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Genetic diversity and structure in Egyptian indigenous sheep populations mirror patterns of anthropological interactions

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ABSTRACT

Human exchange networks are instrumental in influencing gene flow among domesticates. Here, we investigated levels of within- and between-population genetic diversity in 289 animals from six indigenous sheep populations in Egypt (Barki, Farafra, Ossimi, Rahmani, Saidi, Souhagi) and 119 individuals of Awassi breed from Egypt, Turkey and Syria using 13 autosomal microsatellites. Although our analysis revealed genetic differentiation between Souhagi and other Egyptian populations, and between the Awassi from Egypt and the ones from Syria and Turkey, most likely due to reproductive isolation, Bayesian analysis identified two gene pools underlying the ancestral genetic diversity while multivariate analysis identified nine genetic clusters which could be subdivided into four broad genetic groups. Further analysis revealed that this genetic structure was the result of the exchange of genetic stocks along the Nile River valley and the Mediterranean Sea coast, but, minimal gene flow between flocks found in the Northern, Central and Southern Egypt across the Western desert. Our results support the fact that human interaction networks have shaped the genetic architecture of domestic animals while harsh environments such as deserts tend to limit human interactions and hence gene flow among domesticates.

1. Introduction

In natural populations, gene flow is influenced by the extent to which landscape topography facilitate interactions among populations (Taylor et al., 1993). Among domesticates, gene flow is mediated by human socio-cultural and economic networks, which may lead to a unique combination of genotypes as well as geospatial patterns of livestock genetic diversity and structure. Such human networks have shaped the genetic structure of several domestic animals including the Lipizzan horse breed (Achmann et al., 2004), local goat populations in Vietnam (Berthouly et al., 2009) and traditional breeds of sheep in Belgium (Dumasy et al., 2012). Warmuth et al. (2013) showed that ancient trading networks influenced the genetic structure of eastern Eurasian horses. Geographic features can constrain human mobility and indirectly gene flow among domesticates. For instance, mountain ranges and the Yangtze River have been associated with increased levels of genetic differentiation in Yak (Xuebin et al., 2005) and Water Buffaloes (Zhang et al., 2007).

In Egypt, livestock play pivotal roles to most communities whose common history may attenuate and/or enhance their interactions which in turn may sculpture the genetic diversity and demographic dynamics of their livestock. Sheep and goats (Shoats) have been an important livestock species in Egypt since the fifth millennium BC (Galal et al., 2005) and offer themselves as biological candidates to analyse the effects of human socio-cultural and economic interactions on livestock genetic diversity and structure. Unlike cattle or water buffaloes, shoats have been raised by most communities and they fulfill various socio-cultural and economic roles to their owners as well as entire villages. Livestock in Egypt offer an extra layer of information, the insights into the historical phenomenon that have shaped livestock biodiversity, because of the country's strategic location at the entry point of most domestic species (animals and plants) into the African continent (Fuller et al., 2011) and its long history of interaction with Eurasia. We therefore investigated the genetic landscape of indigenous sheep populations in Egypt using autosomal microsatellites to better understand the influence of different factors (such as founder effect, reproductive isolation, admixture) in shaping their genetic diversity and structure. To account for the possible complex scenario that may arise in reconstructing the genetic profiles of the indigenous sheep populations, we included in the study three populations of Awassi, a breed of sheep that occurs in central Asia and the Middle East. Data generated here provides a fine-scale overview on the knowledge

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Table 1
Measures of genetic diversity for the nine sheep breeds/populations that were analysed in the current study.

Population				Allelic diversity				Genetic diversity			
		N	TNA	AR (SD)	MNA (SD)	ENA (SD)	Pa	He (SD)	Ho (SD)	F_{IS}	F_{ij}
Awassi-Egypt	EA	70	142	7.72 (2.17)	10.92 (3.25)	5.80 (2.27)	5	0.79 (0.035)	0.72 (0.015)	0.093	0.393
Awassi-Syria	SA	27	92	6.22 (1.71)	7.08 (2.22)	4.15 (1.24)	1	0.74 (0.041)	0.70 (0.024)	0.047	0.354
Awassi-Turkey	TA	22	87	6.06 (1.22)	6.69 (1.55)	4.01 (1.08)	3	0.75 (0.022)	0.67 (0.028)	0.103	0.377
All Awassi's		*119*	*155*	*8.62 (2.29)*	*11.92 (3.62)*	*6.01 (1.96)*	*9*	*0.82 (0.021)*	*0.71 (0.012)*	*0.085*	
Barki	BK	40	141	8.55 (2.25)	10.85 (3.34)	5.74 (1.80)	4	0.82 (0.019)	0.64 (0.023)	0.226	0.437
Farafra	FF	20	101	7.31 (1.59)	7.77 (1.79)	5.04 (1.52)	2	0.81 (0.018)	0.59 (0.032)	0.281	0.464
Ossimi	OS	58	147	8.25 (1.88)	11.31 (2.90)	6.14 (2.41)	10	0.82 (0.021)	0.76 (0.019)	0.076	0.397
Rahmani	RH	70	159	8.46 (2.08)	12.23 (3.14)	6.29 (2.45)	10	0.82 (0.019)	0.74 (0.015)	0.103	0.417
Saidi	SD	64	153	8.20 (2.09)	11.77 (2.83)	6.08 (2.68)	6	0.80 (0.031)	0.59 (0.018)	0.265	0.467
Souhagi	SH	37	119	7.12 (2.05)	9.15 (2.76)	5.09 (2.03)	3	0.75 (0.048)	0.68 (0.022)	0.105	0.386
All Egyptian		*289*	*220*	*9.12 (2.20)*	*16.92 (5.71)*	*7.33 (3.27)*	*35*	*0.84 (0.018)*	*0.68 (0.008)*	*0.160*	
Overall		408	229	9.12 (2.38)	17.62 (5.88)	7.51 (3.31)	44	0.84 (0.018)	0.69 (0.007)	0.138	

Key: *N*: Sample size; TNA: total number of alleles; AR: allelic richness; SD: standard deviation; MNA: mean number of alleles; ENA: effective number of alleles; Pa: private alleles; He: expected heterozygosity; Ho: observed heterozygosity; F_{IS}: inbreeding coefficient; F_{ij}: coancestry coefficicent.

of current and historical gene flow in a domestic animal species including insights into past and recent anthropogenic interactions within an early livestock dispersal area in Africa.

2. Materials and methods

Blood samples were collected from 168 animals from three "main" populations of indigenous sheep in Egypt; Barki (BK, $n = 40$), Ossimi (OS, $n = 58$), Rahmani (RA, $n = 70$) and 121 animals from three "minor" populations; Souhagi (SO, $n = 37$), Saidi (SD, $n = 64$) and Farafra (FA, $n = 20$) (Table 1; Fig. S1). To capture the largest possible representation of the existing genetic diversity, we sampled unrelated animals from several flocks in each population. In addition, 119 individuals of Awassi breed were sampled from Turkey (TA, $n = 22$), Syria (SA, $n = 27$) and Egypt (EA, $n = 70$). The flock that was sampled in Egypt was introduced to the country from Syria in 1994 and 1997. In total, 90 animals bred for the production of milk and wool were imported. The flock has remained closed ever since, but the breeding goal has shifted to selection for fertility and growth performance. The Syrian and Turkish Awassi were sampled at the International Center for Agricultural Research in the Dry Areas (ICARDA) experimental farm in Tel Hadya (northern Syria) where the breeding goal is to select for milk production.

Thirteen microsatellites selected from the 30 ISAG/FAO panel (Table S1) were used for genotyping. DNA targets were amplified via PCR on a C1000 Thermal Cycler (Biorad, USA) in two multiplex reactions. Each 25 μl reactions contained 100–150 ng DNA, 1X Platinum® Multiplex PCR Master Mix (Lifetechnologies, USA) and 10 pM of each primer. PCR products were size fractionated using the GeneScan™-600 Liz® (Applied Biosystems) internal lane size standard. Genotyping was performed with the ABI 3100 automatic capillary sequencer (Applied Biosystems) and allele sizes were called with GeneMapper v3.5 (Applied Biosystems) applying the 2nd order Least Squares Method.

2.1. Statistical analysis

We computed several statistics representing measures of allelic and genetic diversity for each population. The total and mean number of alleles (TNA, MNA), observed (Ho) and expected (He) heterozygosity were computed with MICROSATELLITE TOOLKIT (Park, 2001); allelic richness (AR) with FSTAT 2.9.4 (Goudet, 2001) and the effective number of alleles (ENA) with POPGENE (Yeh et al., 1997). The total genetic variation (F_{IT}) was partitioned into within (F_{IS}) and among (F_{ST}) components following Weir and Cockerham (1984) in FSTAT 2.9.4. A hierarchical analysis of molecular variance was performed to partition the total genetic variance into components attributable to individual, population and group differences using the AMOVA (Analysis of Molecular Variance) module in ARLEQUIN 3.5 (Excoffier and Lischer, 2010).

The proportion of mixed ancestry for each animal was evaluated with the Bayesian clustering algorithm implemented in STRUCTURE v.2.5.5 (Pritchard et al., 2000). We ran STRUCTURE for $1 \leq K \leq 15$ with the mixed ancestry and correlated allele frequency independent of sampling information. Ten runs of 200,000 iterations following a burn-in of 100,000 were performed for each K. The number of clusters (K) was assessed with ΔK (Evanno et al., 2005). The results were graphically displayed with DISTRUCT (Rosenberg, 2004) after processing with CLUMPP (Jacobsson and Rosenberg, 2007) and STRUCTUREHARVESTER (Dent and von Holdt, 2012).

The algorithm implemented in STRUCTURE is model based, requires populations to meet Hardy–Weinberg expectations and linkage disequilibrium between loci. Furthermore, assigning individuals to groups may likely be inappropriate if they are genetically structured along a cline (Guillot et al., 2009). A cline structure was expected in our data set because we sampled four populations (Rahmani, Ossimi, Saidi, Souhagi) along the Nile River basin (Fig. S1). To circumvent these drawbacks and investigate further population structure, we used the multivariate based Discriminant Analysis of Principle Components (DAPC) (Jombart et al., 2010) executed in ADEGENET v1.3-9.2 (Jombart, 2008) in the R environment v2.15.3 (http://www.R-project.org). We identified the number of genetic clusters in the dataset using the Bayesian Information Criterion (BIC) statistic.

A comparison of genetic distances can provide information on the evolutionary processes that shape population structure and dynamics (Álvarez et al., 2005). For instance, the D_R genetic distance (Reynolds et al., 1983) illustrates the effect of genetic drift on population structure while the molecular kinship distance, D_K, (Eding et al., 2002) provides information on recent between-breed differentiation corrected for allele frequency differences in the founder population. We therefore computed D_K and D_R using MOLKIN 2.0 (Gutiérrez et al., 2005) and POPULATION 1.2.28 (http://bioinformatics.org/~tryphon/populations/), respectively. To visualize population proximities and infer evolutionary processes, we performed a multidimensional scaling (MDS) analysis for each distance matrix using R v2.15.3.

3. Results

In total 229 alleles were observed at the 13 loci genotyped in 408 individuals with an average value of 17.62 (5.88) alleles per population (Table 1). The average expected and observed heterozygosity were 0.84 (0.018) and 0.69 (0.007) (Table 1). The Awassi