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Sheep diversity of five Egyptian breeds: Genetic proximity revealed between desert breeds
Local sheep breeds diversity in Egypt

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ABSTRACT

This study investigated genetic diversity within and among five Egyptian breeds, the three major ones (Barki, Ossimi and Rahmani) and two minor (Sohagi and Saidi), by use of 22 microsatellite markers. The sampling design allowed paying particular attention to desert breeds (Barki, Sohagi and Saidi). Moreover two Algerian breeds (Ouled-Djellal and Rembi) were genotyped with the same set of microsatellites in order to expand the results at a larger scale. Our results showed substantial genetic diversity (average gene diversity ranging 0.64 to 0.79) and very low values of F_{IS} were recorded. A clear genetic structuration was observed, with genetic proximity between the desert breeds. In particular, Sohagi appeared as a mixture between Barki and Saidi, even if the level of admixture was not critical. Hence, this picture of the Egyptian sheep diversity suggests that the situation of the Sohagi have to be carefully monitored; moreover at least six other breeds have to be studied to gain a comprehensive view of the genetic diversity of Egyptian sheep.

1. Introduction

Egypt represents a critical step in the sheep domestication process; as, not long after their domestication in the Fertile Crescent, approximately eight thousand years ago (Ryder, 1984), sheep entered and spread in Africa, *via* the Isthmus of Suez (Muigai and Hanotte, 2013). Archaeological evidence certifies their presence in ancient Egyptian society between seven and eight thousand years ago (Muigai and Hanotte, 2013) while rams were an object of worship. The outcome of this long history was the emergence of a substantial diversity of local breeds presenting unique combinations of adaptive traits to particular environments (Buduram, 2004) widely misunderstood so far.

Sheep of Egypt (around 5,488,000 heads) are raised mainly in three regions: the Nile Delta, Upper Egypt and in the desert rangelands (particularly in the north-west coastal zone and in the Sinai Peninsula (Iniguez, 2005)). Given the lack of rainfall, Egypt shows poor rangeland, that provides only 5% of the feed requirement of the total small ruminant population (El-Nahrawy, 2011). As a consequence rearing is mainly intensive, except in the desert where 1.4 million sheep are kept in extensive/transhumant systems (Al-Keraby, 1997). In Upper Egypt, about 1.5 million sheep are reared, mainly in mixed flocks, with some goats, kept as household animals, in less extensive systems. Livestock plays an important role in the livelihoods of rural people, particularly in Upper Egypt and constitutes a major part of the Bedouin's income (North West Coast of Egypt and Sinai).

Three major sheep breeds are recorded: Rahmani (in the north of the Delta), Ossimi (in Middle Egypt) and Barki (mainly in the northeastern coastal areas); furthermore, eight minor sheep breeds are recorded mainly in the southern part of the country and the

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oases: Abidi, Abudeleik, Farafra, Kanzi, Maenit, Saidi, Sanabawi and Sohagi.

In spite of its importance as a world cultural and genetic heritage very little is known about Egyptian sheep. The three main breeds (Barki, Ossimi and Rahmani) were studied from a genetic point of view with microsatellites markers by ElFawal et al. (2008), El Nahas et al. (2008) and Ghazy et al. (2013); however PCR reaction products were analyzed directly on polyacrylamide gels which makes the results difficult to compare between studies. Sallam et al. (2012) have realized a study centered on the genetic diversity of the Barki, using nine microsatellites. Othman et al. (2015) compared the three major breeds with Italian breeds in a phylogenetic analysis. One study (Eman et al., 2008), attempted to understand the genetic diversity of the three main breeds added with two minor ones (Saidi and Sohagi), using the RAPD-PCR technique. Results highlighted the existence of two clusters, the first including Ossimi and Rahmani, and the second one gathering Barki, Saidi and Sohagi.

Our goal was to provide a first picture of the genetic diversity of the Egyptian livestock, using a fair number of relevant markers (*i.e.* microsatellites recommended by the FAO), in such a way as to obtain comparable results. Five breeds were considered. Our efforts, during the sampling procedure, have focused on the Barki (the Bedouin's breed) and the two minor breeds (Saidi and Sohagi, in the Upper Egypt) as these breeds play key roles in the livelihood of Egyptian rural people and show strong adaptation to desert conditions (FAO, 2011), and hence have to be studied urgently in a climate change context. Unmanaged cross-breeding (*i.e.* crosses realized outside the scope of selection schemes) are current practices in North-African countries (Iniguez, 2005); the main objective of the study was to assess if genetic originality of Egyptian desert breeds is threaten by admixture and genetic erosion.

2. Material and methods

2.1. Ethics statement

The blood used for all of the analyses was collected by veterinarians during routine blood sampling on commercial farm animals (for medical care or follow up). Those animals were not linked to any experimental design and blood sampling was not performed specifically for this study. All the samples and data processed in our study were obtained with the breeders and breeding organizations' consent.

2.2. Breeds and samples

The five Egyptian sheep breeds considered are all of the fat-tailed, coarse-wool group. Ossimi, showing a coat colour white with reddish brown head, probably originated in Giza (near Cairo), and would have been descended from the Awassi (Mason, 1967). The breed inhabits the middle Egypt (Nile valley and south Delta area). The large brown Rahmani originated in northern Syria and northern Turkey, was introduced into Egypt in the 9th century (Galal, 1987). This breed, mainly kept in the north and middle Delta region, shows particular adaptation to hot and humid climate whereas it is not good walker. Barki, the smallest of the three major breeds is reared by Bedouin in the north-west coastal area and is very similar with the fat-tailed sheep of Libya (Mason, 1967; Epstein, 1971). Saidi, a long fat tailed breed is found mostly in its native area, around Beni-Adi in the Asyut province, but its range covers around 79,152 km^2 along the Nile Valley, in the northern part of Upper Egypt (Mason, 1967; De Pauw et al., 2011) while for each of the three major breeds, distribution areas are only around 12,633 km^2. This breed would be the oldest Egyptian breed. Sohagi, inhabits southern part of Upper Egypt, with a distribution area of 34,725 km^2 along the Nil Valley; among the five breeds considered it is one of the smallest (with Barki and Saidi even smaller). Barki, Saidi and Sohagi are adapted to desert conditions, solar radiations and sandy substrate (Mason, 1967; De Pauw et al., 2011).

The sample dataset (N = 107) collected by veterinarians during routine blood sampling on commercial farm animals, carried out in a span of time that goes from 2013 to 2015. In order to maximize sample representativeness and minimize genetic relationship among individuals, different farms were visited for each breed, and individuals were chosen according to their genealogy. Blood samples were cryopreserved until DNA extraction and analysis. Details about sampling are reported in Table 1. Additional data from two Algerian sheep breeds (Ouled-Djellal and Rembi), 25 individuals for each breed, were also used as a reference, by the use of the F_{ST} metric. These values, provided calibration points for the interpretation of the genetic division between the Egyptian native populations, and were obtained from the same set of 22 microsatellites, in the same conditions of genotyping.

2.3. DNA extraction, polymerase chain reaction (PCR) and fragment analysis

Blood was collected into EDTA tubes. DNA was extracted from the blood samples according to established protocol (Miller et al., 1988). Twenty-three microsatellites were amplified; all the microsatellites except CSSM66 and INRA35 were chosen through the panel of microsatellites proposed for sheep characterization by the Food and Agriculture Organization of the United Nations/International Society for Animal Genetics (FAO/ISAG) (2011) (Table 2). Microsatellites were amplified according to three multiplex reactions (annotated 1, 2 and 3 in Table 2), carried out using QIAGEN Multiplex PCR Master Mix with fluorescently labeled primers. PCR amplification of microsatellite loci was carried out in 25 μL volume and included 12.5 μL 2X QIAGEN Multiplex PCR Master-Mix (QIAGEN Multiplex PCR Buffer, containing dNTPs, QIAGEN HotStar Taq DNA Polymerase, 3 mM $MgCl_2$, and 0.3 μM of each primer) 1.25 μL of double-distilled water and 4 μL of template DNA (25 ng/μL). Amplifications were performed in a GeneAmp PCR System 9600 Thermal Cycler with the following program: 5 min at 95 °C; 30 cycles of 30 s at 94 °C, 90 s at the annealing temperature (see Table 2), 30 s at 72 °C; and a final extension of 30 min at 60 °C. Amplification products were loaded on an ABI 3730 Genetic Analyzer using LIZ-600 as internal size standard (Applied Biosystems). Amplified fragment lengths were assigned to allelic sizes with GeneMapper v.4.0 (Applied Biosystems).

2.4. Data analysis

The mean number of alleles per breed, the average observed (H_o) and expected (H_e) heterozygosity over loci per breed were estimated using ARLEQUIN 3.5 (Excoffier and Lischer, 2010). To calculate allelic richness and the richness of private alleles, we used the rarefaction method (Kalinowski, 2004) implemented in HP-RARE (Kalinowski, 2005) adopting a sample of 26 genes, corresponding to 13 individuals. Polymorphic Information Content (PIC) and effective number of alleles (Na_e) were estimated for all markers using the Molkin software (version 2.0) (Gutiérrez et al., 2005).

Departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) among loci were estimated using the program GENEPOP v4.0 (Rousset, 2008). Levels of significance were adjusted using the false discovery rate (FDR) procedure (Benjamini and Hochberg, 1995).

Frequencies of null alleles at each locus and for each breed were estimated with INEST (http://genetyka.ukw.edu.pl/INEst10_setup.exe), in order to take into account simultaneously null allele frequencies at each locus and the average level of the intra-population inbreeding as a multi-locus parameter (Chybicki and Burczyk, 2009).

The unbiased estimator of Wright inbreeding coefficient, F_{IS}, was calculated following Weir and Cockerham (1984) (f estimator). Its significance was assessed using a permutation method (10,000 permutations) implemented in GDA (Lewis and Zaykin, 1999).

The extent of population subdivision was examined by calculating the global multi-locus F_{ST} value. The index of pair-wise F_{ST} of Weir and Cockerham (1984) and their associated 95% confidence intervals were determined using GDA (Lewis and Zaykin, 1999).

A Bayesian model-based clustering approach was used to search for the occurrence of genetic groups (*i.e.*, clusters, K) in our dataset (as implemented in STRUCTURE 2.3.3, Pritchard et al., 2000; Falush et al., 2003, 2007; Hubisz et al., 2009). The burn-in length of the Markov Chain Monte Carlo (MCMC) was set to 50,000 followed by 200,000 iterations. The admixture model and the correlated allele frequencies model were used without priors on sampling information. Fifteen runs were conducted for each K value, with K ranging from 1 to 6. CLUMPP (v. 1.1.1) (Jakobsson and Rosenberg, 2007) was used to align the repetitions for each K and the visualization was made by the program DISTRUCT (v.1.1) (Rosenberg, 2004).

To assess the degree to which breeds differ from each other when adopting an approach without assumptions about HWE or LD, we performed Discriminant Analysis of Principal Components (DAPC), using the approach implemented in the ADEGENET package (Jombart, 2008) within the statistical package R version 3.0.1 (R Core Team, 2013).

Finally we conducted a spatial Analysis of Principal Components (sPCA) (Jombart, 2008), a multivariate method optimizing the identification of spatial genetic patterns. sPCA was performed with the package ADEGENET (Jombart, 2008) using as connection network, the Delaunay triangulation (Upton and Fingleton, 1985). The existence of global and/or local spatial structure was tested using Monte Carlo procedure for 10,000 iterations. The sPCA result was visualized by plotting the samples according to their geographic coordinates and colouring them according to their respective scores along the first sPCA components.

3. Results

The twenty-three microsatellites loci were highly polymorphic; a total of 260 different alleles (mean = 11.30 per locus, s.d. = 4.21) were found in the five breeds, effective number of alleles ranged