

Full Length Research Paper

Determination of genotype differences through restriction endonuclease in Camels (*Camelus dromedarius*)

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Tyrosinase gene or C locus has long been implicated in the coat colour determination. This gene a copper-containing enzyme located on chromosome 11q14.3 is expressed in melanocytes and controls the major steps in pigment production. In camel, C locus a restriction site provoked by the T variant of the mutation was used in a special restriction fragment length polymorphism analysis (PCR-RFLP) for genotyping of camels from six different Pakistani camel breeds (Marecha, Dhatti, Larri, Kohi, Campbelpuri and Sakrai). Significant differences in the genotype frequency between the breeds were estimated. The Sakrai breed showed in comparison to other studied breeds a distinctly higher frequency of the homozygous with restriction genotype. The objective of the present study was to screen the camel breeds using modern genetic technique that have been so far classified on the basis of performance and tribal ownership.

Key words: Camel, genotype, restriction endonuclease.

INTRODUCTION

Restriction endonucleases have become an indispensable tool for the manipulation of DNA (Szalay et al., 1979). These enzymes readily recognize the sequence in the genome and cleave the DNA at the particular sequence called recognition site. The gene (TYR) or C locus has long been implicated in the coat colour determination. This gene, which is a copper-containing enzyme located on chromosome 11q14.3 (Tomita et al., 1989), is expressed in melanocytes and controls the major steps in pigment production (Renugadevi et al., 2010). This gene is of interest in farm animals because of its role in coat colour production. It has been shown to cause a range of dilution phenotypes

including complete albinism in cattle, humans, mice and chickens (Schmidtz et al., 2001). C locus gene has been reported to be consisting of 5 exons and 4 introns in mammals (Giebel et al., 1991). Traditionally, camels have been classified according to their function (for example, riding or pack types). They have also been classified according to their habitat for example, riverine, desert, mountain etc (Wilson, 1997). Camels have been further categorised into conventional types comparable to those applied to cattle that is, beef, dairy, dual purpose and racing (Wardeh et al., 1991). Many modern classifications have advance little beyond these concepts and are usually simply tribal or location names (Hoste et al., 1985), with little attempt to assign the quantitative production parameters, that are now so important in other domestic species, to breed description. The objective of the present study was to screen the camel breeds that

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Table 1. Camel breeds with coat colour.

S/N	Breed	Coat color
1	Marecha	Brown and dark brown
2	Dhatti	Light and dark fawn
3	Larri	Reddish or brown
4	Kohi	Cream or dark brown
5	Campbelpuri	Reddish brown
6	Sakrai	Dark brown to blackish

have been so far classified on the basis of performance, and tribal ownership etc, to sequence the genes of interest in the camel and to find some genetic differences in six already conventionally characterized camel breeds of Pakistan.

MATERIALS AND METHODS

Camels ($n = 140$) belonging to six different Pakistani camel breeds were included in this study. These animals belonged to different ecological zones of Pakistan and presented a high variation in their coat colours (Table 1). Genomic DNA was isolated from their hairs using NucleoSpin Tissue kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. Isolated DNA of at least two animals from each breed was used for sequencing exon 1 of the tyrosinase gene.

Polymerase chain reaction (PCR) amplification

Four primer pairs were designed for part of the coding sequence of exon 1 of the tyrosinase gene. The raw sequence based on four PCRs using primers from homolog regions of cattle, pig, horse, human and mouse (GenBank accession numbers NM18100, AF2556101, AF252540, NM000372 and NM011661, respectively) to amplify a main part of the coding region of the camel C locus gene. The sequence of the resulting fragment was used for primer design for a part of exon 1 of dromedary camel (dTYR) in 5' and 3' direction. The first primer pair D-TY-A up 5' > AGC CTG TGC CTC CTC CAA GAA < 3' and D-TY-A low5' > TGC ATC CAT ACA AAG AAG TCA TAA < 3' yielded a 474 bp fragment. Second pair C1-A11 up 5' > AAT GCT CCT GGC TGT TTT GTA < 3' and C1-A11 Low5' > CTG CCA GGA GGA GAA GGA TGC T < 3' was used to amplified 819 bp fragment, third pair C1 up 5' > TGC CTG CTG TGG AGT TTC < 3' and C1 Low5' > GCC GAA GCC CTG GTG GAT G < 3' amplified 516 bp fragment and fourth pair KC1 up5' > CCA GCT CTC AGG CAG GGG T < 3' and KC1 Low 5' > GAC TCT TCT TGT TGC TGT GGG A < 3' yielded 516 bp fragment. The fragments of these three primer pairs overlapped and resulted in a 770 bp total sequence. PCR reactions were carried out using UNO thermo cycler (Biometra, Germany) in a total volume of 25 μ l containing 2.5 mM, MgCl₂, 0.2 mM dNTP 1U Taq DNA Polymerase (Genaxxon, Germany) 0.2 μ M of forward and reverse primer and 100 ng genomic DNA. After an initial denaturation with 94°C for 2 min 35 cycles were done each consisting with 94°C for 1 min, annealing at 56°C (primer pairs D-TY-A up, D-TY-A low; C1-A11 up, C1-A11 Low and C1 up and C1 low), 60°C (KC1 up and KC1 low) for 30 s and extension at 72°C for 40 s. The final step lasted for 10 min at 72°C. The PCR amplified fragments were excise from 2% agarose gel and purified using Gene Clean II Kit (Q BIO gene, Canada). Each fragment was sequenced in both directions using BigDye Terminator v1.1 Cycle Sequencing chemistry on an ABI Prism 310

Genetic Analyzer (Applied Biosystems, USA). All sequence alignments and distance calculations were made by Lasergene software (DNASar, USA).

Polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP)

PCR reactions for a Ddel restriction were carried out in a total volume of 25 μ l with the primer pair D-TY-A up and D-TY-A low to amplify a 474 bp fragment at 56°C. PCR product (15 μ l) was mixed with master mix (2.0 μ l buffer, 0.2 μ l Bovine Serum Albumin (BSA), 0.2 μ l Ddel enzyme (2 U, Promega Madison WI, USA) and 2.6 μ l water) and incubated at 37°C for 3 h. After incubation 5 μ l loading dye buffer was added and electrophoresed for 2 h.

RESULTS AND DISCUSSION

A part of exon 1 (779 bp) of dromedary camel (dTYR) was amplified using four primer pairs namely C1A11up, C1A11 low, C1 up, C1 low, D-TY-A up, D-TY-A low, KC1 up, KC1 low. With the D-TY-A up and D-TY-A low-primer pair a 474 bp fragment was amplified, that contained a T / C variation and creating a Ddel restriction site. PCR-Ddel restriction was used to analyze the genotype variation at tyrosinase gene in 140 dromedary camels included in the present study (Table 2). Sakrai breed possessed a significantly ($P < 0.05$) higher number of homozygous without restriction (TT) and heterozygous (CT) animals, a significantly lower number homozygous with restriction (CC) animals than that of Marecha, Dhatti, Larri and Kohi breeds. No significant difference ($P > 0.05$) was observed among Marecha, Dhatti, Larri, Kohi and Campbelpuri breeds (Table 3).

The resulting camel tyrosinase sequence showed a high homology 84.2, 88.7, 89.3, 90.1, and 91.9% to the corresponding sequences of mouse (Lavado et al., 2005), human (Gotoh et al., 2004), horse (Wagner and Reissmann, 2000), cattle (Schmutz et al., 2004) and pig (Siebel et al., 2000), respectively. About 150 different TYR mutations have been characterized in humans (Oetting and King, 1999), cattle (Schmutz et al., 2004), rabbit (Aigner et al., 2000) and mice (Beermann et al., 2004). This gene was of interest in farm animals because of its role in coat colour production. In this study, a 779 bp fragment was sequenced in the exon 1 of the camel tyrosinase gene. By sequencing camels of different

Table 2. Genotype frequency observed through restriction enzyme.

Breed	Number	Genotype frequency		
		TT	CT	CC
Marecha	0.192	0.192	0.462	0.346
Dhatti	0.208	0.208	0.334	0.458
Larri	0.185	0.185	0.370	0.445
Kohi	0.200	0.200	0.367	0.433
Campbelpuri	0.320	0.320	0.520	0.160
Sakrai	0.400	0.400	0.560	0.040

TT = Homozygous with restriction, CT= heterozygous and CC = homozygous without restriction.

Table 3. Breed differences (t-Value) in camels screened through restriction enzyme.

Breed	Number	Marecha	Dhatti	Larri	Kohi	Camp-belpuri	Sakrai
Marecha	23	--	1.96	0.68	0.77	2.32	7.26*
Dhatti	22	--	--	0.35	0.33	7.06	14.20*
Larri	24	--	--	--	0.02	4.71	10.96*
Kohi	26	--	--	--	--	5.13	11.57*
Campbelpuri	22	--	--	--	--	--	1.55
Sakrai	23	--	--	--	--	--	--

* = significant at P<0.05.

breeds a single nucleotide polymorphism (C/T) on position 200 after ATG causing an amino acid substitution (Pro/Leu) was detected. A restriction site for Ddel provoked by the "C" variant of this mutation that was used in a special restriction fragment length polymorphism analysis (PCR-RFLP) for genotyping of 140 animals from all six breeds of camels. Statistically significant differences in the genotype frequency between the breeds were estimated. The Sakrai breed showed higher frequency ($P < 0.05$) of homozygote without restriction (TT = 0.40) than the Marecha, Dhatti, Larri and Kohi breeds.

CONCLUSION AND SUGGESTIONS

Among camel breeds significant differences were observed in the genotype frequencies. So it is possible to use this mutation in a complex system of breed description. The significant correlations between the genotypes created by the Ddel restriction and some performance traits should be controlled in a greater number of animals. If further investigation corroborate the first results it would be possible to use this mutation in a marker assisted selection.

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