencies of RAPD alleles in the Kachchhi breed presented an interesting feature. Except for the 1.07 kb band in primer G-2, most of the bands which exhibited probable specificity for the Bikaneri or Jaisalmeri breed were present with the frequency of about 0.2 in the Kachchhi breed.

### Within-breed genetic similarity

Within-breed genetic similarity was estimated as band frequency ( $W^f$ ) and band sharing ( $W^s$ ) for the three breeds (Table 3). The Jaisalmeri breed exhibited the highest within-breed similarity, pooled over primers, with band frequencies of  $0.87 \pm 0.05$  and band sharing of  $0.93 \pm 0.02$ . This degree of similarity within the Jaisalmeri breed was observed in all primers, except G-2, when considered individually and estimated as band frequency.

The Kachchhi camels exhibited less genetic similarity as compared to the Jaisalmeri when estimated as band frequency ( $0.84 \pm 0.06$ ) and band sharing ( $0.93 \pm 0.02$ ) with pooled over primers and when primers were considered individually and estimated as band frequency. The lowest within-breed similarity among the three breeds of camel was observed in the Bikaneri breed as

$0.80 \pm 0.05$  (band frequency) and  $0.92 \pm 0.01$  (band sharing).

This within-breed genetic similarity reflects the history of breeding, selection and population size of the concerned genetic group. In the present study the within-breed genetic similarity was of a very high order. The genetic variability exhibited was the highest in the Bikaneri breed followed by the Kachchhi and Jaisalmeri breeds, which could be attributed to the difference in the population of the breeds in their respective breeding tracts. The population of camels in the breeding tract of the Bikaneri, Jaisalmeri and Kachchhi breeds was 239 000, 127 000 and 53 000 respectively (Livestock Census, 2003). Shereif and Alhadrami, (1996) reported a higher genetic similarity based on band frequency in a local camel breed of U.A.E. Higher within breed similarity was also expected from the observed lack of intrabreed variation in cytogenetic (Sahai and Vijh, 1993) and biochemical studies (Tandon *et al.*, 1997a, b; Tandon, 1998).

### Between-breed genetic similarity

Of the five random oligonucleotide primers, four (GC-10, GT-10, G-2 and OP-08) were used for the estimation of between-breed genetic similarities as they amplified at least one polymorphic band in each of the three breeds. The primer G-1 did not amplify any polymorphic band between the Jaisalmeri and Kachchhi breeds and thus it was excluded from further analyses (Table 1).

The between-breed genetic similarity was estimated as band frequency ( $B^f$ ) and band sharing ( $B^s$ ) based on the method described by Kuhnlein *et al.* (1989). It is evident from the genetic similarity data of each primer that Bikaneri-Kachchhi exhibited highest between-breed similarity followed by Jaisalmeri-Kachchhi and Bikaneri-Jaisalmeri in terms of  $B^f$  and  $B^s$ . The pooled primer information verified identical relationships between breeds in both types of estimates ( $B^f$  and  $B^s$ ). The overall between-breed



Table 3. Within breed similarity estimated as band frequency ( $W^f$ ) and band sharing ( $W^s$ ) in three breeds of camel from five oligonucleotide primers.

| Breed      | Similarity estimates | Primer     |            |            |            |            | Pooled    |
|------------|----------------------|------------|------------|------------|------------|------------|-----------|
|            |                      | GT-10      | GC-10      | G-2        | OP-08      | G-1        |           |
| Bikaneri   | $W^f$                | 0.76       | 0.63       | 0.83       | 0.83       | 0.93       | 0.80±0.05 |
|            | $W^s$                | 0.92±0.004 | 0.88±0.008 | 0.93±0.003 | 0.92±0.004 | 0.97±0.004 | 0.92±0.01 |
| Jaisalmeri | $W^f$                | 0.88       | 0.67       | 0.88       | 0.9        | 1.00       | 0.87±0.05 |
|            | $W^s$                | 0.93±0.003 | 0.86±0.009 | 0.93±0.003 | 0.93±0.003 | 1.00±0.000 | 0.93±0.02 |
| Kachchhi   | $W^f$                | 0.82       | 0.64       | 0.89       | 0.87       | 1.00       | 0.84±0.06 |
|            | $W^s$                | 0.93±0.003 | 0.87±0.008 | 0.96±0.002 | 0.90±0.005 | 1.00±0.000 | 0.93±0.02 |

Table 4. Between breed genetic similarity, Genetic distance and Population subdivision among three camel breeds.

| Primer         | Breed      | Genetic similarity <sup>1</sup> |            |          | Genetic distance <sup>2</sup> |            |          | Population subdivision <sup>3</sup> |            |          |
|----------------|------------|---------------------------------|------------|----------|-------------------------------|------------|----------|-------------------------------------|------------|----------|
|                |            | Bikaneri                        | Jaisalmeri | Kachchhi | Bikaneri                      | Jaisalmeri | Kachchhi | Bikaneri                            | Jaisalmeri | Kachchhi |
| Pooled primers | Bikaneri   | -                               | 0.879      | 0.929    | -                             | 0.132      | 0.075    | -                                   | 0.978      | 0.990    |
|                | Jaisalmeri | 0.890                           | -          | 0.901    | 0.025                         | -          | 0.106    | 0.566                               | -          | 0.980    |
|                | Kachchhi   | 0.904                           | 0.894      | -        | 0.011                         | 0.022      | -        | 0.529                               | 0.558      | -        |

<sup>1</sup>Above diagonal : as band frequency (B), Below diagonal: as band sharing rate (B<sup>s</sup>).

<sup>2</sup>Above diagonal: as band frequency (D<sup>f</sup>), Below diagonal: as band sharing rate (D<sup>s</sup>).

<sup>3</sup>Above diagonal: S<sub>ij</sub> (Lynch,1990), Below diagonal: F<sub>ST</sub> (Lynch,1991).

genetic similarity is of very high order and it indicates low levels of genetic variation among the three breeds of camel (Table 4).

### Genetic distance between breeds

Genetic distance between the three breeds of camel was estimated using four primers for the reasons explained above. The genetic distance estimates pooled over primers indicated the lowest genetic distance between Bikaneri-Kachchhi ( $D^f=0.0745$ ,  $D^s=0.011$ ) followed by Jaisalmeri-Kachchhi ( $D^f=0.1060$ ,  $D^s=0.0220$ ). The Bikaneri-Jaisalmeri breeds had the highest genetic distance ( $D^f=0.1315$ ,  $D^s=0.0245$ ) among the breed pairs studied (Table 4).

Estimation of genetic similarity within and between breeds and genetic distance among different breeds of livestock is an important application of the DNA based genetic markers. The above information is of immense importance in breed characterisation and conservation studies as well as in selection programmes as it is essential for efficient sampling and utilization of germ plasm resources and for making decisions regarding choice of parents (Smith *et al.*, 1990; Nienhuis and Sills, 1992). In the present investigation, two measures of genetic relatedness i.e. genetic similarity and genetic distance were estimated as band frequency and band sharing.

The within breed similarity estimated as band frequency (0.63-1.00) showed a much greater variability than band sharing (0.86-1.00), though both estimates revealed higher within breed genetic similarity in camel breeds. The between breed genetic similarity estimated as band frequency (0.803-0.959) and band sharing (0.860-0.935) indicated close relationships among the camel breeds. The genetic distance, which is the second measure of genetic relatedness among the camel breeds, when measured as band frequency, presented a wider range relatively (0.042-0.219) as compared with the band sharing estimates (0.001-0.042). The band frequency estimates can therefore be

considered as better measures of the genetic relatedness within and between breeds.

### Population subdivision

The three camel populations were considered as three sub-populations of a single breed and the degree of subdivision was measured as  $S_{ij}$  (Lynch, 1990) and  $F_{ST}$  (Lynch, 1991). The pooled over primers differences among the  $S_{ij}$  values of the three breeds (0.978, 0.98 and 0.99) were observed to be very small. However, when the population subdivision was estimated by the modified Wright's index of  $F_{ST}$  values the differences among the three populations (0.529, 0.558 and 0.566) widened (Table 4).

The values of  $S_{ij}$  per primer were in the range of 0.962-0.999, which indicated that the tested sub-populations are nearly homogeneous. It was considered that the similarity index might underestimate the population heterozygosity, especially if alleles are rare (Lynch, 1991). The modified Wright's index of population subdivision ( $F_{ST}$ ) was therefore used. The values obtained were in the range of 0.511-0.608, which indicated an existence of heterozygosity among the three camel populations/breeds.

Thus, the PCR-RAPD technique could discern the underlying genetic variation among Indian camel breeds which was otherwise difficult to quantify (Sahai and Vijn, 1993; Tandon *et al.*, 1997a, b; Tandon, 1998). Due to automation, ease, cost effectiveness and capability to estimate the genetic distance and other related parameters in Indian camel breeds, the technique could be utilized for the characterisation of camel populations which in turn might act as backbone for *in situ* conservation of different camel breeds whose populations are facing a severe decline.

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