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Genetic characterization of Tunisian donkey with DNA microsatellites

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ABSTRACT

The genetic characterization of Tunisian donkeys with microsatellite markers led to the identification of a total of 214 alleles, with an average of 14.27 alleles per locus. The number of effective alleles per locus (N_e) indicates a rich genetic polymorphism for the used markers. The number of alleles per locus (N_a) is higher than Ne,confirming the high genetic diversity in the investigated population. Observed heterozygosis(H_o) for all the studied loci inthe entire population equals 0.744. The FIS index was significantly different from zero, pointing toan excess of heterozygotes. The mean of the number of effective migrants exchanged per generation (Nm) was 3.05 indicating a high level of gene exchange. Genetic distances and the principal coordinate analysis showed three distinct population groups/breeds, with a commongenetic structure for all the individuals. This genetic characterization of the Tunisian donkey population constitutes a valuable basis for further investigations, and to the elaboration of adequate conservation strategies.

Key words: Diversity, Donkey, Genetic, Microsatellites, Tunisia

The ancestor of the donkey (*Equus asinus*) originated from northeastern Africa. Archeological findings state that the domestication of the Nubian and Somalian wild ass (*E. africanus*) began in Egypt around 4000–6000 BC, and later in the Middle East, around 100 BC (Blench 2000; Beja-Pereira *et al.* 2004).

The domestication of the donkey allowed its use for the hard physical activities, as a pack animal or for draught works (Tapsoba 2012). Nowadays, in spite of mechanization, donkey still remains a primary work stock, especially in the Saharian and mountainous regions in Africa. In Tunisia, scientists assume that the domestic donkey has evolved from the north African wild ancestor,as represented in the Roman mosaics in Sousse museum.

The Tunisian donkey population is 1,23,067 distributed in the North (31.5%), Central part (51%) and South (16.5%).

The Tunisian donkey breeds did not receive enough scientific attention like the other animal species with limited literature available (Denjean 1950).

Several studies have reported the genetic diversity among donkey breeds using microsatellite markers (Zhang *et al.* 2016, Yun and Cho 2017).This study was undertaken to genetically characterize the Tunisian donkey populations with FAO recommended microsatellite markers and estimate the genetic similarity and distances among these populations.

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MATERIALS AND METHODS

Blood samples (94) were randomly collected from Tunisian indigenous donkeys, bred in 8 governorates of Tunisia (Le Kef, Jendouba, Seliana, Beja, Kasserine, Zaghouan, La Manouba and Bizerte). The sampled animals belong to the Northern and central parts of the country.

Genomic DNA was isolated from the blood sample using Invitrogen PureLink® Genomic DNA Mini-Kit, according to the manufacturer's instructions.

Genomic DNA was amplified using 15 microsatellites loci (Table 1) according to Yun and Cho (2017) and the FAO guidelines (2011). The PCR conditions were optimized with 15 min of initial denaturation at 95°C, followed by 30 cycles of 30 sec at 94°C, with the annealing temperature optimized at 58°C for 90 sec, and the extension for 1 min at 72°C. A final extension step was carried out at 60°C during 30 min to amplify the used microsatellites. The amplified products were denatured with formamide (8.3 µl) and Gene Scan-500LIZ (0.3 µl). PCR products (2 µl) were separated by capillary electrophoresis using an ABI Prism 3130® DNA genetic analyzer (Applied Biosystems™, USA), and the size analysis of DNA fragments was performed using the Gene Mapper Software® (Applied Biosystems™, Ver. 4.0).

Genetic diversity within breeds, genetic variation, and relationships among breeds were assessed using different

Table 1. Primer sequences and PCR product size range for the studied microsatellite

Loci	Microsatellite sequences	PCR products (bp)	References
AHT4	AACCGCCTGAGCAAGGAAGT CCCAGAGAGTTTACCCT	148 – 164	Binns *et al.* (1995)
AHT5	ACGGACACATCCCTGCCTGC GCAGGCTAAGGGGGCTCAGC	130 – 146	Binns *et al.*(1995)
ASB17	GAGGGCGGTACCTTTGTACC ACCAGTCAGGATCTCCACCG	91 – 109	Breen *et al.*(1997)
ASB2	CCTTCCGTAGTTTAAGCTTCTG CACAACTGAGTTCTCTGATAGG	222 – 254	Breen *et al.* (1997)
CA425	GCTGCCTCGTTAATTCACTCATGTCCGCTTGTCTC	226–246	Eggleston-Stott *et al.* (1997)
HMS1	CATCACTCTTCATGTCTGCTTGGTTGACATAAATGCTTATCCTATGGC	170–186	Guérin *et al.* (1994)
HMS2	ACGGTGGCAACTGCCAAGGAAG CTTGCAGTCGAATGTGTATTAAATG	218–238	Guérin *et al.* (1994)
HMS3	CCAACTCTTTGTCACATAACAAGA CCATCCTCACTTTTTCACTTTGTT	150–170	Guérin *et al.* (1994)
HMS6	GAAGCTGCCAGTATTCAACCATTG CTCCATCTTGTGAAGTGTAACTCA	153–169	Guérin *et al.* (1994)
HMS7	CAGGAAACTCATGTTGATACCATC TGTTGTTGAAACATACCTTGACTGT	165–183	Guérin *et al.* (1994)
HTG10	CAATTCCCGCCCCACCCCCGGCA TTTTTATTCTGATCTGTCACATTT	93–113	Marklund *et al.* (1994)
HTG4	CTATCTCAGTCTTGATTGCAGGACCTCCCTCCCTCCCTCTGTTCTC	127–139	Ellegren *et al.* (1992)
HTG6	CCTGCTTGGAGGCTGTGATAAGAT GTTCACTGAATGTCAAATTCTGCT	84–106	Ellegren *et al.* (1992)
LEX3	ACATCTAACCAGTGCTGAGACTGAAGGAAAAAAAGGAGGAAGAC	142–164	Coogle *et al.* (1994)
VHL20	TCTTACATCCTTCCATTACAACTA TGATACATATGTACGTGAAAGGAT	84–96	Van Hearingen *et al.* (1994)

softwares: GenAlex® 6.2 to calculate gene diversity indexes for each population, Genetix® v4.04 software to screen allelic frequencies and the number of alleles per locus. Observed heterozygosis (Ho), expected heterozygosis (He), and unbiased expected heterozygosis (UH_e) were calculated across loci and populations as well as the effective number of migrants per generation (Nm) (Wright 1969). Wright's F statistics (F_{ST}, F_{IS} and F_{IT}) (Wright 1965) as proposed by Weir and Cockerham (1984) were computed using Genetix® software.

The representation of the genetic relationships among tested populations was established according to the Principal Component Analyses (PCA) using GenAlex® v6.2 and Genetix® v4.04. The dendrogram was constructed through the Unweighted pair group method with average means (UPGMA), based on Nei's genetic distances (Nei 1972). The genetic structure of the sampled animals was analyzed with Structure software® (Pritchard *et al.* 2000). The best k value corresponding to the number of subpopulation is estimated (Jemmali *et al.* 2017).

RESULTS AND DISCUSSION

In our experiment, all primer pairs amplified well and were polymorphic and reproducible. A total of 214 alleles were isolated as shown in Table 2, and the average observed number of alleles per locus (N_a) was 14.266±7.35, ranging from 5 (HMS7) to 36 (ASB2).

The number of effective alleles per locus (N_e) indicates a genetic diversity. $N_e$varied between 1.322 (HMS7) and 23.15 (ASB2), with a mean of 6.3±5.1. For all the analyzed samples, the N_a is higher than N_e, which indicates a high genetic diversity.

The most polymorphic loci were ASB2 (36 alleles), LEX3 (20 alleles) and HMS2 (19 alleles), whereas the least polymorphic were HMS07 (5 alleles), VHL20 (7 alleles) and HTG06 (8 alleles).These results were in agreement to other similar studies (Colli *et al.* 2013, Matassino *et al.* 2014, Yun and Cho 2017).

Allele frequency varied from 0.022 to 0.917, with the highest value recorded for the gene HMS7 (170bp) (Fig. 1).

The H_o varied from 0.26 (HMS7) to 0.928 (HTG06), with a mean of 0.744±0.19, while the H_e varied from 0.24 (HMS7) to 0.96 (ASB2) with a mean of 0.768±017.

Compared to other donkey populations, the expected mean heterozygosity is higher in the Tunisian breeds than reported in other studies, particularly in South Korean, Catalonian, Croatian and Italian donkeys (Matassino *et al.* 2014; Yun and Cho 2017).The F_{IS} varied between –0.327 (HTG06) to 0.295 (VHL20), with a mean of –0.076. This index is significantly different from zero ($P<0.05$), which clearly indicates an excess of heterozygotes (Wright 1978) (Table 2).

Table 2. Observed (N_o) and effective (N_e) alleles, observed (H_o) and expected (H_e) heterozygosity, F_{IS} and N_m across all populations for each locus

Locus	N_a	N_e	H_o	H_e	F_{IS}	N_m
AHT4	11	4.671	0.7949	0.791	–0.082	4.343
AHT5	15	6.2082	0.9103	0.8443	–0.185	2.452
ASB17	11	4.6637	0.6579	0.7908	0.035	2.387
ASB2	36	23.1543	0.8649	0.9633	0.021	3.489
CA245	16	3.8373	0.6923	0.7442	–0.016	2.396
HMS1	12	5.0418	0.8571	0.8162	–0.296	1.979
HMS2	19	7.4788	0.8974	0.8719	–0.113	2.972
HMS3	16	6.6315	0.8026	0.8548	–0.017	2.761
HMS6	14	3.6584	0.8333	0.7317	–0.194	2.852
HMS7	5	1.322	0.2692	0.2452	–0.178	3.851
HTG10	10	4.1487	0.9103	0.7639	–0.276	3.963
HTG4	14	9.5258	0.68	0.901	–0.091	2.823
HTG6	8	3.7956	0.9298	0.7431	–0.327	2.793
LEX3	20	8.0901	0.6957	0.8828	0.099	2.135
VHL20	7	2.3342	0.3699	0.5755	0.295	4.911
Mean	14.2667	6.3041	0.7444	0.768	–0.076	3.056
SD	7.3530	5.1447	0,1958	0,171	0.044	0.216

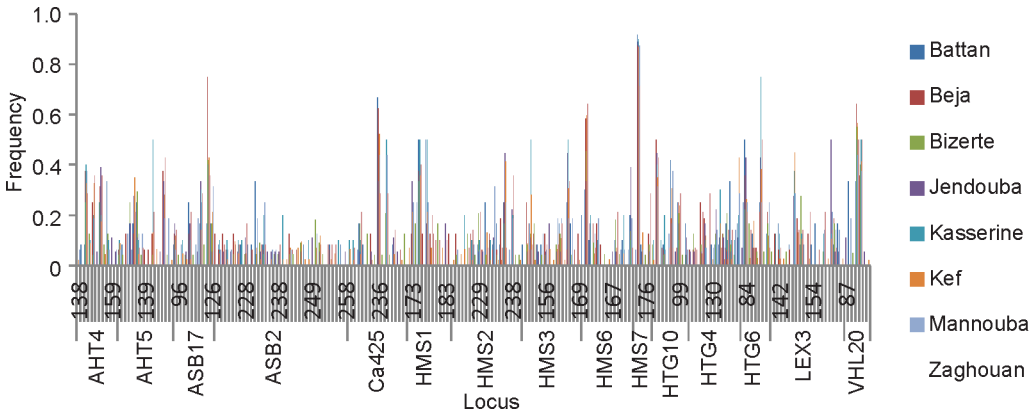

Fig. 1. Allelic frequency for individuals from different regions.

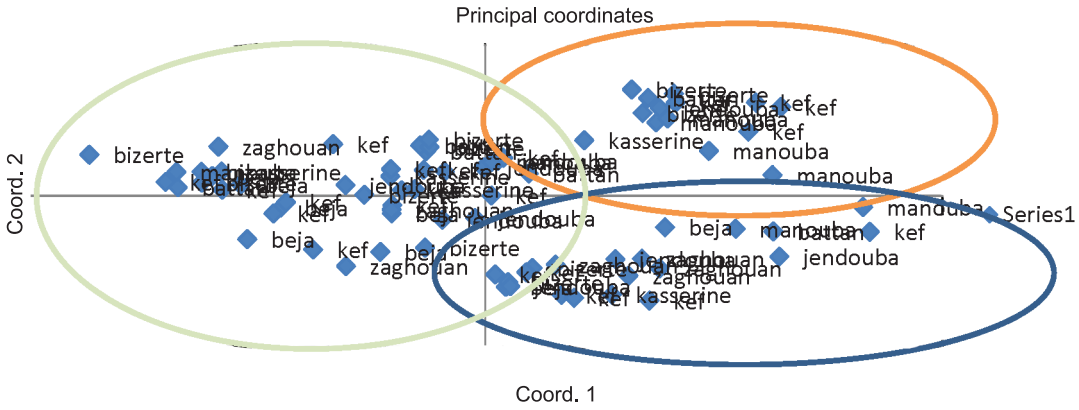

Fig. 2. Principal component analysis for the analyzed donkey populations.

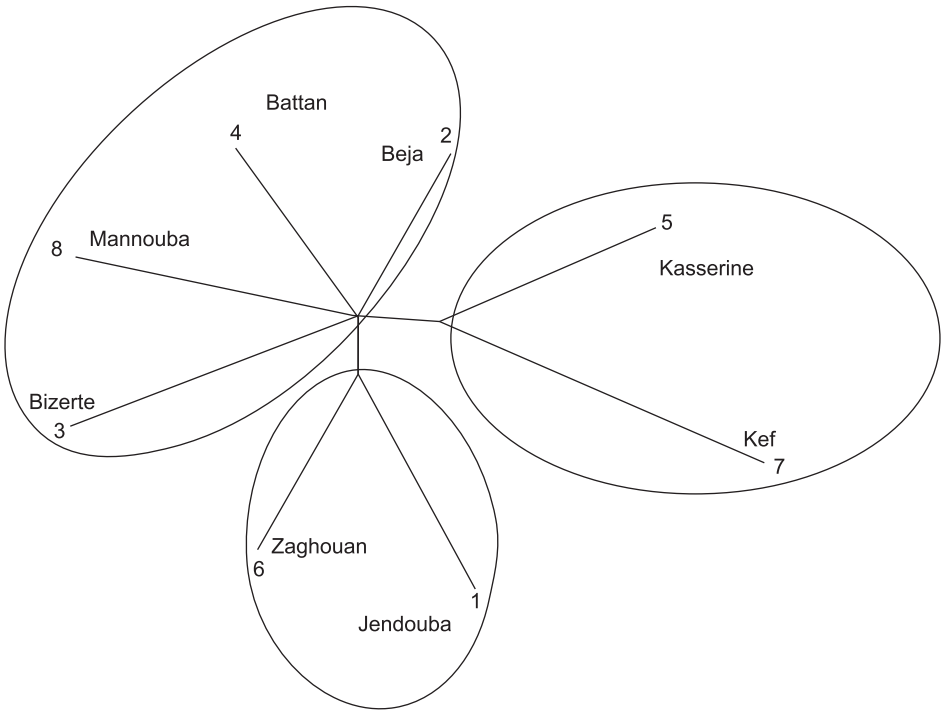

Fig. 3. UPGMA clustering of analyzed breeds.

The Nm values ranged from 1.9 to 4.9, with an average of 3.05, signaling a high level of genes exchange (Slatkin and Barton 1989).

The results of principal component analysis are shown in Fig. 2. The first three axes account almost 60.4% of the total inertia, represented by the first three factors 22.35%, 19.35%, and 18.73% respectively. The spatial distribution shows 3 distinctive groups with a significant difference.

Nei's genetic distance between animals of Mannouba and the other regions of the country (Le Kef,Jendouba, Beja Kasserine) is higher than 0.2. The smallest genetic distance between two populations is recorded between the animals reared in the governorate of Le Kef and the ones bred in Kasserine, Jendouba and Bizerte. This indicates that the donkeys of these regions have a close genetic relationship and in this case, the UPGMA clustering also divides the sampled animals in three groups (Fig. 3):the first closely related population is composed of donkeys bred in the border regions (Le Kef, Kasserine), mountainous and difficult to access; second group/breed consist of donkeys from other mountainous regions, Zaghouan and Jendouba, while the third group gathers animals from the northern part of the country, bizerte, beja and la manouba.

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