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Genetic diversity and structure of goats within an early livestock dispersal area in Eastern North Africa

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In this study we genotyped 14 microsatellites to assess genetic diversity, population stratification and demographic dynamics using Egyptian local goats (Zaraibi, Baladi, Saidi and Barki) and the Shami (Damascus) goat from the Middle East and West Asia close to the geographic center of goat domestication. We observed high levels of allelic and genetic diversity that was partitioned into six gene pools. Cluster analyses separated Zaraibi and Shami, which were identified with independent gene pools of potential ancestral backgrounds. The analyses provided further evidence for extensive sharing of genetic variation, revealing, varying levels of admixture among the study populations. This finding was supported by AMOVA analysis, which indicated that the proportion of genetic variation due to differences among populations was 7.06%. Our results most likely indicate that multiple waves of introduction of diverse gene pools and recent flock intermixing has created and maintained a unique set of caprine biodiversity in Eastern North Africa emphasizing the importance of the region as one of the hotbeds of African animal biodiversity.

Key words: Admixture, Bayesian clustering, bottleneck, expansion, Egypt.

INTRODUCTION

The goat (*Capra hircus*) was the first livestock species to be domesticated for purposes of human consumption. Archaeological evidence points to two possible centers of goat domestication; one in the Euphrates valley in southeastern Anatolia dating around 10,500 years before present (YBP) (Peters et al., 2005) and the other in the Zagros mountains dating to 9,900 to 9,500 YBP (Zeder and Hesse, 2000; Zeder et al., 2006). The occurrence of this phenomenon, close to the geographic center(s) of the first known ancient civilizations (Mesopotamia, Egypt, Indus valley etc) ensured a direct and tight connection between goats and most aspects of human socio-cultural

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and economic life (Boyazoglu et al., 2005). Today, there are more than 300 breeds of goats found in diverse agro-ecologies (Luikart et al., 2006) where they are a critical component of the agro-biodiversity.

Eastern North Africa (Egypt and Sudan) and the Maghreb (Algeria, Morocco, Tunisia and Libya) are important regions in the history of African goats. Radiocarbon dates suggest a rapid dispersal of goats to Africa from Southwest Asia via two routes; one along the North African Mediterranean Sea coast, and the other via the Red Sea Hills region of the Egyptian Red Sea coast (Zeder, 2008). These routes have been corroborated by mtDNA data which also support multiple waves of introduction (Naderi et al., 2008). Wetterstrom (1993) suggested a third and more recent terrestrial route via the Sinai Peninsula and Nile Delta into the Nile Valley. The genetics of local goats in Eastern North Africa and the Maghreb may have been influenced by multiple waves of introduction of goats from different genetic backgrounds and therefore are important in understanding the genetic foundation, demographic dynamics and evolutionary history of African goats.

Goats have contributed significantly to Egypt's gross national livestock product since approximately 5000 BC (Galal et al., 2005). The local goats are raised as multipurpose animals, have remained nondescript since ancient times and, a systematic assessment of their inherent genetic variation remains to be done. Nevertheless, six breeds are currently recognized, three main (Baladi, Barki, Zaraibi) and three minor (Wahati, Saidi, Black Sinai) ones, respectively (Galal et al., 2005). Some local goat breeds have been of particular interest due to their recognized features and characteristics. The Barki goat derives its name from Barka region in Libya where it is common and possibly derived from. It occurs in the Northwestern coastal desert where it is known as "Saharawi" or "of the desert"; due to its excellent adaptation to hot arid desert conditions (Galal et al., 2005). The Baladi (synonym to local or indigenous) is the native goat of Egypt. It shows high phenotypic variation among subpopulations from different agro-ecologies. It is widespread in the Nile Delta and along the Nile Valley where they are called "Local" or "Sharkawi" while in southern Egypt, they are called "Saidi". The Zaraibi is regarded as the most promising goat breed in Egypt. It occurs within a restricted geographic area in the fringes of the Northeastern Nile Delta. It is also called "Nubian" or "Nubi", after the Nuba area of Southern Egypt, where it is presumed to originate from. It is also presumed to be the progenitor of the Anglo-Nubian breed. However, these presumptions were disputed in interviews with flock owners during sampling. The Saidi is found in Southern Egypt. Phenotypically it resembles the Baladi, except that it has a larger head and body. It has a better tolerance to high temperatures due to the introgression of Sudanese goats from the South of Egypt. This introgression however, remains a matter of speculation.

Although the intensification of livestock production in the second half of the 20th century saw the widespread introduction of exotic breeds in the tropics and subtropics, the inhospitable desert conditions in Egypt, precluded the introduction of such breeds and favored the proliferation of better adapted local populations. The gene pool of local goats has therefore remained in its "pure unadulterated form" and local goats are so far the only ones that are found, and, have been described in Egypt (Galal et al., 2005). However, breeds/populations are not static entities and in the absence of stringent artificial selection, populations evolve and diverge over time to fit the diversity of local environments. Traditional management systems, (transhumance, nomadic pastoralism) as well as commercial and socio-cultural exchanges provide opportunities for intermixing of flocks from different regions and genetic backgrounds.

This study was undertaken to obtain an understanding of the degree and pattern of genetic variability among local goats from Egypt as a window to understanding the diversity of African goats. For this purpose, 14 microsatellites were genotyped in 221 individuals to: (1) Assess the within- and among population genetic diversity and (2) Investigate population structure and extent of admixture between Egyptian local goats and between them and the Shami (Damascus) goat, found across the Middle East and West Asia close to the geographic centre(s) of goat domestication.

MATERIALS AND METHODS

Sample collection and DNA extraction

We sampled 163 animals from four Egyptian goat populations (Table 1). We also sampled 58 individuals of Shami goat, which is native to the Middle East (Syria, Turkey, Lebanon, Jordan, Israel, and the Palestinian territories). The five study populations belong to the Lop-eared goat group (Porter, 2002). Similar types also occur in North Africa, the Western Mediterranean region, the Middle East, the Arabian Peninsula and the Indian subcontinent (Porter, 2002). Sampling of Egyptian goats was approval by, and analysis was done within the research premises of the Animal Production Research Institute (APRI) as the National Focal Point of animal genetic resources of Egypt.

The Shami individuals were sampled in Syria; 29 each from Hmemeh Shami Goat Research Station in Aleppo and Karahta Shami Goat Research Station in Damascus. Nine individuals of Zaraibi were sampled from a research station of the Animal Production Research Institute where selection for milk production and fecundity traits is done. The rest of the Zaraibi (31) together with the other Egyptian goat populations were sampled from farmers' flocks. In these flocks, veterinary health care and animal nutrition management are rarely practiced, mating is uncontrolled and performance recording and artificial selection are rare. Two mature animals were sampled per flock; and two flocks were sampled per village to avoid closely related individuals. All samples were collected in form of total blood with EDTA as the anticoagulant. Genomic DNA was extracted from whole blood using DNeasy® Blood and Tissue Kit (Qiagen GmbH, Germany). DNA concentration and purity were assessed using the BioPhotometer Plus (Eppendorf, GmbH, Germany).

Table 1. Indicators of allelic and genetic diversity in Egyptian and Shami goat populations analyzed using 14 microsatellite markers.

Population	N	TNA	Allelic diversity				Genetic diversity			Number of loci deviating from HWE
			AR (SD)	MNA (SD)	ENA	P_A	He (SD)	Ho (SD)	F_{IS}	
Zaraibi	40	137	7.89 (1.837)	9.79 (2.83)	4.82 (2.00)	9	0.76 (0.035)	0.64 (0.021)	0.16***	4
Baladi	28	127	8.27 (2.276)	9.07 (2.84)	5.37 (1.97)	3	0.81 (0.021)	0.65 (0.027)	0.20***	2
Saidi	47	150	8.55 (2.271)	10.71 (3.47)	5.99 (2.60)	5	0.81 (0.026)	0.67 (0.019)	0.17***	4
Barki	48	146	8.28 (2.235)	10.43 (3.39)	5.32 (2.29)	7	0.78 (0.031)	0.67 (0.019)	0.14***	1
Egyptian goats	163	190	9.26 (2.171)	13.57 (4.48)	6.70 (2.64)	24	0.82 (0.022)	0.66 (0.010)	0.20***	
Shami	58	143	8.04 (2.065)	10.21 (3.53)	5.56 (2.16)	11	0.79 (0.029)	0.71 (0.017)	0.11***	6
Overall	221	201	9.53 (2.232)	14.36 (4.58)	7.22 (2.76)	35	0.84 (0.021)	0.67 (0.009)	0.19***	

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. Significant difference was at P<0.001.

DNA amplification and genotyping

We genotyped 14 autosomal microsatellites out of the 30 recommended by the ISAG/FAO Panel on Domestic Animal Genetic Diversity (Table S1). The microsatellites were amplified in two multiplex PCR reactions each containing 100 to 150 ng DNA, 1X Platinum® Multiplex PCR Master Mix (Lifetechnologies, USA) and 10 pM of each primer in 25 ul reaction volumes. The thermal profile was as recommended by the ISAG/FAO Panel and was run on a C1000 Thermal Cycler (Biorad, USA). Genotyping was performed with the ABI3500 Genetic Analyzer (Lifetechnologies, USA) using GeneScan™ 600 LIZ® (Applied Biosystems) internal lane size standard. Allele size calling and binning were carried out with GeneMapper v3.5 (Applied Biosystems).

Statistical analyses

Total number of alleles (TNA), mean number of alleles (MNA), allelic richness (Ar) standardized for a minimum of 16 diploid individuals per population, effective number of alleles (ENA), number of private alleles (Pa) and expected (He) and observed (Ho) heterozygosity, as well as, nuclear pairwise F_{ST} values corrected for multiple testing, were calculated from allele frequencies with FSTAT 2.9.3.2 (Goudet, 2001), MICROSATELLITE TOOLKIT (Park, 2001) and POPGENE 1.31 (Yeh et al., 1997). Genetic inbreeding coefficients F_{IS} (Weir and Cockerham, 1984) were inferred in FSTAT 2.9.3.2. A nuclear AMOVA implemented in ARLEQUIN v3.11 (Excoffier and Lischer, 2010) was used to estimate and partition genetic variation within and among breeds.

The Bayesian clustering algorithm implemented in STRUCTURE v2.3.3 (Pritchard et al., 2000) was used to assess the genotypic composition of the genetic backgrounds of the populations analyzed and proportion of mixed ancestry. We performed 140,000 iterations following a burn-in of 70,000 Markov Chain Monte Carlo replications with an admixture model that allowed for correlation among allele frequencies. Ten independent simulations for each K (1 - 10) were performed to identify the most probable clustering solution by examining the modal distribution of Delta K (Evanno et al., 2005). Graphical representations of these statistics were obtained with STRUCTURE HARVESTER v0.68 (Earl and von Holdt, 2012). The outputs from multiple runs for each K were concatenated with CLUMPP (Jacobsson and Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004) was used to display the assignment probabilities. To further investigate individual clustering profiles, we carried out the multivariate based Discriminant Analysis of Principal Components (DAPC) with ADEGENET v1.3-9.2 (Jombart, 2008) in R 2.15.3 (R Development Core Team, 2006). DAPC is a non model-based method provides an efficient description of genetic clusters using the discriminant functions. This multivariate analysis seeks linear combinations of the original alleles, which show linear combinations of the original alleles, maximizing differences between pre-defined clusters and minimizing variation within clusters. Based on the retained discriminant functions, the analysis derives probabilities for each individual of membership in each of the cluster. This coefficient can be interpreted as “genetic proximity” of individuals to the different clusters. These coefficients provide an “assignment measure” of individuals to predefined clusters, comparable with ancestry value derived by the structure analysis. For this analysis, we ran *K* between 1 and 40 and inferred its most optimal value using the Bayesian Information Criterion (BIC) statistic generated with DAPC in ADEGENET. We also constructed a neighbor joining (NJ) tree of phylogenetic relationships of individuals with POPULATIONS 1.2.32 (Langella, 2002) using the allele sharing distance (DAS) with 1000 bootstrap replications over loci.

We inferred excess/deficiency of nuclear heterozygosity to search for signals of population decline with BOTTLENECK 1.2.0.2 (Cornuet and Luikart, 1997) applying 1000 replications. We performed the evaluation using the stepwise mutation (SMM) and two-phase (TPM) models of microsatellite evolution. We set the proportion of SMM and its variance to 85 and 12% respectively. The significance of the tests was assessed using Wilcoxon sign-rank test (Piry et al., 1999). The mode-shift indicator test, although not a statistical test *per se*, was also performed because stable populations are expected to show larger proportions of alleles at low frequency (Cornuet and Luikart, 1997). We used the intra-locus kurtosis test (*k*-test) and the inter-locus variance test (*g*-test) (Reich and Goldstein, 1998; Reich et al., 1999) to

search for signatures of population expansions. Both tests (*k* and *g*) were performed using the macro program 'KGTESTS' (Bilgin, 2007) implemented in Microsoft Excel®.

RESULTS AND DISCUSSION

Allelic and genetic diversity

Measures of allelic and genetic diversity computed across 14 loci for each population are shown in Table 1. MNA per population had an average value of 14.36 ± 4.58, ranging from a minimum of 9.07 ± 2.84 (Baladi) to a maximum of 10.71 ± 3.47 (Saidi). The effective number of alleles was very similar in the populations studied with means ranging from 5.32 ± 2.29 (Barki) to 5.99 ± 2.60 (Saidi), except for the Zaraibi, which had a mean of 4.28 ± 2.00. The ratio between the effective and mean number of alleles per population ranged from 0.492 (Zaraibi) to 0.592 (Baladi) indicating that the distribution of allele frequencies had a minimal difference in the populations studied. The number of loci with exclusive alleles was highest in Shami (11) and lowest in Baladi (3) while the proportion of loci not in HWE was highest in Shami (6) and lowest in Barki (1). H_e had a mean value across populations of 0.84 ± 0.021 with the lowest mean in Zaraibi (0.76 ± 0.035) and the highest in Baladi and Saidi (0.81). H_o ranged between 0.64 ± 0.021 (Zaraibi) and 0.71 ± 0.019 (Shami) with a global mean of 0.67 ± 0.009, and the mean allelic richness was 9.53 ± 2.23. The mean expected heterozygosity that we observe is higher than that reported for different goat breeds and populations. They ranged from an average of 0.52 in Southeast Asian populations (Barker et al., 2001) and 0.59 in Swiss breeds (Saitbekova et al., 1999) to 0.82 in Chinese breeds (Qi et al., 2009). It is higher than the mean value of 0.69 reported for a diverse group of goats from Europe and the Middle East (Canon et al., 2006). In particular, the average allelic and genetic diversity found in our work exceeds that reported for a caprine gene pool from the geographic center of goat domestication in Iran and Pakistan (Di et al., 2011; Vahidi et al., 2014). This result was unexpected because genetic diversity, for most livestock species, tends to be negatively correlated with geographic distance from the center of domestication (Groeneveld et al., 2010; Wiener and Wilkinson, 2011). Pereira et al. (2009) reported high maternal (mtDNA) and paternal (Y-chromosome) genetic diversity among goat populations from Northern Africa. Together with our findings, these results suggest that northern Africa most likely witnessed the introduction of a diverse gene pool of goats from Southwest Asia which created a large caprine biodiversity in the region which still exists today.

A significant ($P \leq 0.001$) of F_{IS} was observed in all the populations studied ranging from 0.11 (Shami) to 0.20 (Baladi) with a mean value across populations of 0.19. Overall, these results indicate that even though the within-population H_e and H_o were not widely different, the deficit found in within-population heterozygosity (F_{IS}) was different among the populations. Inbreeding detected in the study populations is very likely to be relevant to population management and conservation. In spite of attempts to avoid sampling closely related individuals, high significant positive F_{IS} values (range = 14 to 20%) was detected indicating heterozygote deficiency confirming that the populations are not entirely panmictic. This high level of inbreeding is not unique to Egyptian goats. The values fall within the range of 4.5 and 29.3% observed in several goat populations from Europe and the Middle East (Canon et al., 2006). With several flocks sampled per population for the 4 Egyptian goats breeds, this could have resulted in fine-scale genetic substructure (Wahlund effect) reflected in positive F_{IS} values. In addition, retaining breeding animals from within-the-flock individuals is a common practice of the Egyptian shepherds which, is very likely, resulted in heterozygosity reduction, in a process called "in-favour-homozygotes selection" (Maudet et al., 2002). In the absence of written records, flock owners most likely are unable to recall accurately the long-term pedigree of their animals. Kugonza et al. (2012) observed that Ankole cattle keepers could correctly assign first-degree relatives more easily than they did for second- and third-degree relatives. Furthermore, herd sizes constrained the number of kinship assignments that could be remembered accurately based on memory recalls. For the Shami goats, as they were sampled from 2 closed governmental farms, in which, breeding of ancestrally related animals is highly expected to occur. Both Wahlunds effect and in-favour-homozygotes selection for long time periods may be the likely cause of the positive F_{IS} values.

Population structure and differentiation

The proportion of shared alleles between individuals was used to construct a NJ dendrogram (Figure 1a). The dendrogram shows that only Zaraibi and Shami are each defined by two clades. Although Baladi also separates into two clades, some of its individuals segregate into other clades as well. Individuals of Barki and Saidi do not separate into clear identifiable clades.

The possible ancestral gene pools underlying the observed genetic diversity were assessed with STRUCTURE and DAPC. As inferred by the method of Evanno et al. (2005), within the range of the number of clusters tested, $K = 1 - 10$, the most likely number of gene pools that contribute to the observed genetic variability in the five populations studied is $K = 6$ (Figure S1a). The contributions of the detected gene pools to the five study populations are graphically presented in Figure 1b. At $K = 6$, Shami and Zaraibi are each identified with two different gene pools. Respectively, the contributions of the two gene pools observed in Shami are 48.43 and 39.44%,

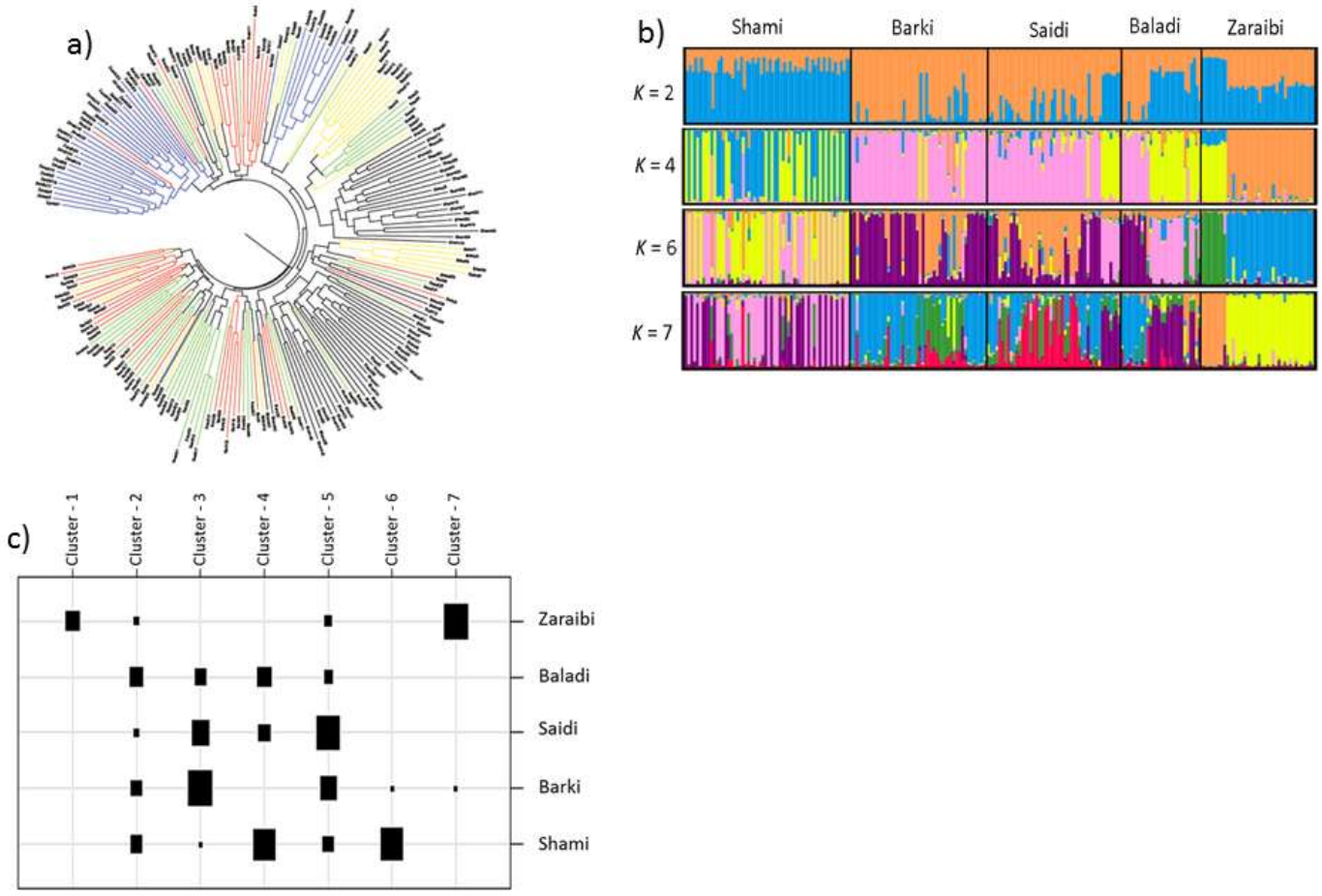

Figure 1. Individual cluster analysis. **(a)** NJ tree cluster of individuals (colour code: Blue = Zaraibi, Yellow = Baladi, Green = Saidi, Red = Barki, Black = Shami). **(b)** individual assignment probabilities generated from STRUCTURE. **(c)** Assignment of individuals to seven clusters based DAPC analyses.

while the contributions of the two gene pools observed in Zaraibi to its genetic makeup are 70.71 and 21.59% respectively. Variable frequencies of four ancestral gene pools define the genetic composition of Barki, Saidi and Baladi. No clear distinctions can be established between these three populations based on the proportions of the four gene pools. One of the gene pool observed in Shami occurs in Saidi and Baladi with a frequency of 14.30 and 41.91% respectively. The Baladi also shares one of the gene pool that is common to Barki and Saidi. This gene pool contributes 29.94, 64.16 and 37.24% of the genetic composition of Baladi, Barki and Saidi respectively. One last gene pool is observed in Barki and Saidi only, at a frequency of 24.77 and 38.88% respectively.

The BIC statistic generated by DAPC indicates that the optimal number of clusters in the data set is $K = 7$ (Figure S1b and c), showing one extra cluster generated by DAPC more than those created by STRUCTURE. Both analyses confirm that the Egyptian goats are admixed. For comparison between the analysis of the two approaches, results correspond to those of STRUCTURE at $K = 7$ are presented (Figure 1b). These results (STRUCTURE, DAPC and NJ) show extensive sharing of genetic variation among Egyptian local goats and between them with Shami. The exception is Zaraibi, which is the most genetically distinct. We therefore took $K = 6$ to represent the optimal number of gene pools that define the genetic backgrounds of the five populations; green and blue (Zaraibi), orange, pink and purple (Baladi, Barki and Saidi), and pink and yellow (Shami).

The results generated by STRUCTURE, DAPC and NJ tree were consistent with those of AMOVA which show 7.10% of the neutral autosomal genetic variation is explained by genetic differentiation between populations and 79.19% is explained by differences within individuals. Excluding Shami from the analysis reduced the variation between Egyptian populations to 6.22%. Excluding Zaraibi, which has two different gene pools, but retaining Shami, reduced the variation between populations to 6.04%. However, excluding both Shami and Zaraibi, based

on the results of STRUCTURE/DAPC/ NJ, reduced the variation between populations to 4.27%. These results indicate that Shami and Zaraibi contribute significantly to the variation present between populations.

F_{ST} values were significantly different from zero in all pairwise comparisons (Table S2). They ranged from 0.032 between Saidi and Barki (which had two common gene pools; Figure 1b and c) to 0.101 between Zaraibi and Shami (which had the most distinct gene pools; Figure 1b and c).

The estimated global F_{ST} which corresponds to the proportion of genetic variability accounted for by differences among populations was 0.071 ± 0.016 indicating that genetic diversity quantified by the neutral autosomal microsatellite markers show little differentiation among the populations analyzed. This value falls within the range of values that have been observed by most studies on goats where F_{ST} ranged between 0.04 (Sechi et al., 2005) and 0.11 (Glowatzki-Mullis et al., 2008). Vahidi et al. (2014) reported an average value of 0.062 ± 0.016 for goats from Iran and Pakistan where goat domestication took place. This comparison however depends on the level of genetic divergence of the populations analyzed. For instance, among well-defined and distinct Swiss goat breeds, the global F_{ST} value was 0.17 (Saitbekova et al., 1999) while in the study by Canon et al. (2006) it was 0.069. The latter is a lower level of genetic differentiation considering that the study analyzed 45 breeds and populations from across Europe and the Middle East. The low level of genetic differentiation in our study populations was confirmed by the poor clustering of individuals on the NJ tree (Figure 1a) generated with allele sharing distance where Zaraibi and Shami grouped in separate clusters. This low level of population differentiation amongst the Egyptian goats can be a result of either common origin as suggested by Naderi et al. (2007) or past admixture among different ancestral genetic stocks. It could also be due to extensive translocation of goats in the recent past following human movements and migrations or as commercial trading items. This has facilitated gene flow among populations and homogenized the caprine gene pool (Luikart et al., 2006; Naderi et al., 2007).

The analysis with STRUCTURE and DAPC confirmed Zaraibi and Shami, which were identified with independent clusters of potential ancestral gene pools, as genetically distinct. For the other three populations, there was clear evidence of genetic admixture, which is the result of variable contributions from four different ancestral gene pools. This result is compatible with the low genetic differentiation between Egyptian populations. It has been suggested that the Zaraibi originates from the Nuba area of southern Egypt and, based on its phenotypic characteristics, has been proposed to be the progenitor of the Anglo-Nubian breed (Galal et al., 2005).

The alternative suggestion, from flock owners, is that Zaraibi was introduced to its current location from around the Mediterranean region and has been maintained ever since as a small flock. We observe no common gene pool between Zaraibi and the other three Egyptian breeds, especially Saidi from southern Egypt, which would otherwise support an origin from southern Egypt. Our results therefore, do not support southern Egypt as a source of Zaraibi. Dispersal from areas around the Mediterranean Sea remains a possibility. Analysis of goats from the Mediterranean countries would be necessary to test this suggestion in view of findings from the analysis of various markers (mtDNA, Y-chromosome and microsatellites) which indicate that bidirectional movement of goats, sheep (Pereira et al., 2005; Canon et al., 2006) and cattle (Cymbron et al., 2005; Anderung et al., 2005) were common between northern Africa and Iberia in medieval times. The sub-clusters observed in Zaraibi and Shami (Figure 1a and b) most likely reveal the effects of reproductive isolation and breeding strategies. Thirty-one samples of Zaraibi, which form the main cluster, came from farmers' flocks while nine, which formed the minor cluster, came from a research station. This also reveals variation in breeding goals between Egyptian Zaraibi holders in East-Northern Delta and research farm. Research farms follow breeding program for genetic improvement of milk production and twinning trait in Zaraibi goats. Meanwhile, Zaraibi goats are found as household goats in the East-Northern delta, where they are kept as a hobby rather than production animal (DAGRIS, http://dagris.ilri.cgiar.org/display.asp?ID=876). Shami were sampled from two separate research stations in Syria and the clustering pattern clearly mimics these two flocks. The reproductive isolation of these two closed flocks, their different breeding management strategies, and the very likely utilization of ancestrally related animals for breeding may explain the clear genetic substructure we observe in the two breeds.

We detected signatures of introgression of Shami in Saidi and Baladi in the form of a shared gene pool (Figure 1b and c). There is no report of crossbreeding Shami with farmers' flocks other than for breeding trials in research stations (Abdelsalam et al., 2000). If the introgression was the result of crossbreeding, we expected to observe the gene pool in Barki as well. This result either reveals the introgression of Shami into Saidi and Baladi prior to their arrival in Eastern North Africa or, a more likely, a common ancestral background between the three populations. This background is gradually being lost in Egyptian populations due possibly to purifying selection and/or genetic drift. It however continues to persist in the Shami, a breed that is found in the Middle East within the proximity of the geographic center(s) of goat domestication. We speculate that the gene pool may still be maintained in the Middle East because it confers a selective advantage in the region.

One gene pool was detected in Barki, Saidi and Baladi while another was observed only in Barki and Saidi. The proportion of these gene pools in the three populations

was variable revealing an admixture trend among Egyptian goats. This may explain their high level of diversity and low differentiation due to either common origin or past admixture among populations of different genetic backgrounds. The common origin could be the hypothesis of choice considering the results of Vermeersch et al. (1994), reporting evidence that domesticated goats appeared in Southern Egypt and Northern Sudan earlier than in Northern Egypt. Therefore, it is likely that Saidi (Southern-Egypt breed) is the common origin of the other Egyptian populations. In addition to high genetic diversity, local goats in Africa and Asia are characterized by extensive phenotypic variation. The traditional management systems still in use today are defined by the absence of stringent artificial selection and by uncontrolled breeding management. These practices may have contributed to the high genetic variation we observe within these populations (Hassanane et al., 2010).

Population dynamics and demographic inferences

We performed four tests to investigate demographic dynamics and trends. We detected significant deficit in heterozygotes ($P \leq 0.05$) under the SMM model but neither a significant deficit nor excess in heterozygotes ($P \geq 0.05$) was detected under the TPM model implemented in BOTTLENECK. The graphical representations of allele frequencies revealed a normal L-shaped distribution. The *k*-test revealed several loci with non-significant ($P > 0.05$) negative *k* values; the *g*-test values were also not significant ($P > 0.29$) (Table S3). These results are characteristic of stable populations under mutation-drift equilibrium. Therefore, *KG*-tests results did not reveal significant patterns of heterozygote excess, and the mode-shift test displayed an allele frequency distribution characteristic of non-declining populations. These results do not support evidence of a recent genetic bottleneck. The *kg*-tests show all the populations to be at demographic equilibrium; none has experienced any expansions in effective population sizes in the recent past. One migrant per generation can counteract the effects of isolation and genetic drift (Mills and Allendorf, 1996). Considering the history of goats and the results of STRUCTURE, we cannot describe the Egyptian goats as being reproductively isolated from each other but, rather, would expect continuous gene flow among them. For the Zaraibi and Shami, in spite of being reproductively isolated from each other and from the other Egyptian populations, from a genetics point of view, they have not reached a critical threshold for them to be considered threatened.

Conclusion

Overall, our study indicates that within Egyptian goats, genetic diversity is high but genetic differentiation among populations is low. Although inbreeding is high, the populations analyzed are at genetic equilibrium. The observation of extensive admixture reinforces the importance of Eastern North Africa as bedrock of African caprine biodiversity while the observed enrichment of the goat gene pool in the area emphasizes the importance of the region in the history of African livestock. Knowledge on population stratification and the distribution of genetic variability within and among breeds, populations and strains are important in formulating strategies for maintaining genetic diversity, in understanding the evolutionary history of breeds and populations and, in generating insights into the history of human populations. Results suggest that northern Africa most likely witnessed the introduction of a diverse gene pool of goats from Southwest Asia that created a large caprine biodiversity in the region, which still exists today. Results clearly indicated that the indigenous goat populations before Barki, Baladi and Saidi) have been admixed and Saidi goat (Southern-Egypt) could be the common origin of other indigenous populations. Zaraibi goats are very likely to be Mediterranean-originated rather than Southern-Egyptian, as it lacks common genetic backgrounds with any of the studied indigenous goat breeds. For the animal genetic resource community in Egypt, it recommended to consider variation between Zaraibi research farm under genetic improvement practices and smallholdings in East-Northern Delta region. Baladi seems to be the most admixed population in Egypt, which also needs to be considered in any plan for genetic conservation and utilization. In spite of the different phenotypes and geo-ecological distribution of Barki and Saidi breeds, they have common genetic backgrounds. The two breeds therefore, need further investigation, probably using high-density genotyping approach.

Conflict of Interests

The authors have not declared any conflict of interests.

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Table S1. Data on microsatellite primers used in the study; microsatellite, minimum and maximum allele size, and total number of alleles detected, and multiplex in which the primer was used for full-size amplification.

Microsatellite	Allele size range		Total number of alleles detected	PCR multiplex
	Minimum	Maximum		
HTC	264	300	18	
ILSTS19	138	154	9	
INRA05	114	126	7	First
SRCRAP05	153	187	17	multiplex
SRCRSP08	202	247	20	
SRCRSP24	133	167	18	
CSRD247	217	245	15	
ILSTS87	133	151	10	
INRA023	191	215	13	
INRA063	163	183	11	Second
MAF065	113	161	22	multiplex
MCM527	148	166	10	
OarFcB20	87	111	13	
SRCRSP23	75	119	18	

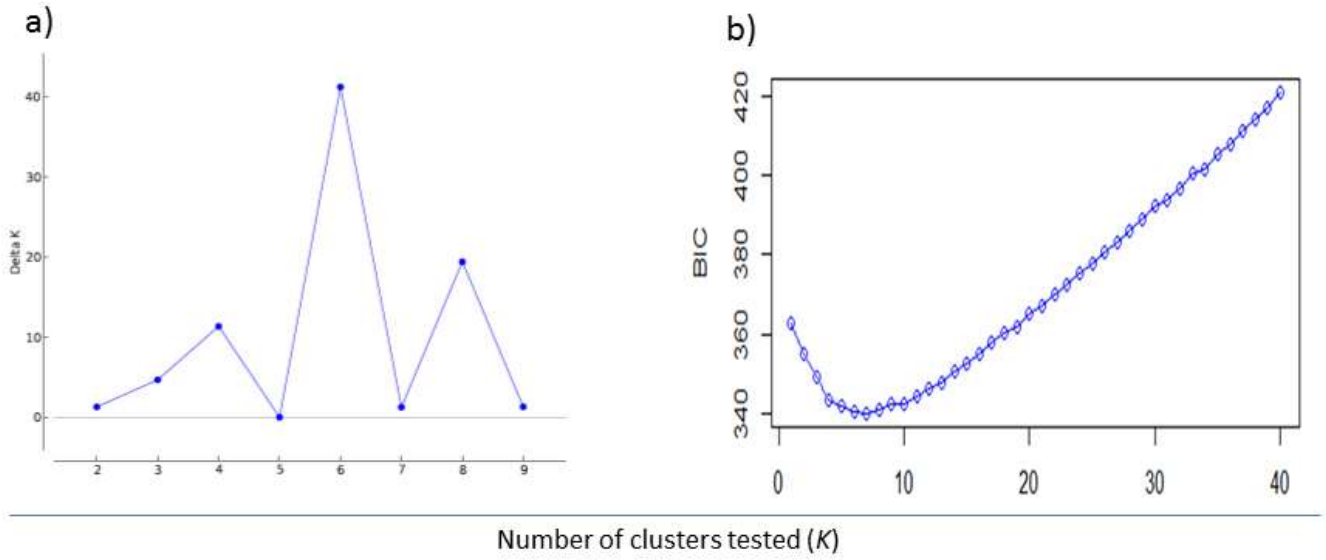

Figure S1. Distribution of (a) Delta *K* and (b) BIC values for Egyptian goats.

Table S2. Estimated pairwise F_{ST} as a measure of genetic differentiation among Egyptian and Shami goats.

Population	Zaraibi	Baladi	Saidi	Barki	Shami
Zaraibi	-	0.092*	0.068*	0.089*	0.101*
Baladi		-	0.049*	0.059*	0.077*
Saidi			-	0.032*	0.062*
Barki				-	0.076*
Shami					-

Significant difference was at $P < 0.05$.

Table S3. KG-tests results for the five populations analyzed in the current study.

Population	Number of loci with negative K value	K-test (P-value) (intra-locus)	g-test value (inter-loci)
Zaraibi	8	0.352012	0.818576
Baladi	5	0.890482	0.390459
Saidi	7	0.560168	0.329173
Barki	9	0.180484	0.32936
Shami	5	0.890482	0.412952
Overall	8	0.352012	0.332722