

REGULAR ARTICLE

Assessing genetic diversity of three Tunisian dromedary camel (*Camelus dromedarius*) sub populations using microsatellite markers

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Abstract

One of the main tasks of the PROCAMED project is to promote research on genetics of dromedary camel. In this regard, and to evaluate the genetic diversity among Tunisian dromedary camel, a total of 62 blood samples were collected from unrelated animals in three different regions (Tataouine, Medenine and Kebili) and belonging to three sub-populations (Ourdhaoui Médenine, Ourdhaoui Tataouine and Merzougui) defined on the basis of morphologic and geographic criterions. From seven microsatellite markers used only four were successfully amplified resulting in a total of 26 alleles observed in the three sub-populations with a mean number of alleles (MNA) of 6.5. Unbiased expected heterozygosity (H_e) ranged from 0.76 to 0.84 whereas the observed heterozygosity was absolute ($H_o = 1$) and an excess of heterozygotes was observed in the three groups for all four loci. The mean estimates of the fixation index F_{ST} was 0.052 showing a moderate genetic structure between the different sub-populations. Little differentiation was observed between Ourdhaoui Médenine and Merzougui sub-populations, compared to Ourdhaoui Tataouine sub-population which seems to be more established. The results showed the limits of camel classification on the basis of only morphologic and regional distribution criterions.

Key words: Camel, Genetic diversity, Microsatellites, Tunisia

Introduction

The PROCAMED project aims to promote camel breeding and products in the southern countries of the Mediterranean area and the genetic characterization of the dromedary camel populations constitutes one of the project's objectives. While a considerable amount of data is available as regard genetic characterization of other livestock species using molecular markers, few studies involved camelid species. According to the FAO statistics, the camel population in Tunisia is around 237,000 heads (FAO, 2012) mostly found in the southern arid zone of the country and its milk and meat constitute an important component of inhabitant diets in these regions. The genetic diversity and the relationships among the Tunisian dromedary camel populations are poorly documented, and camel populations are often

classified according to the tribal affiliation, body measurements (Chniter et al., 2013), ecotypes and socio-geographic origins as it was done in other countries of camel origin (Abdallah and Faye, 2012). Three main sub-populations are found in southern Tunisia and known as Ourdhaoui Médenine, Ourdhaoui Tataouine, and Merzougui (region of Kebili). Whether these camels are truly differentiated and established breeds or a single admixed group remains to be clarified. Thus, the objective of this work was to generate preliminary data concerning the genetic structure and relationships of these sub-populations and the level of their genetic differentiation using microsatellite markers.

Materials and Methods

A total of 62 blood samples were collected from healthy unrelated dromedary camels from three governorates: Tataouine, Medenine and Kebili (Figure 1) and belonging to three known sub-populations (Merzougui $n=20$, Ourdhaoui Tataouine $n=21$ and Ourdhaoui Medenine $n=21$). DNA was extracted following the standard phenol chloroform protocol (Sambrook et al., 1989). Seven microsatellites (Volp03, Cvr10, Cvr102, Volp08, Volp10, Volp32 and Cvr105) were tested and PCR amplifications were carried out using primer pairs

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as previously described (Sasse et al., 2000; Mburu et al., 2003) in 25 μ l reaction volume containing 50-100 ng of genomic DNA, 400 nM of each primer, 0.2 mM each dNTP, 2 mM MgCl₂, 1X PCR buffer, and 0.5 unit of Taq DNA polymerase (Promega, USA). Cycling profile consisted of an initial denaturation at 94°C for 2 min followed by 35 cycles of 45 s at 94°C, 1 min at hybridization temperature of the primer pair, 1 min at 72°C and a final step of extension at 72°C for 1 min using Eppendorf thermal cycler (Eppendorf, Germany). PCR products were separated by electrophoresis on 8% polyacrylamide gel and band sizes were analyzed using Gel Pro Analyser v3.

Exact tests for deviation from Hardy–Weinberg equilibrium (HWE) were applied using the Markov Chain Monte Carlo simulation (100 batches, 1000 iterations per batch, and a dememorization number of 10000) as implemented in GENEPop version 4.1.2 (Raymond and Rousset, 1995). Sub-population comparisons using F-statistics (Weir et al., 1984), observed, expected heterozygosity (H_o and H_e , respectively), and Shannon's information index were generated using GenAlEx 6.501 (Peakall and Smouse, 2006). The neighbor-joining tree was drawn based on Nei's genetic distances (DA) calculated between individuals (Nei et al., 1983) with the POPULATIONS 1.2.32 software (Langella, 1999).

Results and Discussion

From the seven tested microsatellite markers, three showed either no amplification (Volp03 and Cvr101) or random non reproducible amplification (Cvr102) whereas the remaining four loci were successfully amplified giving sharp and specific bands (Volp08, Volp10, Volp32 and Cvr105). These four loci were analysed in a total of 62 unrelated individuals and proved to be polymorphic with a total of 69 alleles detected.

The number of alleles per locus varied from 6 to 7, similar to the range previously observed for the same markers in South African population (Sasse et al., 2000) but higher than that reported in the Saudi camel population (Mahmoud et al., 2012) and lower than that reported in a comparative study of dromedary camel populations in India and South Africa (Banerjee et al., 2012). Generally, a minimum of four alleles per locus is required to reduce the standard error of distance estimates in genetic distance studies (Li et al., 2002), therefore, the four loci (Cvr105, Volp08, Volp10 and Volp32) are suitable for the genetic diversity of the dromedary camel populations in Tunisia. The total number of alleles observed in the studied sub-

populations; AB (Ourdhaoui Medenine), AT (Ourdhaoui Tataouine) and ME (Merzougui) were respectively: 23, 21 and 25. Only 2 alleles of Cvr105 were private and were only observed in the Merzougui group.

The characteristics of the microsatellites in the three studied sub-populations are shown in Table 1. Shannon's Information index I varied from 1.47 (Volp32) to 1.83 (Volp08) and showed a positive correlation with the effective number of alleles (N_e) and the unbiased expected heterozygosity (H_e) indicating the suitability of these microsatellite markers for the assessment of genetic diversity in camels.

All genotyped samples were heterozygotes for the loci studied; therefore H_o was equal to 1 in each group, for each locus and for all loci among the three groups. The observed heterozygosity values were significantly higher than expected and the lowest value of expected heterozygosity (0.752) was detected in AT. The mean H_e (0.78) was higher than that reported (0.6) by Ould Ahmed et al. (2010). This excess in heterozygotes can be explained by an isolate-breaking resulting from possession herd belonging to three different tribal regions. All loci showed significant ($p < 0.05$) departures from the Hardy-Weinberg proportions in the whole population. When considering groups separately and testing for the four loci or per locus for each group or for all loci among the groups, Hardy–Weinberg disequilibrium was verified ($p < 0.05$).

Sub-population differentiation was estimated by calculating the fixation index (FIT, FIS, and FST) for the four analysed loci across the three groups (Table 2). Mean estimates of F-statistics obtained over loci were $FIT = -0.226 \pm 0.019$, $FIS = -0.295 \pm 0.030$ and $FST = 0.052 \pm 0.020$. FIT indicated an excess of 22% in heterozygotes. Negative FIS and FIT values may be explained by random mating which increased the heterozygosity. Overall, the studied groups exhibited a moderate genetic differentiation of 5.2%, while the remaining 94.8% of the genetic variability was attributed to the differentiation within sub-populations (between individuals). This value was comparable to the genetic differentiation level previously reported (8.3%) for Tunisian camel population (Ould Ahmed et al., 2010), for Indian camel population (8.2%) (Vijh et al., 2007) and for Majorero camel in Canary Islands (3.1%) (Shulz et al., 2005). The within sub-population inbreeding estimate, per group across loci, were -0.369 (AT), -0.258 (AB) and -0.265 (ME). When considering loci separately, FST values for Volp08, Volp10 and Cvr105 (Table

2) indicate a relatively high gene flow which may prevented genetic drift from causing local genetic differentiation. For Volp32 locus results suggest possible linkage with other loci which are probably under natural selection and thus favouring different alleles in three sub populations. Evidence of gene flow between camel populations was also corroborated by the mean frequency of private alleles and the number of migrants (N_m) after correction for size, 0.087 and 0.994 respectively, indicating a relatively high gene flow among studied groups.

The genetic distances (Nei) between the studied sub-populations, as well as F_{ST} estimates are presented in Table 3. The genetic differentiation between AB and ME groups was rather low (0.015) and this could be mainly due to the cross breeding between the two sub populations which share the same grazing area (Dhahar rangelands) in the transhumance periods. On the contrary, F_{ST} is the highest (0.061) for Merzougui and Ourdhaoui Tataouine groups, indicating limited genetic exchange and reflecting the relative geographical isolation between these two regions. Nei's genetic distance values pointed to a genetic resemblance between AT and AB groups but especially between AB and ME groups, while ME and AT groups were genetically more distant. Neighbour-Joining phylogenetic tree (Figure 2) reveals the genetic relationship among the three groups and its topology reflects their geographic distribution.

Table 1. Characteristics of the microsatellites loci in the analyzed populations. N_A : number of alleles, N_e : effective number of alleles, H_o : observed heterozygosity, H_e : unbiased expected heterozygosity, I : Shannon's index.

Locus	Size range (bp)	N_A	N_e	H_o	H_e	I
Volp10	245-273	6	4.37	1	0.78	1.58
Volp08	136-158	7	5.82	1	0.84	1.83
Volp32	245-282	6	4.04	1	0.76	1.47
Cvrl05	151-191	7	4.13	1	0.77	1.48

Table 2. F statistics at each locus across the three groups.

Locus	F_{IS}	F_{IT}	F_{ST}
Volp10	-0.304	-0.273	0.024
Volp08	-0.208	-0.188	0.016
Volp32	-0.340	-0.206	0.100
Cvrl05	-0.327	-0.239	0.066
Mean	-0.295±0.030	-0.226±0.019	0.052±0.020



Figure 1. Geographic repartition of the three dromedary camel sub-populations in southern Tunisia.

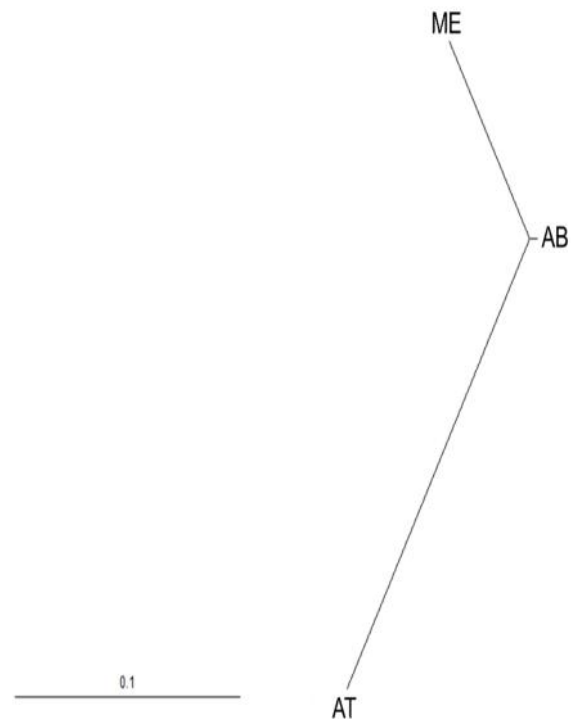


Figure 2. Neighbour-Joining phylogenetic tree based on Nei's standard genetic distance.

Conclusions

The four microsatellite markers (Volp08, Volp10, Volp32 and Cvrl05) showed to be suitable for future genetic diversity studies on Tunisian dromedary. The results concerning the genetic variability and the relationships between them provide important information on the genetic structure of camel populations in the studied regions and showed the limits of camel classification on the basis of only morphologic and regional distribution criterions. However, these results are considered as preliminary due to the reduced number of microsatellite markers used. Further investigation is required to define if the Tunisian camel population presents separate genetic entities. A study involving a larger number of samples covering more regions of southern Tunisia and a larger number of microsatellite markers is underway in the framework of the PROCAMED project.

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Author Contributions

Conceived and designed the experiments: M. H. Y. Performed the experiments: R. B. S. Analyzed the data: G. N. and S. K. Contributed reagents / materials / analysis tools: M. H. and T. K. Wrote the paper: G. N., S. K. and M. H. Y.

References

- Abdallah, H. R. and B. Faye. 2012. Phenotypic classification of Saudi Arabian camel (*Camelus dromedarius*) by their body measurements. Emir. J. Food Agric. 24(3):272-280.
- Banerjee, P., J. Joshi, U. Sharma and R. K. Vijh. 2012. Population differentiation in dromedarian camel: A comparative study of camel inhabiting extremes of geographical distribution. Internat. J. Anim. Vet. Adv. 4(2):84-92.
- Chniter, M., M. Hammadi, T. Khorchani, R. Krit, A. Benwahada and M. Ben Hamouda. 2013. Classification of Maghrebi Camels (*Camelus dromedarius*) according to their tribal affiliation and body traits in southern Tunisia. Emir. J. Food Agric. 25 (8):625-634.
- FAO- statistics. 2012. <http://faostat.fao.org/>
- Langella, O. 1999. Populations, 1.2.32. Available via <http://bioinformatics.org/~tryphon/populations/>
- Li, M., S. Zhao, C. Bian, H. Wang, H. Wei, B. Liu, M. Yu, B. Fan, S. Chen, M. Zhu, S. Li, T. Xiong and K. Li. 2002. Genetic relationships among twelve Chinese indigenous goat populations based on microsatellite analysis. Genet. Select. Evol. 34:729-744.
- Mahmoud, A. H., M. A. Alshaikh, R. S. Aljumaah and O. B. Mohammed. 2012. Genetic variability of camel (*Camelus dromedarius*) populations in Saudi Arabia based on microsatellites analysis. Afr. J. Biotech. 11(51):11173-11180.
- Mburu, D. N., J. W. Ocheing, S. G. Kuria, H. Jianlin, B. Kaufmann, J. E. O. Rege and O. Hanotte. 2003. Genetic diversity and relationships of indigenous Kenzan camel (*Camelus dromedarius*) population. Anim. Gen. 34:26-32.
- Nei, M., F. Tajima and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19:153-170.
- Ould Ahmed, M., F. Ben Salem, S. Bedhief, B. Rekik and M. Djemali. 2010. Genetic diversity in Tunisian dromedary (*Camelus dromedarius*) populations using microsatellite markers. Livestock Sci. 132:182-185.
- Peakall, R. and P. E. Smouse. 2006. GENALEX 6.501 genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes. 6:288-295.
- Raymond, M. and F. Rousset. 1995. GENEPOP (version 4): population genetics software for exact tests and ecumenicism. J. Hered. 86:248-249.
- Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Sasse, J., M. Mariasegarm, R. Babu, J. Kinne and U. Wernery. 2000. South American camel microsatellite amplification in camelus dromedarius. Anim. Gen. 31:75-76.
- Shulz, U., Y. Minguez, M. L. Checa, P. Garcia-Atance, S. Dunner, D. Garica and J. Canon. 2005. The Majorero camel (*Camelus dromedarius*) breed. Anim. Gen. Res. Inform. 36:61-71.

Vijh, R. K., M. S. Tantia, B. Mishra and S. T. Bharani Kumar. 2007. Genetic diversity and differentiation of dromedarian camel of India. *Anim. Biotechnol.* 18:81–90.

Weir, B. S. and C. C. Cockerman. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.