Genetic structure and assignment of Tunisian meat sheep breeds

Article · January 2023

CITATIONS
0

READS
47

9 authors, including:

Samia Kdidi
Institut des Régions Arides
23 PUBLICATIONS **222** CITATIONS
SEE PROFILE

Jorge H Calvo
Centro de Investigación y Tecnología Agroalimentaria de Aragón
187 PUBLICATIONS **2,591** CITATIONS
SEE PROFILE

Semir Bechir Suheil Gaouar
Abou Bakr Belkaid University of Tlemcen
330 PUBLICATIONS **813** CITATIONS
SEE PROFILE

Mohamed Dbara
Institut des Régions Arides
17 PUBLICATIONS **63** CITATIONS
SEE PROFILE

Genetics & Biodiversity Journal

Journal homepage: https://journals.univ tlemcen.dz/GABJ/index.php/GABJ

ISSN: 2588-185X

Original Research Paper

Genetic structure and assignment of Tunisian meat sheep breeds

Samia KDIDI [1*], Jorge HUGO CALVO [2], Semir Bechir Suheil GAOUAR [3], Mohamed DBARA [1], Slah BELHADJ [4], Ezzeddine BELFEKIH [4], Mohamed HAMMADI [1], Touhami KHORCHANI [1], Mohamed Habib YAHYAOUI [1]

[1] Livestock and Wildlife Laboratory, Arid Lands Institute, Route Djorf km 22- 4119 Medenine, University of Gabes, Tunisia.;

[2] Unidad de Producción y Sanidad Animal, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)- Instituto Agroalimentario de Aragón (IA2) (CITA-Universidad de Zaragoza), Zaragoza, Spain

[3] Laboratory of Applied Genetic in Agronomy, ecology and public health. Biology department. University Abou Bekr Belkaid. Tlemcen. 13.000. Algeria

4 Office de l'Elevage et des Pâturages 30, rue Alain Savary, 1002 Tunis, Tunisia

***Corresponding Author**: Samia Kdidi, Medenine, University of Gabes, Tunisia;
Email: kdidi_samia@yahoo:fr

Article history: Received: November 13th 2022; Revised: January 1st 2022; Accepted: .January 18th 2023

Abstract

Microsatellite markers succeeded to reveal different population genetic parameters. The present work aimed to investigate the genetic assignment and structure of the Tunisian meat sheep breeds. (Barbarin (BB), Western Thin Tail (WTT), and Black Thibar (BT)).The current study also opted for testing different methods of assignment implemented in several programs for genetic identification and traceability purposes of these breeds and for assessing whether these markers could be useful for an efficient genetic assignment of these ovine breeds. The genotypes of 90 animals (30 samples per breed) were typed for 22 microsatellite markers. All the loci displayed a high polymorphic content (between 0.561 and 0.884). The GENECLASS2 and the WHICHLOCI programs were used to choose the most powerful markers (17 microsatellites). The FLOCK program was more efficient with 22 markers. Genetic differentiation tests (FST = 0.0127) and assignment of individuals to populations revealed the highest level of misassignment in BB and WTT breeds, while the BT breed revealed the highest percentage of individuals assigned to itself. The reduction of the number of microsatellites (from 22 to 17) does not affect the assessment of the genetic structure of Tunisian sheep breeds. This result shed the light on the importance of the shift towards lambs with thin tails imposed by the butchers. It also revealed the unfitness of microsatellite markers in genetic identification analysis for studied sheep breeds.

Keywords: Genetic assignment; microsatellites; breeds; sheep; structure, Tunisia.

الملخص

نجحت المورثات الواسمة في الكشف عن المعلمات الوراثية الجماعية المختلفة. كان الهدف من هذا العمل هو التحقيق في التمييز الجيني والتركيب الجيني لسلالات الأغنام التونسية المنتجة للحم (البربري (BB)، الغربي (WTT) وسوداء تيبار (BT)). علاوة على ذلك، تختار الدراسة الحالية اختبار طرق التمييز المختلفة المنفذة في العديد من البرامج لأغراض التمييز الجيني والتتبع لهذه السلالات ولتقييم ما إذا كانت هذه المورثات يمكن أن تكون مفيدة في التمييز الجيني الفعال لهذه السلالات من الأغنام. تم تحليل الأنماط الجينية لـ 90 حيوانًا (30 عينة لكل سلالة) لـ 22 مورثا واسما. جميع المورثات تضمن مستوى عال من التنوع (بين 0.561 و0.884). تم استخدام برامج GENECLASS2 وWHICHLOCI لاختيار أنجع مورثات (17 مورثا). كان برنامج FLOCK أكثر كفاءة مع 22 مورثا. كشفت اختبارات التمايز الجيني (FST= 0.0127) وتخصيص الأفراد للمجموعات عن أعلى مستوى من سوء التمييز في سلالات BB و WTT ؛ بينما كشفت سلالة BT أعلى نسبة من الأفراد المنتمين لنفسها. إن تقليل عدد المورثات الواسمة (من 22 إلى 17) ليس له تأثير على تقييم التركيب الوراثي لسلالات الأغنام التونسية. سلطت هذه النتيجة الضوء على أهمية التحول نحو الحملان ذات الذيل الرفيع التي يفرضها الجزارون. كما كشفت عن عدم صلاحية المورثات الواسمة في تحليل التمييز الجيني لسلالات الأغنام المدروسة.

الكلمات المفتاحية التمييز الوراثي، المورثات الواسمة، السلالات، الأغنام، التركية، تونس

Introduction

In Tunisia, sheep farming is an important economic and social activity contributing to more than 41% of the total red meat production (OEP, 2017). There are more than 6.4 million sheep heads,

and 3.7 million ewes (ONAGRI, 2017) that belong to four different breeds: Barbarin (60.3% of the total), Western Thin Tail (34.6%), Black Thibar (2.1%) and Sicilo Sarde (0.7%). The Barbarin fat-tailed sheep is the common breed in the different regions of the country and it is reared with the two thin-tailed breeds Western Thin Tail (WTT) and Black Thibar (BT) for meat production.

Several studies have shown that consumers are concerned with the origin of the meat they eat (Quagrainie et al.1998; Hoffmann, 2000; Iaccarino et al.2006; Verbeke et al.2010). Within Tunisia, there has been a marked rise over the past decade in meat sold by specie. Lambs meat is attracting the highest price. Consumption of this meat is closely linked to eating habits and meat quality. The Barbarin lamb meat remains the favorite across the country (Djemali et al.,2006). However, an interesting shift towards lambs with the thin tail is shown by Butchers since they are finding difficulties in selling the fat of the Barbarin lamb tail, which represents 15% of the lamb carcass (Bedhiaf-Romdhani et al., 2008).

Over the last decade, the genetic diversity and structure of Tunisian sheep breeds have been intensively investigated using microsatellites (Sassi-Zaidyet al., 2014a; Sassi-Zaidy et al., 2014b; Kdidi et al; 2015; Ben Sassi-Zaidy et al., 2022) and SNP markers (BenJemaa et al., 2019; Bedhiaf-Romdhani et al., 2020; Baazaouiet al., 2021). All cited works revealed a high level of genetic admixture between these breeds. The authors seem to agree that such mixing is attributable to the occurrence of a gene flow between breeds. Several reasons were suggested by these authors, namely, the breeder management practices (Bedhiaf-Romdhani et al., 2008; Kdidi et al., 2015). According to these authors, these practices consist of either uncontrolled mating; all for the shift towards muttons with a thin tail. As a result, the Barbarin sheep breed is subjected to a high risk of genetic erosion (Sassi-Zaidy et al., 2014b, Kdidi et al., 2015). Moreover, the absence of genetic improvement programs and the historical origin of the breeds could also be suggested as reasons (Kdidi et al., 2015. Ben Jemaa et al., 2019).

In this context, the genetic assignment of an animal to its breed of origin seems to have a crucial role to protect consumer preferences. The methods of identification of breeds are focusing on genetic variability detection within closely related populations. Short sequence repeat markers (microsatellites) have been used in sheep (Bramante et al.2011), in cattle (Rogberg-Muñoz et al.,2014; Mateus Russo-Almeida, 2015), in pigs (Oh et al.2014) and in chicken (Nakamura et al.,2006; Rikimaru Takahashi, 2007) for tracing meat or meat products at the breed level. This tracing is based on the assignment test of animals or animal products to their breeds of origin (Shackell et al.,2001; Lenstra, 2005; Manel et al., 2005; Dalvit et al.,2008).

The present study uses a set of microsatellite markers, recommended by the FAO, and commonly used for genetic diversity and traceability purposes. We aimed to investigate the genetic assignment and structure of the Tunisian meat sheep breeds. Different methods of assignment implemented in several programs for genetic identification and traceability purposes of these breeds have been tested to assess whether these markers could be useful for efficient genetic identification of ovine breeds raised in Tunisia for meat production.

Materials and Methods

Samples and genotypes

The present work concerned the three Tunisian meat sheep breeds (Barbarin, Western Thin Tail, and Black Thibar). The genotypes of 90 animals (30 samples per breed) typed for 22 microsatellite markers belonging to the data generated by Kdidi et al. (2015) were used for the statistical analyses of the current work.

The twenty-two microsatellites were chosen as the most informative ones, whereas, the samples were the individuals most typed for each of the 22 selected loci. These microsatellites were: *OARHH47, MCM527, MAF65, ILSTS005, OARCP38, MAF209, OARFCB304, INRA63, MAF214, SRCRS09, MAF70, OARVH72, BM1824, OARJMP58, OARJMP29, DYMS1, OARFCB193, MCM140, ILSTS28,*

DOI: 10.46325/gabj.v7i1.325

HUJ616, OARFCB226, MAF33. More information and details of these microsatellites are shown in Table 1.

DOI: 10.46325/gabj.v7i1.325

Table 1. Details of the 22 microsatellite markers used; Loci, Chromosome location (Ch. Location), primer sequences, GenBank Accession (Accession number), groups for multiplexing (Gp), thermocycling conditions (annealing temperature: Ta), fluorescent dye, allele size range, alleles number (Na), Number of individuals typed for each locus (N), Polymorphic Content Information (PIC), observed heterozygosity (Ho), expected heterozygosity (He) computed by Cervus v. 3.0.3 (Marshall et al., 1998) program and mean genetic diversity(Hs) performed using FSTAT V. 2.9.3.2 (Goudet,1995). Loci in bold were selected by GENECLASS 2 (Piry et al, 2004) and WHICHLOCI programs (Banks et al. 2003) as the most powerful microsatellite markers**.**

Loci	Ch. location	Primer sequences (5'-3'): Forward/ Reverse	Accession number	Gp	Ta (°C)	Labeling dye	Allele size (bp)	Na	N	PIC	H_o	H_e	H_s
MAF65	OAR15	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	M67437	1	55	NED	123-127	10	90	0.726	0.678	0.763	0.760
ILSTS005	OAR7	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	L23481	1	55	NED	174-218	11	74	0.712	0.392	0.754	0.757
MAF209	OAR17	GATCACAAAAAGTTGGATACAACCGTGG TCATGCACTTAAGTATGTAGGATGCTG	M80358	2	55-53	6-FAM	109-135	13	89	0.780	0.798	0.807	0.796
OarFCB304	OAR19	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	L01535	2	55-53	VIC	150-188	16	89	0.874	0.910	0.889	0.886
INRA063	OAR14	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	X71507	2	55-53	6-FAM	163-199	22	89	0.882	0.764	0.896	0.892
MAF214	OAR16	GGGTGATCTTAGGGAGGTTTTGGAGG AATGCAGGAGATCTGAGGCAGGGACG	M88160	2	55-53	NED	174-214	13	88	0.643	0.420	0.681	0.675
SRCRSP09	OAR12	AGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	L22201	2	55-53	NED	96-130	8	85	0.561	0.424	0.601	0.577
OarHH47	OAR18	TTTATTGACAAACTCTCTTCCTAACTCCACC GTAGTTATTTAAAAAAATATCATACCTCTT AAGG	L12557	2	55-53	PET	130-152	16	89	0.836	0.618	0.857	0.858
MCM527	OAR5	GTCCATTGCCTCAAATCAATTC AAACCACTTGACTACTCCCCAA	L34277	2	55-53	PET	165-187	17	85	0.856	0.600	0.875	0.879
MAF70	OAR4	CACGGAGTCACAAAGAGTCAGACC GCAGGACTCTACGGGGCCTTTGC	M77199	3	53	NED	120-170	20	90	0.884	0.667	0.898	0.893
OarVH72	OAR25	GGCCTCTCAAGGGGCAAGAGCAGG CTCTAGAGGATCTGGAATGCAAAGCTC	L12548	3	53	VIC	118-148	14	90	0.692	0.644	0.721	0.719

BM1824	OAR1	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	G18394	3	53	VIC	170-200	6	86	0.660	0.593	0.714	0.709
OarJMP58	OAR26	GAAGTCATTGAGGGGTCGCTAACC CTTCATGTTCACAGGACTTTCTCTG	U35058	3	53	6-FAM	145-169	15	90	0.803	0.822	0.823	0.818
OarJMP29	OAR24	GTATACACGTGGACACCGCTTTGTAC GAAGTGGCAAGATTCAGAGGGGAAG	U30893	3	53	PET	96-150	16	90	0.777	0.767	0.804	0.807
DYMS1	OAR20	AACAACATCAAACAGTAAGAG CATAGTAACAGATCTTCCTACA	AJ621046	3	53	PET	59-211	16	90	0.843	0.800	0.862	0.857
OarFCB193	OAR11	TTCATCTCAGACTGGGATTCAGAAAGGC GCTTGGAAATAACCCTCCTGCATCCC	L01533	4	57	PET	96-136	16	90	0.774	0.778	0.803	0.787
MCM140	OAR6	GTTCGTACTTCTGGGTACTGGTCTC GTCCATGGATTTGCAGAGTCAG	L38979	4	57	PET	167-193	14	89	0.798	0.854	0.823	0.812
ILSTS28	OAR3	TCCAGATTTTGTACCAGACC GTCATGTCATACCTTTGAGC	L37211	4	57	VIC	114-160	16	90	0.845	0.844	0.865	0.860
HUJ616	OAR13	TTCAAACTACACATTGACAGGG GGACCTTTGGCAATGGAAGG	M88250	4	57	6-FAM	117-195	17	90	0.720	0.656	0.747	0.715
OarFCB226	OAR2	CTATATGTTGCCTTTCCCTTCCTGC GTGAGTCCCATAGAGCATAAGCTC	L20006	5	60-52	NED	119-160	17	89	0.823	0.506	0.841	0.842
MAF33	OAR9	GATCTTTGTTTCAATCTATTCCAATTTC GATCATCTGAGTGTGAGTATATACAG	M77200	5	60-52	VIC	121-141	13	78	0.820	0.564	0.845	0.835
OarCP38	OAR10	CAACTTTGGTGCATATTCAAGGTTGC GCAGTCGCAGCAGGCTGAAGAGG	U15700	sole	53	PET	117-129	8	78	0.681	0.449	0.717	0.721

Data analysis

We determined for each locus number of individuals typed (N) the number of alleles (Na), the observed heterozygosity, (Ho) and the expected unbiased heterozygosity (He) using CERVUS version 3.0.3 software(Marshall et al.1998). GENETIX 4.05 (Belkhir et al.2004) was used to determine the FST values for pairwise comparisons of the breeds, to compute Wright's FIT, inbreeding estimator (FIS;Weir Cockerham, 1984), and to assess FIS significance using 1000 random permutations of alleles in each breed. Mean genetic diversity (Hs) was estimated using FSTAT V. 2.9.3.2 (Goudet, 1995). Analysis of molecular variance (AMOVA) was performed with ARLEQUIN 3.5.1.2 (Excoffier et al.,2005).

The relative discriminatory power of each locus to assign animals to breeds was determined using the GENECLASS 2 program (Piry et al.,2004) and WHICHLOCI program (Banks et al., 2003) with the 1000 resampled data with a sample size of 90, allele frequency differential for population assignment, 95% assignment accuracy, and assignment stringency of LOD 1.0 (Banks et al., 2003). This step also allows the reduction of microsatellites and the cost of the analysis. The assignment of animals to their breed of origin was carried out using different ways: the first two ways were based on the Bayesian method proposed by Rannala and Mountain (1997) and the frequency method proposed by Paetkau et al. (1995) developed by Piry et al. (2004) in GENECLASS 2 program. The third one was based on the Jackknife method implemented in WHICHRUN 4.1

Software (Banks and Eichert, 2000). Moreover, we applied the allocation approach of Duchesne and Turgeon (Duchesne Turgeon, 2012) implemented in the FLOCK program to assign each analyzed animal to each breed. This approach was used for the genotype data of 22 microsatellites and 17 microsatellites. For this purpose, 50 runs were performed, and the number of iterations per run was 20.

Data from six Spanish sheep breeds, the mouflon, and the SiciloSarde (N=30) Tunisian sheep breeds (Calvo et al., 2011; Kdidi et al., 2015, respectively) has been added. The Spanish breeds consist of Churra Tensina (CT, N=65), Churra (CH, N=60), ChurraLebrijana (CL, N=65), Latxa (LX, N=51), Merino (Me, N=29) and Spanish mouflon (M, N=39). All added genotypes were of the same microsatellite markers.

The genetic structure and differentiation level of analyzed sheep breeds were studied in the two cases (22 microsatellites and 17 microsatellites). Also, a comparison between the genetic structure of Tunisian and Spanish sheep breeds was accomplished. For this purpose, several programs were used.

The hierarchical structure of all the breeds was analyzed using the program Populations 1.2.32 (Langella 1999), which initially determines genetic distances with the Reynolds' genetic distance (Reynolds et al., 1983) method for all pairs studied breeds and subsequently constructs an unrooted tree with bootstrapping permutations over loci (1,000 permutations in this study). These trees were visualized with the software program the Interactive Tree Of Life(iTOL) v5 (Letunic& Bork, 2021).

Four R packages were used: adegenet (Jombart, 2008)., ade4 (Drayet al., 2007), ape (Paradis et al., 2004), and RColorBrewer (Neuwirth & Neuwirth,2014) of R program 4.1.2 to draw individual unrooted trees, Discriminant Analysis of Principal Components (DAPC) scatterplots and membership probabilities bar plots.

Results

Ninety unrelated animals belonging to three native sheep breeds of Tunisia were genotyped for 22 microsatellites, and 314 alleles were found. The mean number of alleles per locus was 14.27. The Polymorphic Information Content (PIC) ranged between 0.561 (SRCRSP09) and 0.884 (MAF70). The overall FST value (after 1000 bootstraps over 22 loci) was 0.0127 (95% Confidence Interval: 0.00710 - 0.01933), besides the estimated values of FIS and FIT was 0.1664 (0.10623 - 0.23118) and 0.1770 (0.11935 - 0.24032) respectively.

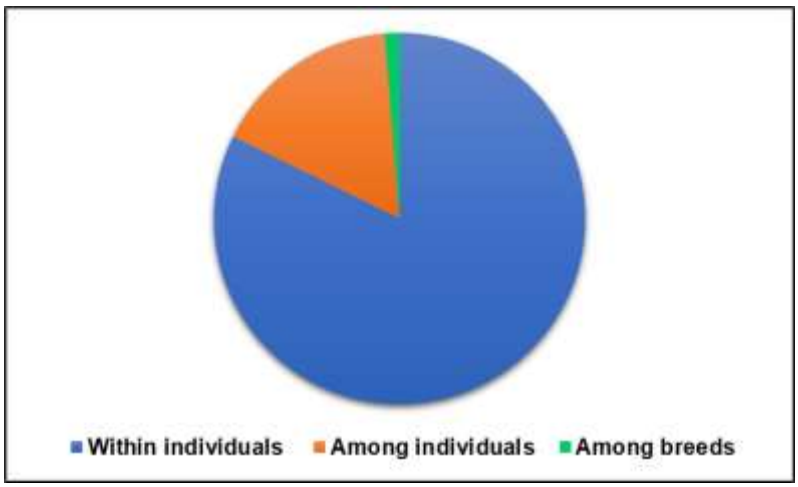

Figure 1. Results of the molecular analysis of variance (AMOVA) for the three studied sheep breeds based on the 22 microsatellites markers. The values were 82.29%; 16.43% and 1.27% for within individuals; among individuals and among breeds, respectively.

An analysis of molecular variance (AMOVA) was performed to describe the distribution percentage of variation at different levels (Fig.1). AMOVA revealed that, while most of the variation (82.29%) was distributed within individuals, a low proportion of the variation was also attributable to

differences among individuals within breeds (16.43%). Only 1.27% of the variation was partitioned among breeds.

BB/WTT pairwise values of genetic differentiation were the lowest (Table 2). The values of the pair WTT/BT were slightly higher than that of the BB/BT pair. Interestingly, the gene flow between BB and WTT was the highest (Table 2), whereas the Nm values of the BB/BT pair seem to be slightly higher than that of the WTT/BT pair.

Table 2. Pairwise FST values (below diagonal) and gene flow (Nm, above diagonal) among breeds. Values in bold were statistically significant (P < 0.05).

	Barbarin	**Western Thin Tail**	**Black Thibar**
Barbarin	-	48.35	14.52
Western Thin Tail	**0.005**	-	14.28
Black Thibar	**0.016**	**0.017**	-

The major problem while using microsatellite genotyping in the routine assay is the cost of the procedure. To find a fast and low-cost genetic test, we have tested the discriminatory power of each locus to assign animals to their breed of origin. This step could allow the proposal of a panel of effective microsatellites in the genetic assignment analysis of the Tunisian sheep. In this context, the most used programs are GENECLASS 2 (Piry et al.,2004) and WHICHLOCI (Banks Eichert, 2000). A set of 17 microsatellites (*OARHH47, MCM527, ILSTS005, MAF209, OARFCB304, INRA63, MAF214, MAF70, OARVH72, OARJMP58, DYMS1, OARFCB193, MCM140, ILSTS28, HUJ616, OARFCB226, and MAF33*) was selected for traceability use in our Tunisian sheep breeds, combining both enough assignment potential the lowest economic cost.

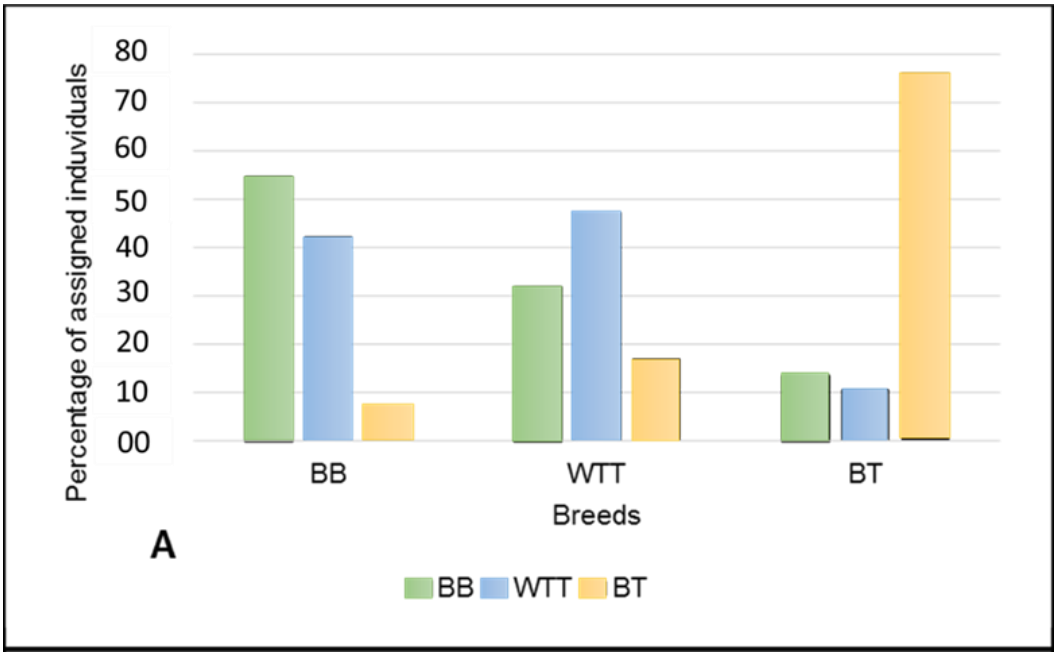

Figure 2. The allocation of individuals to their populations of origin by the Bayesian method proposed by Rannala and Mountain (1997) and implemented in GENECLASS 2 program

The result of the assignment test using the Bayesian method was reported in Fig.2. BT had the fewest individuals assigned to other breeds, with 76.10% of individuals assigned to it and 13.49% and 10.74% assigned to BB and WTT, respectively. The BB breed had 54.62% of individuals assigned to it, with 42.18% assigned to WTT and 7.04% assigned to BT. WTT breed had 47.08% of individuals assigned to it, 31.74% to BB and 16.86% to BT. Fig. 3displays the result of the assignment based on the frequency method. Herein also BT breed had the fewest individuals assigned to other breeds, with 74.42% of individuals assigned to it and 14.72% and 12.55% assigned to BB and WTT, respectively. The BB breed had 60.59% of individuals assigned to it, with 32.18% assigned to WTT and 9.93% assigned to BT. WTT breed had 55.27% of individuals assigned to it, 24.69% to BB, and 15.65'% to BT.

Overall, the rate of misassignment was high: many individuals sampled in a breed tended to be assigned with a relatively high probability in other breeds, which is consistent with a low level of differentiation among breeds when 17 microsatellites are used (FST = 1.39%). The two methods of the GENCLASS 2 program revealed that the most important misassignment was observed in the BB breed, and it is due to a relatively high number of individuals assigned to WTT (Fig.2, Fig.3).

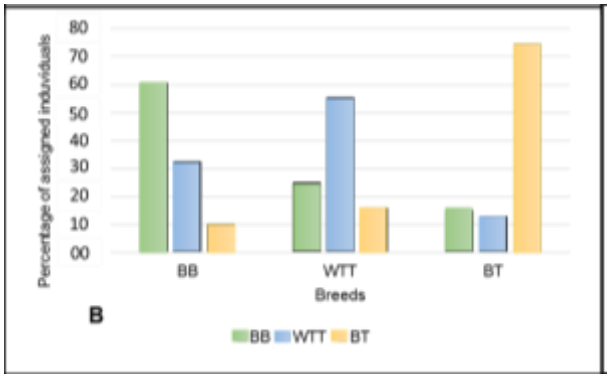

Figure 3. The allocation of individuals to their populations of origin by frequency method (Paetkau et al.1995) developed under GENECLASS 2 program.

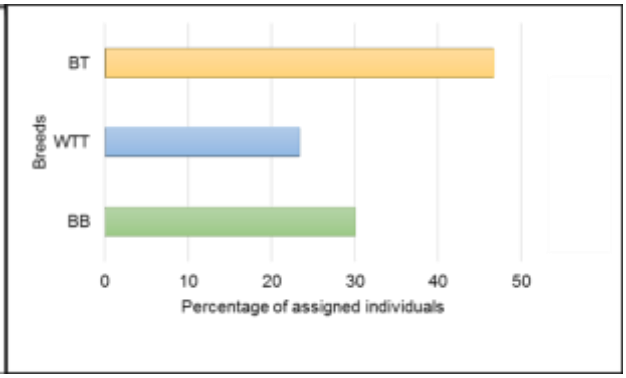

Figure 4. The percentage of individual assignment for each breed to itself using WHICHRUN program

The tests of individual allocation performed by the WHICHRUN program resulted in a relatively low percentage (< 50%) of correct classification for the three studied breeds (Fig.4). BT has the most individuals assigned to it (46.66%), whereas only 30% of individuals belonging to BB have been assigned to it, and 23.33% of individual sampled were assigned to WTT.

The results of the FLOCK program using 22 microsatellites agreed with the result obtained from other used programs, whereas data from 17 microsatellites resulted in a very different assignment from the reality and the history of breeds. Thus, only the output of Flock applied on 22 microsatellite genotypes was considered.

The result of the FLOCK program runs based on 22 microsatellite markers was shown in Fig5. BT had the fewest individuals assigned to other breeds, with 73% of individuals assigned to it and 10% and 17% assigned to BB and WTT, respectively. The WTT breed had 57% of individuals assigned to it, with 40% assigned to BB and 3% assigned to BT. BB breed had 53% of individuals assigned to it, 27% to WTT, and 20% to BT.

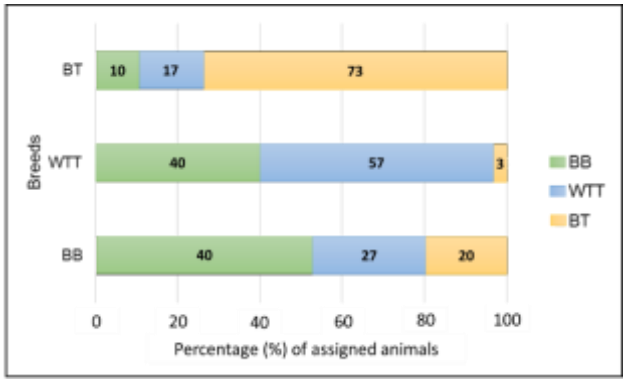

Figure 5. Percentage of individual of each breed assigned to three investigated sheep breeds computed in FLOCK program using 22 microsatellites.

The effect of the reduction of microsatellites from 22 to 17 on the estimation of the genetic relationship of different breeds and their genetic structure and differentiation was also studied. For this reason, several methods were used. Unrooted Neighbour-joining phylogenetic trees (Fig 6 and

Fig 7) revealed the absence of difference between 17 and 20 microsatellite markers. In fact, the trees were similar in the two cases. Moreover, both Discriminant Analysis of Principal Components (DAPC) scatterplots and membership probabilities (Fig 8 and Fig 9) showed the same level of genetic admixture between analyzed breeds.

Discussion

Genetic assignment

In several previous studies, unrelated animals belonging to genetically differentiated breeds have been successfully used for traceability (Bramante et al., 2011; Rogberg-Muñoz et al.2014; Mateus Russo-Almeida, 2015). In this work, we examined the amount of information obtained from microsatellite markers for genetic traceability within three native sheep breeds raised in Tunisia. We tried to reduce the number of used loci in order to reduce the costs of analysis. Highly significant (< 0.001) FIS value (0.1664) revealed a rather high inbreeding degree within breeds. Furthermore, the AMOVA analysis results displayed that the genetic structure was almost absent, resulting from a very low level of differentiation. The main variation was shown within individuals (82.29%).

WTT is a common breed in Tunisia and Algeria, where it is known as the "Ouled Djellal" (Thin Tailed breed (Iñiguez, 2006). Besides, BT originated at the beginning of the 20th century by crossing native Algerian thin-tail sheep with the French Merinos d'Arles(Chalh et al.,2007), suggesting that the genetic relationship between BT and WTT is closer than BT with other Tunisian sheep breeds. However, our analysis showed that BT had more individuals assigned to BB than WTT, according to the results obtained on GENECLASS 2 program using the two methods (Fig.2 and Fig.3). These results were in agreement with those revealed by the pairwise genetic differentiation and gene flow, and can be explained by the importance of the crossing between BB and BT in the latest decades.

On the other hand, the results of the FLOCK program (Fig.5) seem to be consistent with the historic crossing of the BT with the Thin Tail breed: BT showed more individuals (17%) assigned to WTT compared to those assigned to BB.

The methods used and implemented in the programs of WHICHRUN and GENCLASS2 revealed that both WTT and BB were most often targeted breeds by the crossing. Moreover, under the two methods of the GENCLASS2 program, the crossing of BT with WTT is more frequent than BT with BB.

FLOCK program (Fig.5) showed also that both BB and WTT underwent crossing, however, the crossing between BT and BB is more common than that between BT and WTT. In addition, the most common crossing was between BB and WT. This result was in agreement with what was reported by Bedhiaf-Romdhani et al. (2008). Because of the difficulty of selling the fat of the tail (that represents up to 15% of the carcass weight) of BB by butchers, farmers are shifting to thin-tail breeds and their crosses (Bedhiaf-Romdhani et al.,2008). They are crossing the local BB (a fat-tailed breed) with other thin-tailed breeds (WTT and BT). Fig 5 showed that the crossing between BB and WTT in BB was more common than that between BB and BT. This result could be explained by the limited distribution area of the BT (only in the northwest of the country) compared with WTT, which shares with the BB most of the geographic area in the country.

The FLOCK program seems to be the most powerful in giving the real picture regarding what happened in different studied sheep breeds of Tunisia. However, it underlines with other used programs the high level of gene flow and genetic admixture, which preclude microsatellite markers to be efficient in traceability analysis in our case.

Genetic structure

The effect of the reduction of microsatellites from 22 to 17 on the estimation of the genetic relationship of different breeds and their genetic structure and differentiation was also studied. This reduction does not affect the genetic distance and the hierarchical structure of analyzed breeds (Fig 6 and Fig 7). The four domestic sheep breeds (CT, CH, CL, LX, Me) formed the Spanish group, localized far more than the Spanish Moufflon. This last breed could be properly identified with a high bootstrap of 92%. The genetic distance of CL and other Churra breeds can be the cause of its genetic isolation as reported by Calvo et al., (2011).

DOI: 10.46325/gabj.v7i1.325

The Tunisian sheep breeds formed two groups. The first one consisted of the SS sheep dairy breed. However, the BB and WTT breeds seem to be very close to each other. BT and SS are revealed in the same plan this can be explained by the European origin of their ancestors. These results are in agreement with those found by Kdidi et al. (2015).

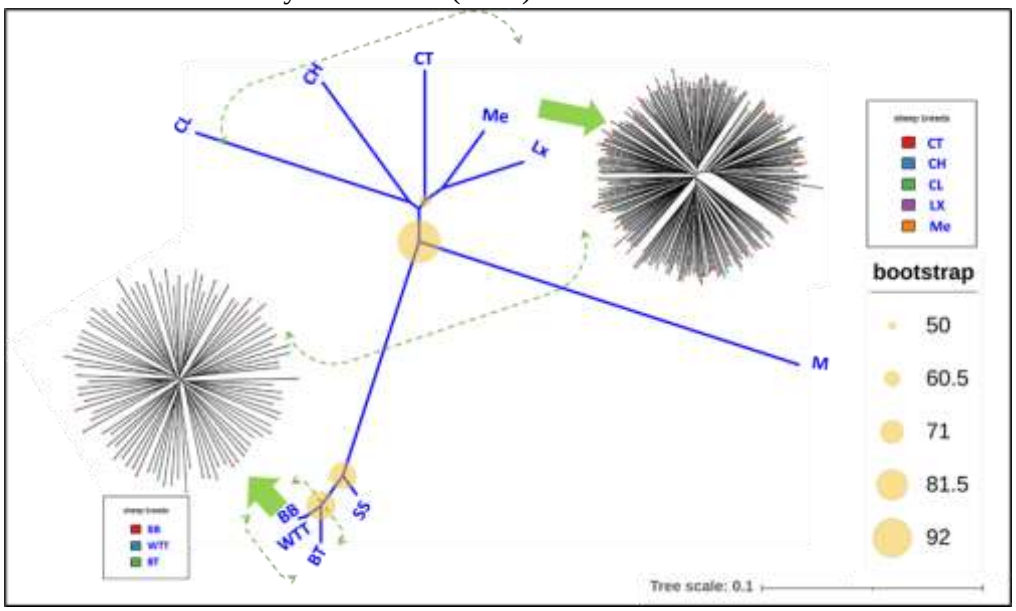

Figure 6. Unrooted Neighbor joining phylogenetic tree of Tunisian and Spanish sheep breeds. Unrooted individual tree of Spanish sheep breeds at the top right and unrooted of the three Tunisian meat sheep breeds at the top left. The three trees are drawn based on 22 microsatellites markers

The individual trees, both in the case of 17 and 22 microsatellites, revealed the absence of the genetic structure. This result is supported by the DAPC and the Barplot found in Figs 7, 8 and 9. In the DAPC breeds are represented by different colors, and dots represent different individuals. These figures showed that the Tunisian sheep breeds are the most admired. CL and the Spanish Moufflon are the most differentiated sheep breeds.

These microsatellites could be used only to identify Tunisian sheep breeds from Spanish sheep breeds. The shifting adopted by sheep farmers has an effective contribution and dangerous consequences for the genetic erosion both of BB and WTT breeds.

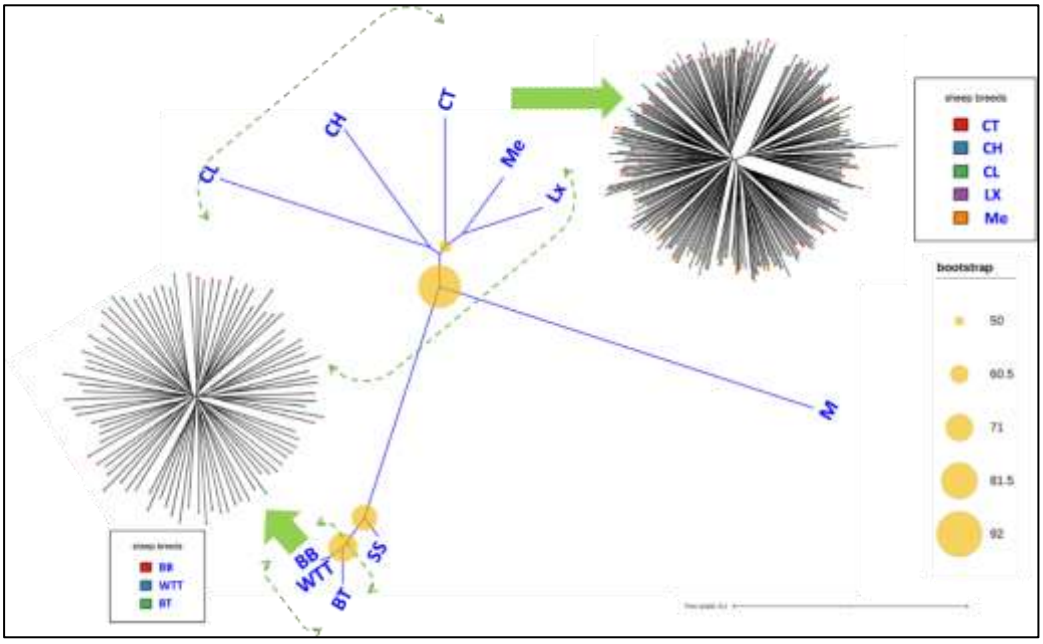

Figure 7. unrooted Neighbor joining phylogenetic tree of Tunisian and Spanish sheep breeds. Unrooted individual tree of Spanish sheep breeds at the top right and unrooted of the three Tunisian meat sheep breeds at the top left. The three trees are drawn based on 17 microsatellites markers

DOI: 10.46325/gabj.v7i1.325

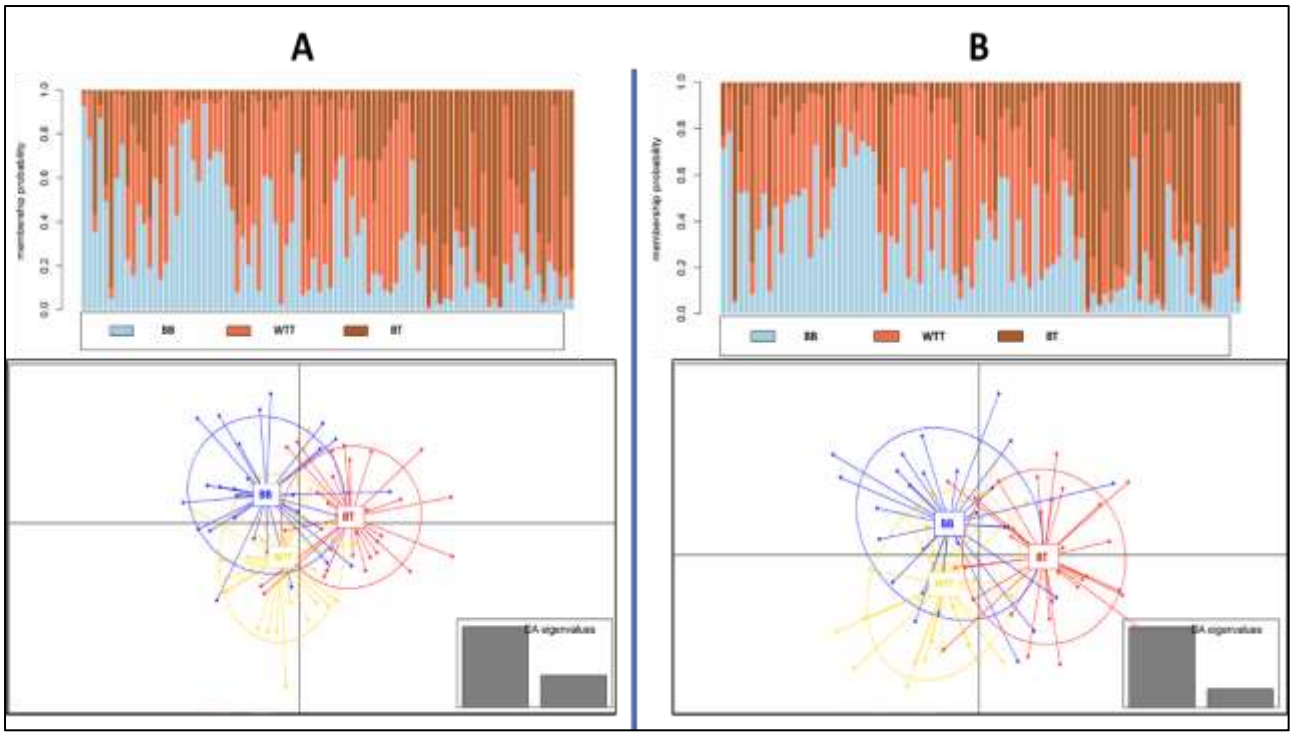

Figure 8. Discriminant analysis of principal components (DAPC) scatterplots and membership probabilities on 17 and 22 microsatellite genotype data (A and B, respectively). of Tunisian meat sheep breeds.

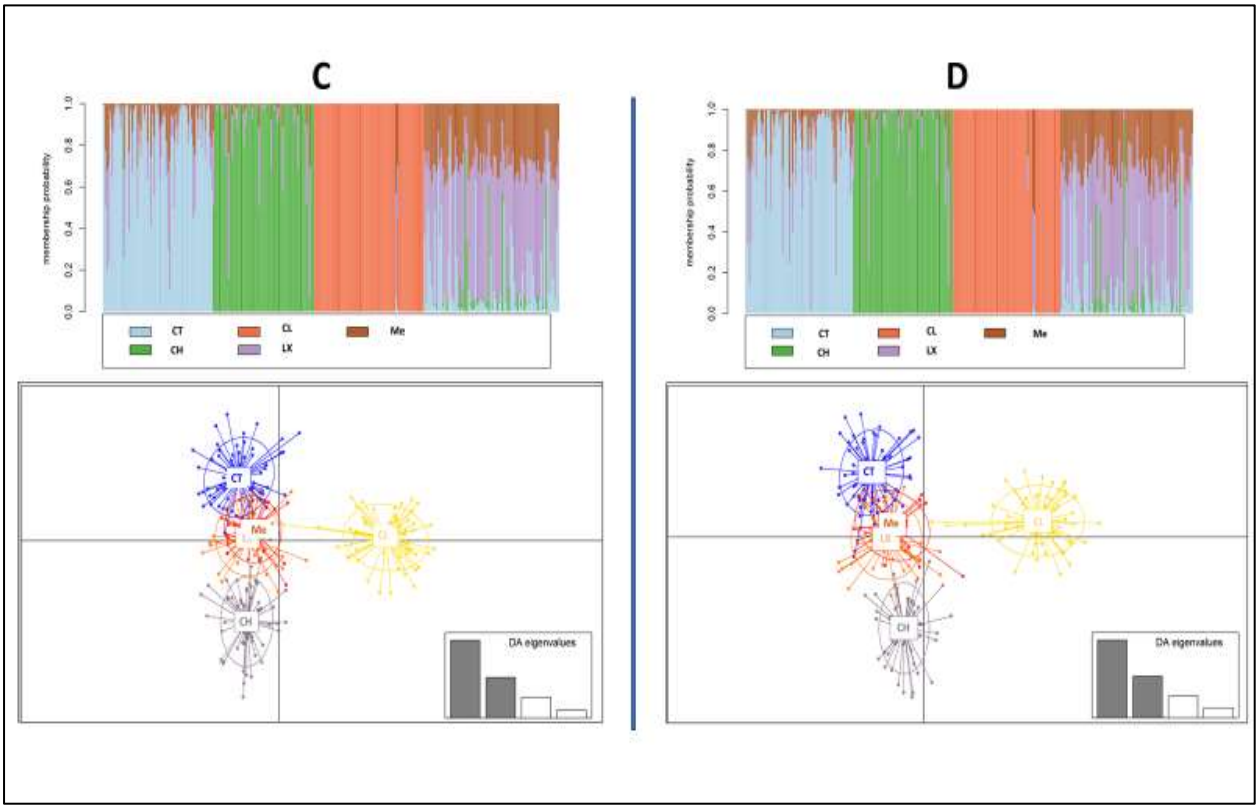

Figure 9. Discriminant analysis of principal components (DAPC) scatterplots and membership probabilities on 17 and 22 microsatellite genotype data (A and B, respectively). of Spanich sheep breeds

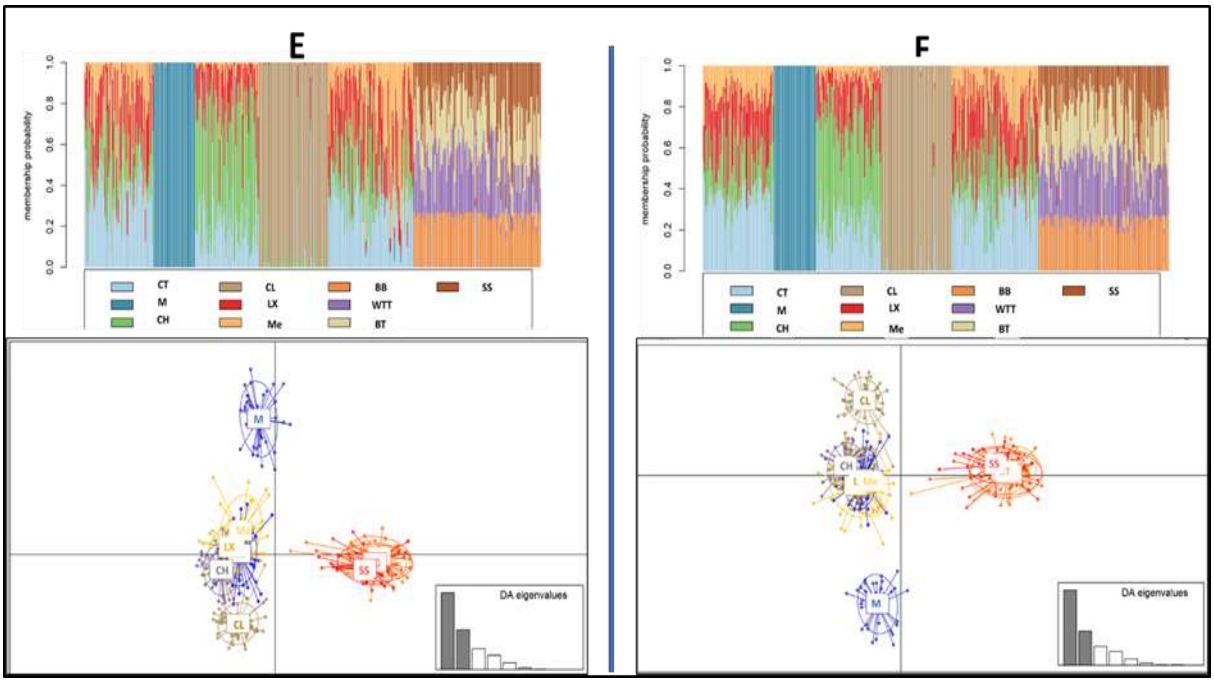

Figure 10. Discriminant analysis of principal components (DAPC) scatterplots and membership probabilities on 17 and 22 microsatellite genotype data (A and B, respectively). of Spanich and Tunisian sheep breeds

Conclusion

In our work, 22 microsatellite loci and several methods implemented in different programs were used to calculate the rate of assignment of individuals in each of the three ovine breeds (BB, WTT and BT) raised in Tunisia for meat production. The seventeen most powerful markers were chosen using WHICHLOCI and GENECLASS2 programs and used for running WHICHRUN and GENECLASS2. FLOCK software, which was more efficient with 22 microsatellites, appeared the most powerful program in showing a real picture regarding what happened in different studied sheep breeds of Tunisia. The most important crossing was between BB and WTT resulting in the highest level of misassignment in these breeds, whereas, the BT breed revealed the highest rate of assignment of individuals. All used programs indicated the unfitness of microsatellite markers in the traceability analysis of our sheep breeds. Other genetic markers, especially SNPs,should be studied to assess their fitness as regards the assignment of muttons in their breeds of origin with a high level of inbreeding and genetic admixture.

Funding

Samia Kdidi was supported by MESRS and by the MOBIDOC scheme, funded by the EU through the EMORI program and managed by the ANPR.

Acknowledgments

We would like to thank the breeders for their cooperation.

Conflicts of Interest

The authors declare no conflict of interest.

Ethics

Not applicable.

Author contributions

Samia KDIDI contributed to writing the original draft preparation, review, editing, conceptualization, formal analysis, and investigation,
Jorge HUGO CALVO contributed to the formal analysis and investigation
Semir Bechir Suheil GAOUAR, Mohamed DBARA, Ezzeddine BELFEKIH and *Slah BELHADJ* contributed to investigation
Mohamed HAMMADI contributed to resources and funding acquisition

Touhami KHORCHANI contributed to resources, supervision, and project administration and funding acquisition
Mohamed Habib YAHYAOUI contributed to writing original draft preparation and review, resources, editing, supervision, project administration and funding acquisition

References

Banks M. A. Eichert, W. 2000. WHICHRUN (version 3.2): a computer program for population assignment of individuals based on multilocus genotype data. Journal of Heredity, 91(1), 87-89. doi:10.1093/jhered/91.1.87.

Banks M. A. Eichert W. Olsen J. B. 2003. Which genetic loci have greater population assignment power?. Bioinformatics, 19(11), 1436-1438. doi:10.1093/bioinformatics/btg172

Bedhiaf-Romdhani S. Djemali M. Zaklouta M. Iniguez L. 2008. Monitoring crossbreeding trends in native Tunisian sheep breeds. Small ruminant research, 74(1-3), 274-278. doi:https://doi.org/10.1016/j.smallrumres.2007.07.008

Belkhir K. 2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. http://www. genetix. univ-montp2. fr/genetix/genetix. htm. doi:https://kimura.univ-montp2.fr/genetix/

Bramante A. Cecchi F. Ciani E. Castellana E. D'Andrea M. S. Pilla F. Ciampolini R. 2011. The breed traceability of sheep meat by using molecular genetics methods: preliminary results. Italian Journal of Food Safety, 1(1), 41-44. doi: https://doi.org/10.4081/ijfs.2011.749

Calvo JH. Alvarez-Rodriguez J. Marcos-Carcavilla A. Serrano M. Sanz A. 2011. Genetic diversity in the Churratensina and Churralebrijana endangered Spanish sheep breeds and relationship with other Churra group breeds and Spanish mouflon. Small ruminant research, 95(1), 34-39.

Chalh A. El Gazzah M. Djemali M. Chalbi N. 2007. Genetic and phenotypic characterization of the Tunisian Noire De Thibar lambs on their growth traits. Journal of Biological Sciences, 7, 1347-1353. doi:10.3923/jbs.2007.1347.1353.

Dalvit C. De Marchi M. Dal Zotto R. Gervaso M. Meuwissen T. Cassandro M. 2008. Breed assignment test in four Italian beef cattle breeds. Meat Science, 80(2), 389-395. doi: https://doi.org/10.1016/j.meatsci.2008.01.001

Djemali M. Romdhani S. Wurzinger M. Iniguez L. 2006. Impact of butchers and market trends on the integrity of small ruminant genetic resources. In: Book of Abstracts of the 57th Annual Meeting of the European Association for Animal Production, van der Honing, Y. (Ed.). Wageningen Academic Pub, Antalya, Turkey, 17-20 September 2006

Dray S. Dufour AB. 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of statistical software, 22, 1-20.

Duchesne P. Turgeon J. 2012. FLOCK provides reliable solutions to the "number of populations" problem. Journal of Heredity, 103(5), 734-743. doi:https://doi.org/10.1093/jhered/ess038

Excoffier L. Laval G. Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary bioinformatics, 1, 117693430500100003. doi: https://doi.org/10.1177/117693430500100003

Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of heredity, 86(6), 485-486. doi: doi:https://doi.org/10.1093/oxfordjournals.jhered.a111627

Hoffmann R. 2000. Country of origin–a consumer perception perspective of fresh meat. British food journal, 102(3), 211-229. doi:https://doi.org/10.1108/00070700010332304.

Iaccarino T. Di Monaco R. Mincione A. Cavella S. Masi P. 2006. Influence of information on origin and technology on the consumer response: The case of soppressata salami. Food Quality and Preference, 17(1-2), 76-84. doi:https://doi.org/10.1016/j.foodqual.2005.08.005

Iñiguez L. 2006. Characterization of small ruminant genetic resources in Central Asia, the Caucasus, West Asia and North Africa. In International Workshop "Options and Strategies for the Conservation of Farm Animal Genetic Resources" (p. 34).

Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24(11), 1403-1405.

Kdidi S. Calvo JH. González-Calvo L. Sassi MB. Khorchani T. Yahyaoui MH. 2015. Genetic relationship and admixture in four Tunisian sheep breeds revealed by microsatellite markers. Small Ruminant Research,131, 64-69.

Langella O. 1999. Populations 1.2.28: Population genetic software. CNRS UPR9034,

http://bioinformatics.org/~tryphon/populations/ Google Scholar

Lenstra J. A. 2005. Primary identification: DNA markers for animal and plant traceability. In: Improving Traceability. In Food Processing and Distribution, Smith I., Furness T. (eds). Woodhead Publ., Cambridge, pp. 147 – 164.

Lenstra J. A. 2006. DNA markers for animal and plant traceability (pp. 147-164). Woodhead Publishing, Cambridge, UK.

Letunic I. Bork P. 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic acids research, 49(W1), W293-W296.

Manel S. Gaggiotti OE. Waples RS. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology & Evolution, 20(3), 136-142. doi:https://doi.org/10.1016/j.tree.2004.12.004

Marshall TC. Slate JB. Kruuk LEB. Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular ecology, 7(5), 639-655. doi:https://doi.org/10.1046/j.1365-294x.1998.00374.x

Mateus J. C. Russo-Almeida P. A. 2015. Traceability of 9 Portuguese cattle breeds with PDO products in the market using microsatellites. Food Control, 47, 487-492. doi:https://doi.org/10.1016/j.foodcont.2014.07.038

Nakamura A. Kino K. Minezawa M. Noda K. Takahashi H. 2006. A method for discriminating a Japanese chicken, the Nagoya breed, using microsatellite markers. Poultry science, 85(12), 2124-2129. doi:https://doi.org/10.1093/ps/85.12.2124

Neuwirth E. Neuwirth M. E. 2014. Package 'RColorBrewer'. ColorBrewer Palettes.

OEP. 2014. http://www.oep.nat.tn/index.php/fr/donnees-sectorielles/41-productions. Accessed June 25, 2014.

Oh JD. Song KD. Seo JH. Kim DK. Kim SH. Seo KS. Lim HT. Lee JB. Park HC. Ryu YC. Kang MS. Cho S. Kim E.S. Choe H.S. Kong H.S. Kang M. S. 2014. Genetic traceability of black pig meats using microsatellite markers. Asian-Australasian journal of animal sciences, 27(7), 926. doi:10.5713/ajas.2013.13829.

ONAGRI (Observatoire National de l'Agriculture en Tunisie), 2012. La production animale en 2012. http://www.onagri.tn/statistiques.

Paetkau D. Calvert W. Stirling I. Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular ecology, 4(3), 347-354. doi: https://doi.org/10.1111/j.1365-294X.1995.tb00227.x.

Paradis E. Claude J. Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics, 20(2), 289-290.

Piry S. Alapetite A. Cornuet J. M. Paetkau D. Baudouin L. Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of heredity, 95(6), 536-539. doi: https://doi.org/10.1093/jhered/esh074.

Quagrainie KK. Unterschultz J. Veeman M. 1998. Effects of product origin and selected demographics on consumer choice of red meats. Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie, 46(2), 201-219. doi: https://doi.org/10.1111/j.1744-7976.1998.tb00363.x.

Rannala B. Mountain JL. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences, 94(17), 9197-9201. doi: https://doi.org/10.1073/pnas.94.17.9197.

Reynolds J. Weir BS. Cockerham CC. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics, 105(3), 767-779.

Rikimaru K. Takahashi H. 2007. A method for discriminating a Japanese brand of chicken, the Hinai-jidori, using microsatellite markers. Poultry Science, 86(9), 1881-1886. doi: https://doi.org/10.1093/ps/86.9.1881.

Rogberg-Muñoz A. Wei S. Ripoli MV. Guo BL. Carino MH. Castillo N. Villegas EE. Castagnasoa EE. Lirón JP. Morales Duranda HF. Meluccic L.Villarrealc E. Peral-García P. Wei YM. Giovambattista G. 2014. Foreign meat identification by DNA breed assignment for the Chinese market. Meat science, 98(4), 822-827. doi: https://doi.org/10.1016/j.meatsci.2014.07.028.

Shackell GH. Tate ML. Anderson R M. 2001. Installing a DNA-based traceability system in the meat industry. In Proceedings of the Association for the Advancement of Animal Breeding and Genetics (Vol. 14, pp. 533-536).

Verbeke W. Pérez-Cueto FJ. de Barcellos MD. Krystallis A. Grunert KG 2010. European citizen and consumer attitudes and preferences regarding beef and pork. Meat science, 84(2), 284-292. doi: https://doi.org/10.1016/j.meatsci.2009.05.001.

Weir BS. Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. evolution, 38(6), 1358-1370.