

GENETIC CHARACTERISATION OF MOROCCAN CAMEL POPULATIONS USING MICROSATELLITES MARKERS

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ABSTRACT

The first genetic characterisation of Moroccan camel populations were performed by using microsatellite markers. A total of 140 dromedary camels belonging to 5 known populations (Guerzni, Marmouri, Khouari, White, and Jebli) were used. DNA was analysed using 7 microsatellites. Seventy nine alleles were observed: 52 in Guerzni type, 47 in Khouari type, 53 in Marmouri type, 40 in Jebli type and 43 in White type. The average number of alleles was 7.4 in Guerzni type, 6.7 in Khouari type, 7.6 in Marmouri type, 5.7 in Jebli type and 6.1 in White type. A new specific allele (210bp) was identified at the locus CVRL6 in Jebli type. Based on average heterozygosity values, variability was relatively lower and similar in White and Jebli types: 0.6406 and 0.6366 respectively, while it was higher and comparable in Guerzni, Khouari and Marmouri types: 0.7619, 0.7162 and 0.6751, respectively. A high similarity was found between White and Jebli populations and between Guerzni, Marmouri and Khouari population after determination of genetic distances, Fst, AFC and phylogenetic tree. Probabilities of percentage exclusion obtained with the 7 markers were higher than 99.99% for the five populations and probabilities of identification of individual camels varied between 1/8.106 and 1/55.106. The estimated percentage of assignment of each animal to his type shows a good percentage of assignment (85 to 89 %) for the white type.

Key words: Dromedary camel, genetic characterisation, individual assignment, microsatellite

Beside its specific morphological and functional particularities, camel (*Camelus dromedarius*), has shown a well adaptation to its environment. Moreover, camel is able to ensure better utilisation of limited resources of its ecosystem eventhough under extreme and harsh environmental conditions. These characteristics have been yet studied by many scientists. However, less interest was given to characterise and identify camel populations in different countries. The first work on this topic in Morocco was carried in 1988 by Bengoumi. The study concerned the biochemistry and clinical polymorphism of albumin and transferrin in the dromedary camel. Thereafter, Achaaban *et al* (1997) have tried to characterise Moroccan camel breeds by using phenotypic criteria and protein system polymorphism. It was possible to distinguish five different types of camels in Morocco based on phenotypic profile, three (Marmouri, Guerzni and Khouari- called also Maldat) in the southern regions and two (Jebli and White type) in the northern part

of the country. The Marmouri is characterised by its large size, a little marked hump and a fine skin with scarce hair. The Guerzni is short, stocky (especially females) and muscular type. It has a marked hump, a thick skin and harsh coat particularly abundant around the neck and trunk. The Khouari has some characteristics of type Guerzni, but the type Marmouri is more dominant and it can easily be confused with it. It seems to be a crossover product of both Guerzni and Marmouri. The Jebli type is characterised by its short stature and a black coat, while the White type is known by its white coat and large size. However, in some cases, the phenotypic characteristics are unable to distinguish between these types of camel, probably because of a deep cross breeding. Therefore, the use of DNA analysis is known to allow a good identification. Few studies have been conducted in the field of genetic characterisation of different camel populations all over the world (Mburu *et al*, 2003; Mehta *et al*, 2006). Such investigation are still lacking in our country. The aim of this study is to perform a genetic

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characterisation of Moroccan camel populations by using polymorphic microsatellite markers.

Materials and Methods

The study was conducted on 140 camels of different types coming from different area of the country, as shown in table 1. Blood samples were collected from jugular vein. Genomic DNA was extracted from blood using an alkaline lysis method (Miller *et al*, 1988).

Primers

Three new world Camelidae microsatellite primer pairs: VOLP03 (Obreque *et al*, 1998) and, YWLL44, YWLL59 (Lang *et al*, 1996), and four dromedary specific primer pairs, CVRL01, CVRL05, CVRL06 and CVRL07 (Sasse *et al*, 2000) were used.

DNA amplification

The polymerase chain reaction (PCR) were carried out in a total volume of 12 µl containing 20-40 ng genomic DNA, 5-10 pmoles of each primer divided in two multiplex depending on the labeled color and the fragment size (YWLL59-VOLP03-CVRL06 and CVRL01-05-07) and one simplex (YWLL44) and a PCR kit (PCR Master Mix Promega) including 3mM MgCl₂, 50 units/ml Taq polymerase and 400µM of each dnTP and nuclease free water. Cycling profile included an initial denaturation step at 95°C for 10 min, followed by 30 cycles of 45 S at 94°C, 1 min at 55-60°C depending on the primer pair used, 1 min at 72°C and a final step of 15 min at 72°C using a GeneAmp 2700 (Applied Biosystems) Thermal cycler.

Fragment analysis

The PCR fragments were resolved on an ABI Prism 310 DNA fragment analyser using the internal size standard Genescan 350-Rox (Applied Biosystems). Data were analysed using the Genescan (version 3.7) and the Genotyper (version 3;7) softwares (Applied Biosystems).

Statistical analysis

Genetic polymorphism for each population was measured as the mean number of alleles per locus (MNA) and the expected heterozygosity (He) assuming Hardy-Weinberg (HW) equilibrium using GENETIX (version 4.03) (Belkhir *et al*, 2001). Genetic distance between pairs of populations (Nei, 1978) and genetic differentiation parameter (Fst) (Weir & Cockerham, 1984) was calculated. Multivariant Correspondence was applied using individual genotypes to produce 3D graphical representation using GENETIX (Belkhir *et al*, 2001). Distance matrices were calculated using Population (version 1.2.28) software package.

Assignment of individual camel to their most likely subpopulation was performed by GeneClass (version 2.0) software package (Piry *et al*, 2004).

Probability of exclusion and identity were calculated using an Excel macro.

Results

Population structure

A total of 79 alleles were observed: 52 in Guerzni type, 47 in Khouari type, 53 in Marmouri type, 40 in Jebli type and 43 in White type. The total number of alleles per locus ranged from 3 (YWLL59) to 16 in CVRL1 locus, the most polymorphic microsatellite system. A new specific allele (210bp) was identified at the locus CVRL6 in Jebli type (table 2).

The average number of alleles was 7.4 in Guerzni type, 6.7 in Khouari type, 7.6 in Marmouri type, 5.7 in Jebli type and 6.1 in White type.

The largest MNA was found in Marmouri type with 7.6 while the lowest mean of alleles was found in the Jebli (MNA=5.7) and white type (MNA= 6.1). Expected average heterozygosity values averaged over loci (He) showed an overall pattern similar to that observed for the MNA per locus. The highest value (0,675) was observed within the Marmouri and Guerzni type where as the lowest value (0,625) was

Table 1. Number and area of camel sampled for each type.

Type	Number	Regions
Guerzni	32	Dakhla (23°43'N, 15°57'O), Laâyoune (27°50'N, 13°12'O) and Tantan (28°27'N, 11°5'O)
Marmouri	30	Dakhla (23°43'N, 15°57'O), Laâyoune (27°50'N, 13°12'O) and Tantan (28°27'N, 11°5'O)
Khouari	29	Dakhla (23°43'N, 15°57'O), Laâyoune (27°50'N, 13°12'O) and Tantan (28°27'N, 11°5'O)
Jebli	25	Ouarzazate (30°56'N, 6°54'O)-Zagora (30°19'N, 5°52'O) -Errachidia (31° 33' N, 4° 15'O) et Essaouira (31°30'N, 9°46'O)
White	24	Ouarzazate (30°56'N, 6°54'O)-Zagora (30°19'N, 5°52'O) -Errachidia (31° 33' N, 4° 15'O) and Essaouira (31°30'N, 9°46'O)

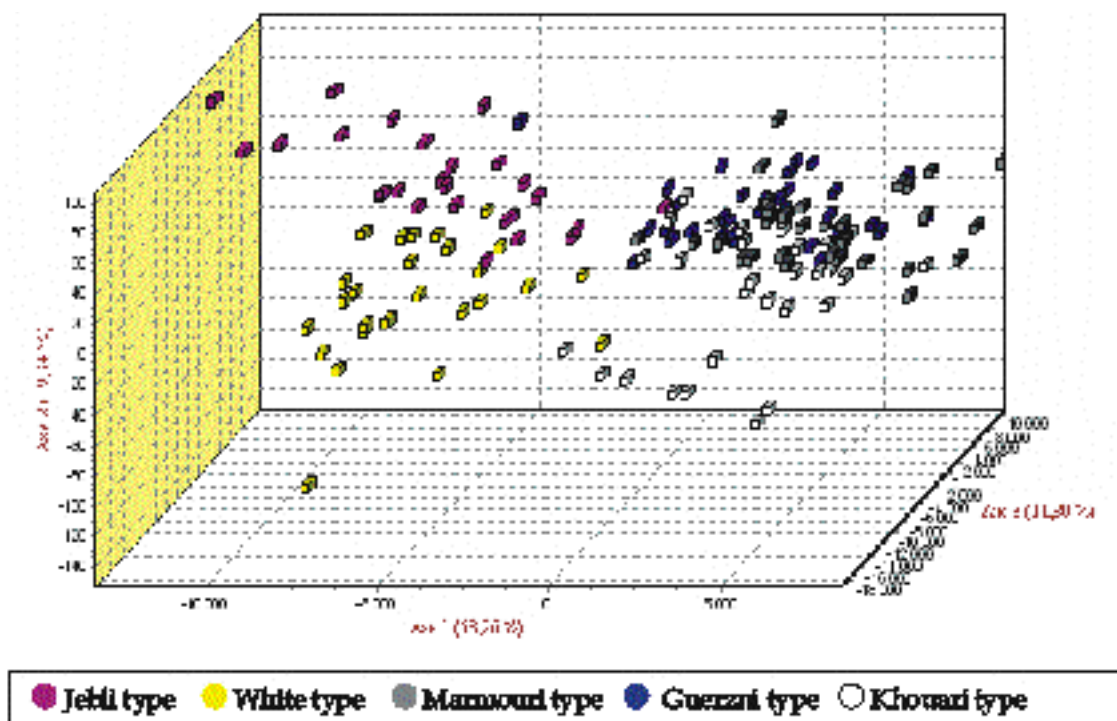


Fig 1. 3D graphical representation of multivariate correspondence analysis for the five camel types using individual genotypes.

observed within the northern types (White and Jebli) (table 3).

Relationships between populations

Genetic differentiation parameter (Fst) from the data estimator of Weir and Cockerham (1984) was calculated after permutation of individuals for each pair of populations. The Fst represent a classical description method for inter and intrapopulations genetic variability. Fst values obtained (Table 4) between the populations studied showed a low genetic differentiation between white and Jebli types (0.0112) and between Guerzni and Marmouri types

(0.0112). Fst was about ten times lower between Khouari and Guerzni (0.00344) and between Khouari and Marmouri (0.00244). Genetic distance between pairs of populations according to Nei *et al* (1978) rapprochement (Table 5) showed also a between White and Jebli types on one hand and between the south populations on the other hand (Guerzni, Khouari and Marmouri). The lowest differentiation was observed between Khouari and Guerzni and between Marmouri and Khouari.

Table 2. Alleles in bp for each microsatellite marker.

Microsatellite	Alleles in bp
CVRL1	198, 200, 202, 204, 208, 210, 212, 214, 216, 218, 222, 224, 226, 228, 232, 234
CVRL5	152, 154, 156, 158, 160, 162, 164, 168, 170, 172, 174, 176, 178, 180
CVRL6	194, 196, 198, 200, 202, 206, 210
CVRL7	272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298
VOLP3	144, 146, 148, 150, 152, 154, 158, 164, 166, 168, 170, 172, 174
YWLL44	82, 84, 86, 88, 90, 100, 102, 104, 106, 108, 110, 112
YWLL59	106, 108, 112

Table 3. Mean number of allele (MNA) and expected average heterozygosity (He) within each of the 5 dromedary populations.

Breed	MNA	He
Guerzni	7.4	0.6742 (± 0.1123)
Khouari	6.7	0.6688 (± 0.1635)
Marmouri	7.6	0.6728 (± 0.1229)
Jebli	5.7	0.6239 (± 0.1121)
White	6.1	0.6274 (± 0.1685)

Table 4. Genetic differentiation parameter.

Breed	Guerzni	Khouari	Marmouri	Jebli
White	0.10216	0.10713	0.13842	0.01188
Guerzni		0.00344	0.01112	0.08837
Khouari			0.00244	0.10173
Marmouri				0.12886

Multivariate Correspondence method was applied using individual genotypes to produce the graph shown in (Fig 1). The five populations formed two groups of dots distinctly distinct: White and Jebli types on one side and Guerzni, Marmouri and Khouari on the other side. This finding confirms the observations made by the two others methods: Fst and genetic distances.

Using Population and Tree view softwares, a phylogenetic tree were established (Fig 2). It appears that five types of camel populations formed two groups: the first one includes the White and Jebli types and the second one is formed by two sub-groups. The 1st sub-group consists of Khouari and Marmouri types and the 2nd subgroup is formed by the Guerzni type.

Probability of parentage exclusion (PE), Probability of identity (PI) and Breed assignment

Probability of parentage exclusion (PE) is an index allowing quantification of the percentage of incorrect detected affiliations. It were obtained using the 7 markers varied between 95 to 97% with one parents and were higher than 99.99% with two parents for the five populations. The probability of identity (PI) of individual camels is the probability to take haphazardly two individuals with the same genotype. PI varied between $1/8.10^6$ and $1/55.10^6$ (Table 6). We noticed that, among the five studied populations, the CVRL1 followed by VOLP3 loci were the most effective loci to exclude the false parents,

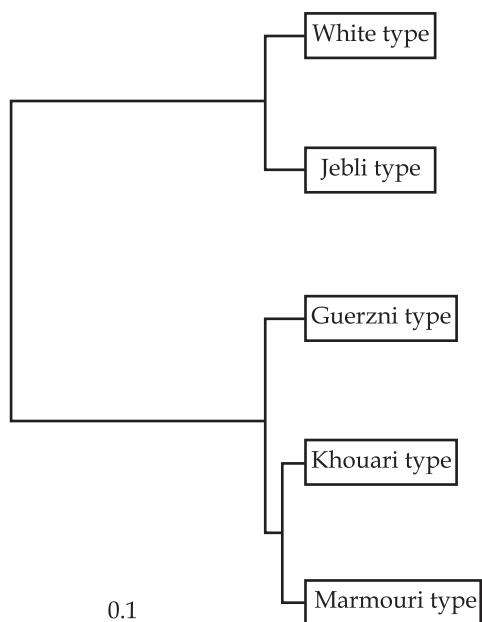


Fig 2. UPGMA dendrogram of the molecular relationship among different dromedary types based on Nei, (1978) genetic distance derived from 7 microsatellite loci.

Table 5. Genetic distance (Nei *et al*, 1978) between pairs of populations.

	Blanc	Guerzni	Khouari	Marmouri	Jebli
White	0.000	0.267	0.280	0.392	0.032
Guerzni	0.267	0.000	0.019	0.034	0.223
Khouari	0.280	0.019	0.000	0.015	0.262
Marmouri	0.392	0.034	0.015	0.000	0.354
Jebli	0.032	0.223	0.262	0.354	0.000

while YWLL44 and YWLL59 loci present the lowest PE and PI.

Breed assignment which is percentage of assignment of each animal to its type was estimated by GENECLASS. A good percentage (85 to 89 %) was observed only for the White type. The Jebli type was poorly assigned (10 to 15%), while these northern types were not assigned to the southern. The Guerzni, Marmouri and Khouari showed a moderate assignment for the southern camel populations ranging from 11 to 45% only.

Discussion

Five different types of Moroccan dromedaries exist and a simple phenotypic description do not allows a good distinction. Furthermore this five types are divide geographically on the northern (Jebli, White) and southern types (Marmouri, Guerzni and Khouari). Genetic characterisation by using microsatellites markers allow to confirm or to reject this finding and to analyse genetic variability within the breed.

Unlike electrophoretic systems (Ouragh and Bengoumi, 1996), the seven amplified microsatellites were all polymorphic. The locus that shows the highest heterozygosity and the highest polymorphism was CVRL1 loci with 16 alleles. Thus, CVRL1 is a very informative locus in Camels. Our results appear similar to those obtained by Mburu *et al* (2003), where the MNA detected in Kenyan and non-Kenyan breeds were 7.00 and 6.64, respectively.

Expected heterozygosity values (He) obtained are relatively high. In the study carried out in Kenya (Mburu *et al* 2003), the expected heterozygosity of the two populations were 0.538 ± 0.057 and 0.610 ± 0.051 , respectively for the Kenyan and non-Kenyan camels. The relatively high He among Moroccan camel populations indicates that they are more heterogeneous but in India (Mehta *et al*, 2006) a more higher heterozygosity are described in the Bikaneri (0.8 ± 0.05), and Kachhi (0.84 ± 0.06) and Jaisalmeri

Table 6. Probabilities of parentage exclusion when only one parents is known (PE1), Probabilities of parentage exclusion when both parents are known (PE2) and Probabilities of identity (PI) for the five populations.

Breed	PE1	PE2	PI
Guerzni	0.9779	0.9999	1/55.106
Khouari	0.9770	0.9999	1/32.106
Marmouri	0.9659	0.9999	1/14.106
Jebli	0.9556	0.9999	1/8.106
White	0.9701	0.9999	1/28.106

breeds (0.87 ± 0.05) by using random oligonucleotide primers.

Genetic distance between pairs of populations, genetic differentiation parameter, and Correspondence factor analysis show a similar structure (Nei, 1978). A non-significant difference on the genetic distance between White and Jebli populations and between Marmouri, Guerzni and Khouari populations was also found. Moreover, the lowest genetic distance was found between Marmouri and Khouari and between Khouari and Guerzni. This result corroborates that reported by Achaaban *et al* (1997), considering the Khouari type as a product of Marmouri and Guerzni.

The UPGMA tree also showed the formation of the same groups but with the appearance of a third sub-group within the second group which includes the Marmouri and Khouari types.

The two groups identified by the analysis of genetic parameters correspond to the two distinct geographical origins. The Guerzni, Marmouri and Khouari group originates from southern Morocco (Dakhla, Laayoune and Tantan) and the White and Jebli group is located in the central and south-east regions (Zagora-Ouarzazate, Errachidia and Essaouira).

The estimation of genetic distance between breeds or types of Moroccan camels is an important application of the DNA genetic markers. This information is of great importance in breed characterisation as well as in selection programmes and parentage.

The results of parentage control are expressed in terms of probability of exclusion and identity. For reminder, this index allows quantifying the percentage of false filiations that can be detected. By using only seven microsatellites markers PE and PI were very high for all population. Sasse *et al* (2002) also reported high PE varying between 98 to 99% while using 10 microsatellite markers.

The breed assignment or affectation was low, especially for the southern populations and the Jebli type. This could be attributed to the weak genetic differentiation between the southern populations and probably cross-breeding. Interestingly, the assignment accuracy was high for only the White type, meanwhile all the white camels were not assigned to the other types. Schulz *et al* (2004) conclude that the molecular information available after analysing genetic variability within Majorero breed and an African camel population does not manage to assign the individuals into clusters corresponding to its population.

The use of microsatellites has been of great help to identify the genetic variability of different Moroccan camel types. Managing the genetic variability of populations requires knowledge of the genetic structure of individuals. Even for breeds of which we are relatively well informed, it may be necessary to establish a parentage control. This study shows the effectiveness of primers in identifying individuals, as well as in detecting false filiations.

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References

- Achaaban MR, Ouragh L and Ouassat M (1997). Characterisation of Moroccan camel's population. Final report of Camel Applied Research and Development Network (CARDN) Project: Study and practical research in the field of camel livestock. 58 pp. Hassan II Institute of Agronomy and Veterinary Medicine. Rabat, Morocco.
- Belkhir K, Borsa P, Chikhi L, Raufaste N and Bonhomme F (2001). GENETIX 4.03, logiciel sous Windows TM pour la génétique des populations, Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.
- Bengoumi M (1988). Unpublished Masters Thesis, In french: Contribution à l'étude de la biochimie clinique et du polymorphisme des albumines et des transferrines chez le dromadaire au Maroc. 86 pp, Faculty of Sciences, Mohamed V University, Rabat, Morocco.
- Cornuet JM, Piry S, Luikart G, Estoup A and Solignac M (1999). New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153:1989-2000.

- Lang KDM, Wang Y and Plante Y (1996). Fifteen polymorphic dinucleotide microsatellites in llamas and alpacas. *Animal Genetics* 27:285-94.
- Mburu DN, Ochieng JW, Kuria SG, Jianlin H, Kaufmann B, Reger O and Hanotte O (2003). Genetic diversity and relationships of indigenous Kenyan camel populations: implications for their classification. *Animal Genetics* 34:26-32.
- Mehta SC, Mishra BP and Sahani MS (2006). Genetic differentiation of Indian Camel (*Camelus dromedarius*) Breeds using random oligonucleotide primers. *Animal Genetic Resources Information* 39:77-88.
- Miller SA, Dykes DO and Polesky HT (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 16:1215.
- Nei M (1978). Destination of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 80:583-590.
- Obreque V, Coogler L, Henney PJ, Bailey E, Mancilla R, Garcia-Huidobro J, Hinrichsen P and Cothran EG (1998). Characterisation of 10 polymorphic alpaca dinucleotide microsatellites. *Animal Genetics* 29:460-467.
- Ouragh L and Bengoumi M (1996). Blood protein polymorphism in the one-humped camel (*Camelus dromedarius*) in Morocco. *Revue Elevage et Médecine des Pays Tropicaux* 49(4):347-348.
- Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L and Estoup A (2004). GeneClass2: A Software for Genetic Assignment and First-Generation Migrant Detection. *Journal of Heredity* 95:536-539.
- Sasse J, Shah R and Pullnayegum J (2002). Paternity test development in dromedary racing camels with DNA microsatellites. *Proceedings of International Conference on Animal Genetics ISAG., Göttingen (Germany), August 11-15.* pp 124-125.
- Sasse J, Mariasegaram M, Babu R, Kinne J and Wernery U (2000). South American camellid microsatellite amplification in *Camelus dromedarius*. *Animal Genetics* 31:68-79.
- Schulz U, Minguez Y, Checa ML, Garcia-Atance P, Dunner S, Garcia D and Canon, J (2004) The Majorero camel (*Camelus dromedarius*) breed. *Animal Genetic Resources Information* 36:61-72.
- Weir BS & Cockerham CC (1984). Estimating F-Statistics for the analysis of population structure. *Evolution* 38:1358-1370.
- Wilson RT (1984). *The Camel*. Longman House, London and New York. pp 223.