# GENETIC VARIATIONS OF TWO EGYPTIAN GOAT BREEDS USING MICROSATELLITE MARKERS

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#### SUMMARY

Two native Egyptian goat breeds: Baladi and Zaraibi were genetically analyzed using three microsatellite markers: INRA005; ILSTS005 and ILSTS087. The results showed that all the microsatellites studied were polymorphic and gave 6, 5 and 8 alleles respectively. The overall heterozygosity and polymorphism information content (PIC) values were 0.9 and 0.755 indicating the presence of high genetic diversity. In the two breeds, low inbreeding rates was observed (mean FIS= -0.114) within the breeds. Genetic differentiation between breeds was moderate with a mean FST value of (4.593). Deviations from Hardy-Weinberg equilibrium were noted for most of the loci. The clusters obtained on the phylogenetic tree generated from Nei's genetic distance matrix agreed with the geographic origin of the breed. The time of divergence in Zaraibi and Baladi breeds was estimated to be 71 generations (284 years). The two breeds showed high degree of admixture. The study analyzed the population structure of these two breeds and contributed to the knowledge and genetic characterization of the Egyptian native goat breeds (Baladi and Zaraibi). In addition, the microsatellites recommended by ISAG proved to be useful for the biodiversity studies in Egyptian goat breeds. Due to the polymorphism observed, the three microsatellites studied could be fruitfully used in paternity tests. Moreover, they could be employed for further researches on mapping quantitative trait loci (QTL) detection and subsequently marker assisted selection (MAS) breeding programs.

Keywords: Genetic variation, Egyptian goats, Baladi and Zaraibi, Microsatellites

#### INTRODUCTION

The goats are hollow-horned ruminants belonging to the mammalian order Artiodactyla, sub- order Ruminantia, family Bovidae and either of the genera Capra or Hemitragus (Herre and Rohrs, 1973). Together with sheep they constitute the tribe Caprini, but the differentiation between goats and sheep is well established.

The Egyptian goats are believed to be domesticated after migration from Asia along the present Iran-Iraq borders then to Africa (Mason, 1981). Rock drawings of goats have been found in Aswan, dated to the old or the Middle Kingdom of Egypt. Those goats were characterized by scimitar-shaped horns, erect ears, a convex facial profile and absence of a beard (Mason and Maule, 1960; and Epstein, 1971). Domestic goats can be traced to Egypt as far back as the Badarian age when goats

were used extensively for meat and their skins were quite often used to wrap the dead. The origin of domestic animals in Africa, including goats has been described and about 70 different breeds and varieties were recognized (Epstein, 1953; Mason and Maule, 1960; and Epstein, 1971).

In Egypt, there are 3.13 million goats raised mainly in three regions: the Nile Delta, Upper Egypt and in the desert rangelands, particularly in the north-west coastal zone. Production systems and breeds in the three zones are different. There are about 700,000 goats in the Nile Delta, where agriculture is very intensive. Goats are usually found in small holdings as mixed flocks with sheep and other farm animals like cattle and buffaloes, and are mainly kept as household dairy animals. In Upper Egypt, which is characterized by mild, dry winters and very hot summers, agriculture is less intense. There are about 1.7 million goats, mainly in mixed flocks, with some goats kept as household animals. In the desert rangelands, 1.4 million sheep and goats are kept in extensive systems (Galal et al., 2005). The Egyptian goats are classified into several breeds differing in color, size, and other morphological features, such as Zaraibi, Baladi, Sinawi or Bedouin, Barki and Saidi.

During recent years, a great interest is focused towards the use of molecular markers in understanding the animal genome and genetic diversity analysis. Molecular markers have been widely used to assess genetic variation since they provide information on every region of the genome. Among the molecular markers, microsatellites are currently most used markers. Microsatellites have been used in studying genetic variability in all domestic species of animals, birds and even fish. Genetic variability within and among populations is often of importance and may contribute to the selection and preservation of genetic resources. Microsatellites, segments of the nuclear genome composed of tandem repeat of short-sequence motifs, have become excellent candidate markers for genetic studies, since they are numerous, highly variable and easy to score.

Best to our knowledge, only one study conducted by Agha et al. (2008) dealt with the microsatellite typing for assessment of the genetic diversity between Egyptian and Italian goat breeds.

The aim of the present study was to analyze the genetic variations within and between Baladi and Zaraibi goats, moreover to evaluate the genetic relationships among them.

# MATERIALS AND METHODS

# Blood samples:

Individual blood samples were drawn from 20 non-relative goats of each breed with a total number of 40 animals, the sample were collected from both sexes. The samples were collected from Baladi breed located in the research farm of the Department of Animal Production, Faculty of Agriculture. Fayoum University, El-Fayoum, and from Zaraibi breed located in the Agriculture research station (El-Serow, Domiatta) of the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture.

#### DNA extraction, PCR and electrophoresis:

Genomic DNA was extracted using standard salting out procedure (Miller et al., 1988). Three microsatellite markers were chosen according to a joint meeting

recommendation between the International Society of animal genetics (ISAG) and FAO (1998) for genetic diversity studies. Details of these markers are in the Table 1:

Table 1. Used microsatellites information

Microsatellite	Chromosomal location	Primer sequence	Reference
ILSTS087	` 6	AGC AGA CAT GAT GAC TCA GC CTG CCT CTT TTC TTG AGA G	Steffen et al. (1993)
ILSTS005	10	GGA AGC AAT TGA AAT CTA TAG CC TGT TCT GTG AGT TTG TAA GC	Steffen et al. (1993)
INRA05	12	CAATCTGCATGAAGTATAAATAT CTTCAGGCATACCCTACACC	Vaiman et al. (1994)

Polymerase chain reaction (PCR) was carried out on 50 ng of genomic DNA in a 25 µl reaction of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 µM dNTP, 1.5 mM MgCl<sub>2</sub>, 1 mM tetra-ammonium-chloride, 0.1% Triton X-100, 0.01% gelatin, 4.5 pmol of each primer and 0.25 U *Taq* DNA polymerase. The standard PCR run cycle was usually as: primary denaturation at 95°C for 3 min., then: 35 cycles at 95°C for 15 sec., 55-60°C for 30-60 sec.; 72°C for 30 sec. Final extension: 72°C for 5 min., Storage 15°C forever. The success of the PCR was detected on 2% agarose after running in horizontal electrophoresis set and staining with ethidium bromide. For optimization of PCR, the temperature and time of annealing were changed.

The products of the successful PCR were characterized under denaturing conditions on 12% polyacrylamide vertical electrophoresis (Sambrook et al., 1989). By the end of the run, the gel was stained in an ethidium bromide solution (0.5 Tig/ml TBE buffer). The gel image was captured electronically using Biometra gel documentation system. The allele sizes were determined using free software named Lab. image V2.7. It is dispersed free from the web site of Proland company (Germany). <a href="http://www.labimaging.com/servlet/engine/home/start.html">http://www.labimaging.com/servlet/engine/home/start.html</a>

### Statistical analysis of the results:

POPGENE software package (Yeh et al., 1999) was used to calculate allele frequencies, observed number of alleles, effective number of alleles (Kimura and Crow, 1964), observed (Ho) and expected (He) heterozygosity at each locus in both breeds. Polymorphism information content (PIC) value for each locus was calculated using the method of Bostein et al. (1980). Pair-wise allele sharing was calculated manually from the raw results.

Using the variance-base method of Weir and Cockerham (1984), population differentiation by F-statistics was computed using FSTAT, version 2.9.3.2, computer program (Goudet, 2002). Means and standard deviations of the F-statistics program, F.f, that are analogue to Wright's (1951, 1978), Fis and Fst, were obtained across breeds by the Jackknifing procedure over loci (Weir, 1990). The extent of global inbreeding was further studied with the same software by estimated Fis value.

The effect of migration and gene flow on the genetic structure of the analyzed populations was estimated between each pair of populations according to an island model under neutrality and negligible mutation (Slatkin, 1985). Genetic distances among populations were estimated using (Ds) standard genetic distance of Nei (1972) and the DA distance of Nei et al. (1983).

#### RESULTS AND DISCUSSION

All the microsatellites studied were shown to be polymorphic and gave many alleles, so they can be used further of mapping quantitative trait loci and subsequently marker assisted selection (MAS) as well as parentage testing.

The microsatellite INRA005 gave 6 alleles with a size range from 114 to 126 bp, the microsatellite ILSTS005 gave 5 alleles with a size range between 164 -188 bp, while the microsatellite ILSTS087 gave 8 alleles with a size range between 136-158 bp. The allele frequencies are presented at Table 2.

Table 2. Different microsatellite results: allele sizes, ranges and frequencies in the two breeds under study

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Microsatellite Allele no.		Allele size Allele frequen			eies
wheresatemite	Allele no.	range	Baladi	Zaraibi	All breeds
	1	114	0.000	0.250	0.125
	2	116	0.125	0.100	0.113
INRA005	3	118	0.450	0.150	0.300
INKAOOS	4	122	0.050	0.225	0.138
	5	124	0.250	0.175	0.213
	6	126	0.125	0.100	0.113
	1	164	0.200	0.175	0.188
	2	166	0.175	0.275	0.225
ILSTS005	3	168	0.125	0.225	0.175
	4	186	0.225	0.075	0.150
	5	188	0.275	0.250	0.263
	1	136	0.300	0.200	0.250
	2	138	0.200	0.225	0.213
	3	140	0.050	0.075	0.063
ILSTS087	4	150	0.000	0.025	0.013
10012001	5	152	0.025	0.050	0.038
	6	154	0.125	0.200	0.163
	7	156	0.150	0.150	0.150
·	. 8	158	0.150	0.075	0.113

# Hardy- Weinberg equilibrium;

The two breeds under study showed a significant deviation from hardy-Weinberg equilibrium for two of the microsatellite studied (INRA005 and ILSTS005), while the third microsatellite ILSTS087 did not show any significance deviation. The overall result proved that the two breeds are not in equilibrium state. These results are presented at Table (3).

Table 3. Estimation of Hardy Weinberg test for microsatellites in the two goat breeds understudy

Desad	Micro	satellite	
Breed —	INRA005	ILSTS087	ILSTS005
Baladi	26.364**	20.713 ns	25.669**
Zaraibi	37.393**	38.500 ns	28.953**

<sup>\*\*</sup> Significant at 1% level, ns: non significant

This deviation from HW equilibrium could be ascribed to the presence of linkage disequilibrium which in turn due to population stratification (ie. selection, migration, mutation and genetic drift). Similar HW un-equilibrium results for these microsatellites were reported by Araújo et al. (2006), who indicated that breeds showed breeds HW un-equilibrium status, may be they did not been subjected for any of the systematic (selection, migration and mutation) and dispersive forces (genetic drift and inbreeding). This microsatellite may be away or not-linked with an economic trait, so no selections for its alleles were occurred.

#### Genetic variation measurements:

Data of measuring of genetic variation for each breed (observed and effective number of alleles); (observed and expected heterozygosity) as well as polymorphism information content (PIC) are presented in Table (4):

Table 4. Microsatellite alleles (No, observed number of alleles; Ne, effective number of alleles), heterozygosity (Ho, observed; He, expected) and polymorphism information content (PIC) at each locus in the different breeds under study

Breed	Locus		INRA005	ILSTS087	ILSTS005	Mean
	Allolog	No	5	7	5	5.667
	Alleles	Ne	3.344	5.161	4.706	4.404
Baladi .	Het.	Ho	0.850	0.900	1.000	0.917
		He	0.719	0.827	0.808	0.785
	PIC		0.657	0.780	0.753	0.730
Zaraibi	Alleles	No	6	8	5	6.333
		Ne	5.369	5.970	4.444	5.261
	Het.	Ho	1.000	1.000	0.650	0.883
		He	0.835	0.854	0.795	0.828
	PIC		0.787	0.812	0.738	0.779

The values of observed heterozygosity were higher than the expected for the three microsatellites that indicated much variability. The lowest value of heterozygosity was 0.650, using ILSTS005 microsatellite in Zaraibi breed, while the highest value was 1.000, using ILSTS005 microsatellite in Baladi breed and using INRA05 and ILSTS087 microsatellites in Zaraibi breed. The difference between the observed and expected values (Chi- square) was significant at (P < 0.05) for all the microsatellites in both breeds. Similar results were observed by Agha *et al.* (2008), who assessed the genetic polymorphism in Egyptian and Italian goats.

The observed high values of heterozygosity in the breeds understudy may be due to the absence of good breeding programs for goats in Egypt. Many scientists reported a correlation between the observed high number of alleles and the absence of selection (Gregorius, 2009). It may also be due to the selection of animals included in the study which were selected as unrelated animals, which may gives more variations (FAO, 1998). The results of heterozygosity confirms this result which usually higher than 0.5. The observed high heterozygosity values are correlated with

of high heterozygosity and consequent non-fixation of alleles at these loci, there is a further scope for improvement of the breed.

The polymorphism information content (PIC) is an expected heterozygosity derived from allele frequencies in a random mating population. PIC is an indicator of how many alleles a certain marker has or how much these alleles divided evenly. For example if a marker has many alleles but only one of them is frequent, the PIC will be low. PIC values were generally high and varied from 0.657 (INRA005) in Baladi breed to 0.812 (ILSTS087) in Zaraibi breed. PIC indicates the genetic variation; microsatellites with high PIC value are considered highly informative microsatellites (Arora et al., 2003). So the three microsatellites were highly informative since the PIC value was more than 0.5. The results agreed with the report of Agha et al. (2008).

Concerning the gene diversity for the three microsatellites in the two breeds, the results are presented in Table (5).

Table 5. Average gene diversity for the microsatellites and breeds

Desail	Microsatellite			Mean	
Breed	INRA05	ILSTS087	ILSTS005	estimate	
Baladi	1.376	1.754	1.578	1.569	
Zaraibi	1.732	1.895	1.537	1.721	
The two breeds	1.715	1.853	1.590	1.719	

Average gene diversity overall loci was 1.719, while for individual loci the average gene diversity ranged between 1.376 (INRA005) in Baladi breed and 1.895 (ILSTS087) in Zaraibi breed. Since the microsatellite INRA005 had only six alleles, the lowest values of gene diversity were noticed.

Total numbers of alleles observed across all the breeds were found to be 19 alleles. In Baladi 17 and in Zaraibi 19 alleles were observed. The highest number of alleles observed across the breeds was 8 for ILSTS087. The mean observed allele numbers across the breeds for all 3 loci was 6.333, indicating the high level of polymorphism of the selected microsatellites. The maximum numbers of alleles observed were 8 for ILSTS087 in Zaraibi. The mean number of the observed alleles in Zaraibi breed was 6.333, where as in Baladi breed was 5.667. The mean number of alleles and the expected heterozygosities detected are good indicators of the genetic polymorphism within the breed. Generally the mean number of alleles is highly dependent on the sample size because of the presence of unique alleles in the populations, which occur in low frequencies and also because the number of the observed alleles tends to increase with increases in breed size. The number of alleles scored for each microsatellite is an invaluable indicator of the future usefulness of the microsatellite for genetic screening (Luikart et al., 1999).

# Inbreeding measurements (F-statistics):

Fixation indexes most currently referred to as F statistics were proposed by Wright (1951) to describe the properties of a subdivided population (inbreeding). Parameters FIT and FIS are the correlation between two uniting gametes with respect to the whole population and to gametes of subpopulations, respectively. Parameter FST is the correlation between random gametes from different individuals within subpopulations with respect to the total breeds and is a measure of the differentiation

of subpopulations. If there is no significant selective advantage of different alleles, FIS can be interpreted as a measure of inbreeding within the breed.

To assess genetic differentiation within the breeds, Fis is a measure of the within breed heterozygote defect (inbreeding). Table (6) is showing within the breeds inbreeding estimates {Fixation index statistics (FIS = f)} in the two breeds under study.

Table 6. Inbreeding estimates (FIS = f) within breeds under study

Microsatellite	Br	- Total	
Microsalemie -	Baladi	Zaraibi	- IOIAI
INRA05	-0.2121	0.2289	-0.1491
ILSTS087	-0.1163	-0.2012	-0.1511
ILSTS005	-0.2698	0.1613	-0.0414

All the inbreeding values within the two breeds studied were either below zero or negative meaning the complete absence of the inbreeding within the same breed (Wright, 1931). Inbreeding is the mating of individuals that have higher relationship coefficient than the breed average. Inbreeding coefficient was developed as the correlation between uniting gametes also called coefficient of consanguinity (Lush, 1994).

# Inbreeding between breeds under study:

Table (7) indicates pair-wise values of genetic differentiation between breeds measured by fixation index (Fst), which varies between zero (meaning no genetic differentiation) and one (meaning complete genetic differentiation). The observed (Fst) is usually much smaller than 1.To help interpreting (Fst), Wright (1978) divided the value of (Fst) into four intervals: (1) from 0 to 0.05, indicating little genetic differentiation; (2) from 0.05 to 0.15, indicating moderate genetic differentiation; (3) from 0.15 to 0.25, indicating great genetic differentiation; (4) from 0.25 to 1, indicating very great genetic differentiation.

Table 7. FST (Fisher statistics) estimate (below diagonal) and effective migration rate (Nm) (above diagonal) (FST=1/4N m+1) (Wright 1951)

 Breed
 Baladi
 Zaraibi

 Baladi
 \*\*\*\*
 9.158

 Zaraibi
 0.027
 \*\*\*\*

#### Gene flow:

To quantify the effects of breeds of migration on the genetic structure the gene flow was estimated by converting Fst to amount of gene flow (Nm). Nm indicates the average number of effective migrants exchanged per generation.

Gene flow was estimated according to Nei (1987) as Nm indicating the ratio of the migrants exchanged from one generation to another. It was estimated using the equation: Gene flow estimated from Nm = 0.25 (1 - Fst)/Fst. The lowest value of genetic differentiation between the breeds is confirmed by the high level of gene flow between each two breeds

The gene flow was also calculated for each microsatellite separately. The results of this analysis are presented at Table (8).

Table 8. Gene flow between the two breeds for each microsatellite

Microsatellite	Gene flow	_
INRA005	3.987	_
ILSTS005	17.857	
ILSTS087	34.500	
Mean	9.158	

The values of the gene flow for the 3 microsatellites studied were: 3.987; 17.857 and 34.500 for the microsatellites: INRA005; ILSTS005 and ILSTS087 respectively.

Immigration is an important force shaping the social structure, evolution, and genetics of breeds. Multilocus genotypes can be used to identify individuals who are immigrants, or have recent immigrant ancestry. The method given by Rannala and Mountain (1997) is appropriate for use with allozymes, microsatellites, or restriction fragment length polymorphisms (RFLPs) and assumes linkage equilibrium among loci. Potential applications of detecting immigrants include studies of dispersal among natural breeds of animals and plants, human evolutionary studies, and typing zoo animals of unknown origin (for use in captive breeding programs).

# Genetic distance and phylogeny evaluations:

Estimation of Nei's standard genetic distances (Ds) and assumed mutation rates of microsatellites loci ( $\alpha$ ) were used to estimate the time of divergence (t, in generations) Where, Ds=  $2\alpha t$ .

The Ds method described by Nei (1972) for determining genetic distances was used. Genetic distance measures the time that has elapsed since breeds were genetically equivalent.

The genetic distance value indicates that the two breeds are evolved from a common ancestor, and this took around 284 years to separate the two breeds from this common ancestor. This is a logical result since the two breeds were evolved and raised from the same area. Moreover the Nei genetic analyses are presented at Table (9).

Table 9. Nei's genetic identity, (above diagonal), and genetic distance (below diagonal)

Breed	Baladi	Zaraibi
Baladi	***	0.1708
Zaraibi	0.8430	***

The relationship among the breeds can be examined using the genetic distances/identities and these are calculated using allele frequencies. In this study three microsatellites were used for the measurement of genetic distances/identities between two goat breeds.

A reliable approach to measure the genetic distances is to estimate the differences in the frequency of different genetic variants (alleles) at the number of microsatellite loci. Breeds which share the same alleles at different frequencies (or different alleles

all together) are farther apart. Distances or relationship between breeds can then be summarized using a phylogenetic tree or by multidimensional scaling. Microsatellite DNA seems to be very useful for clarifying the evolutionary relationships of closely related breeds (Takezaki and Nei, 1996). The majority of mutations that the microsatellite loci exhibits are stepwise in nature, changing allele size by one or a very few number of repeats, and thus distances that are designed specifically to apply to microsatellite generally assume Kimura and Ota, (1975) stepwise mutation model and one of its generalization. However the genetic distances which are based on IAM (Infinite allele model) and purely geometrical and or mathematical considerations perform well for phylogenetic reconstruction. All the genetic distances estimated in the study are those from IAM. This is the major reason for using these distances for phylogenetic reconstruction. In the present study genetic distance estimates viz. proportion of shared alleles (Dps), fuzzy set similarity (Dfs), absolute difference algorithm (Dad), Goldstein distance (δu) 2 Ddm, average square D1 (ASD), Nei's chord distance (1983) (Da), Nei's standard genetic distance (corrected for sample size) (D), Cavalli-Sforza and Edwards (1967) chord distance (Dc) were used to estimate the relationship within Baladi and Zaraibi goat breeds. The estimates of distances measured using proportion of shared alleles, Nei's chord distance; Nei's standard genetic distances Cavalli Sforza and Edwards chord distance are found relative to one another. The standard genetic distance of Nei's (1978) and Cavalli-Sforza and Edwards (1967) chord distance, calculated from the allele frequencies, demonstrated their superior performance in phylogenetic tree construction when the microsatellite markers were used.

Estimated divergence time between the two breeds understudy are were calculated according to Nei standard genetic distance and the mammalian mutation rate are presented in Table 10.

Table 10. Estimated divergence time of the two breeds under study on the basis of the 3 microsatellites loci studied (Nei, 1978)

	Nei's standard	Mutation	Divergence time	
Breed	genetic distance (Ds)	rate (α)	Generations	Years
Baladi & Zaraibi	0.1708	1.2 X10 <sup>-3</sup>	71	284

#### CONCLUSION

The values obtained for allele diversity, heterozygosity, inbreeding measurements and gene diversity showed that Baladi and Zaraibi goat breeds possessed substantial amount of genetic diversity. This variability can be a good tool for further improvement of goat performance in Egypt. From another side the results from the present study, point to the usefulness of evaluations of diversity using molecular markers for the choice of breeds worthy of conservation.

# REFERENCES

Agha, S.H., F. Pilla, S. Galal, I. Shaat, M. D'Andrea, S. Reale, A.Z.A. Abdelsalam, and M.H. Li, 2008. Genetic diversity in Egyptian and Italian goat breeds

- measured with microsatellite polymorphism. J. Anim. Breed. Genet., 125: 194-200.
- Araújo, A.M., S.E.F. Guimarães, T.M.M. Machado, P.S. Lopes, C.S Pereira, F.L.R. Silva, M.T. Rodrigues, V.S. Columbiano and C.G. Fonseca, 2006. Genetic diversity between herds of Alpine and Saanen dairy goats and the naturalized Brazilian Moxotó breed. Genetics and Molecular Biology, 29 (1): 67-74.
- Arora, R., B.D. Lakhchaura, R.B. Prasad, A. Chauhan, R.K.S. Bais, M.S. Tantia and R.K. Vijh, 2003. Buffalo Newsletter, 19: 12-14.
- Bostein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of genetic linkage maps in man using restriction fragment length polymorphism. Am. J. Hum. Genet. 32: 314–331.
- Cavalli-Sforza, L. and W. Edwards, 1967. Phylogenetic analysis: models and estimation procedures, Evolution, 21: 550-570.
- Epstein, H., 1953. The dwarf goats of Africa. E. Afr. Agric. J., 18: 123-132.
- Epstein, H., 1971. The Origin of the Domestic Animals of Africa, Vol. 1, pp. 185–555. Africana Publishing Corporation, New York.
- FAO, 1998. Food and Agriculture Organization of the United Nations, Rome: Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (Mo-DAD): Original Working Group Report. http://dad.fao.org/en/refer/library/guidelin/workgrp.pdf
- Goudet, J., 2002. FSTAT Computer Package for PCs. Institute of Ecology, UNIL, Lausanne, Switzerland.
- Gregorius, H.R., 2009. Relational diversity. J Theor Biol., 257(1): 150-158.
- Herre, W. and M. Rohrs, 1973. Hausturezooligsch geshern. G. Fischer: Stuttgart.
- Kimura, M. and J.F. Crow, 1964. The number of alleles that can be maintained in a finite population. Genetics 68: 47.
- Kimura, M. and T. Ota, 1975. Distribution of allelic frequencies in a finite population under stepwise production of neutral alleles. Proc. Natl. Acad. Sci., 72: 2761-2764.
- Luikart, G., M.P. Biju-Duval, O. Ertugrul, Y. Zagdsuren, C. Maudet. and P. Taberlet, 1999. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). Anim. Genet., 30(6): 431-438.
- Lush, J.L., 1994. The Genetics of Populations. Special Report No. 94, Iowa State University, Ames, Iowa.
- Mason I.L., 1981. Classification and distribution of goat breeds. In: K. Maijala (ed.), Genetic Resources of Pig, Sheep and Goats. World Animal Science B8. Elsevier, Amsterdam, pp.8.
- Mason, I.L. and J.P. Maule, 1960. The indigenous livestock of eastern and southern Africa. Tech. Commun. No. 14. Commonw. Bur. Anim. Breed. Genet., Commonwealth Agricultural Bureaux: Farnham Royal, UK.
- Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research, 16: 12-15.
- Nei, M., 1972. Genetic distance between populations. Am. Nat., 106: 283-292.
- Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.
- Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

- Nei, M., 1983. Genetic polymorphism and the role of nutrition in evolution. In: Evolution of genes and proteins (Ed. M. Nei and R. Koehn). pp. 165-190. Sunderland, MA: Sinauer Associates.
- Nei, M., F. Tajima and Y. Tateno, 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19: 153–170.
- Rannala, B. and J.L. Mountain, 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. U.S.A., 94: 9197–9201.
- Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989. Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press.
- Slatkin, M., 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst., 16: 393-430.
- Steffen, P., A. Eggen, A.B. Dietz, J.E. Womack, G. Stranzinger and R. Fries, 1993. Isolation and mapping of polymorphic microsatellites in cattle. Anim. Genet., 24(2): 121-124.
- Takezaki N. and M. Nei, 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA, Genetics, 144: 389–399.
- Vaiman, D., D. Mercier, K. Moazami-Goudarzi, A. Eggen, R. Ciampolini, A. Lepingle, R. Velmala, J. Kaukinen, S.L. Varvio and P. Martin, 1994. A set of 99 cattle microsatellites: characterization, synteny mapping, and polymorphism. Mamm. Genome, 5(5): 288-297.
- Weir, B. S., 1990. Genetic Data Analysis. Sinauer, Sunderland. MA.
- Weir, B. S. and Cockerham, C. C. (1984): Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.
- Wright, S., 1931. Evolution in Mendeleian populations. Genetics, 16: 97-159.
- Wright, S., 1951. The genetic structure of populations. Ann. Eugen. 15: 323-354.
- Wright, S., 1978. Evolution and the genetics of population. Variability Within and Among Natural Population, vol. 4. University of Chicago Press, Chicago, IL.
- Yeh, F.C., T. Boyle, Y. Rongcai, Z. Ye and J.M. Xian, 1999. POPGENE, Version 1.31. A Microsoft Window Based Free Ware for Population Genetic Analysis. University of Alberta, Edmonton. Canada.

# الإختلافات الوراثية لمعلالتين من الماعز باستخدام التوابع الوراثية الدقيقة محمد صابر حساتين ، أحمد فريد الخولي ، على ربيع عبد الرحمن ، رشا محمد صميدة ق

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تناولت هذه الدراسة تحليل التباين الوراثى لسلالتين من سلالات المعـــز المـــصرية وهمـــا: البلـــدى والزرايبي باستخدام ثلاثة من واسمات التتابعات الوراثية الدقيقة هي:

(INRA005, ILSTS005, ILSTS087)

ولإجراء هذا البحث تم تجميع اربعين عينة دم من معز ايس بينها درجة قرابة 20 (عينة لكل سلالة) ثم عزل ونتقية المادة الوراثية (DNA) من عينات الدم التي تم جمعها. أجري تفاعل إنزيم البلمسرة المتسلسل (Polymerase Chain Reaction (PCR) للواسمات المراد دراستها وذلك مسع بادئسات المتسلسل (Polymerase Chain Reaction وذلك مسع بادئسات نتابعات دقيقة متخصصة لكل واسم على حدة، هذا وقد تم تغريد ناتج نفاعل انزيم البلمرة المتحصل عليه على جهاز تفريد كهربائي رأسي و علي مادة البولي أكريلميد تحت ظرف denaturing وذلك افك الالبيلين عسن بعضهما في وجود مادة وراثية معلومة الطول (DNA size marker). وبعد التقريد تم الصبغ بمادة بروميد الايثيديوم ثم التصوير بكاميرا مخصصة لذلك ثم إدخال الصور المتحصل عليها على برنسامج كمبيونر متخصص متخصص لتحليلها و تحديد أطوال الاليلات لهذه الحيوانات. وبعد الحصول على قياسات هذه الأطسوال تسم حدولة النتائج وتحليلها إحصائيا باستخدام برامج TSTAT & POPGENE و هي برامج متخصصة في مجال وراثة العشائر.

أظهرت جميع واسمات النتابعات الوراثية الدقيقة التي شملتها الدراسة نتائج عالية في تعدد الأشكال المظهرية وقد أعطت أكثر من أليل حيث أعطت الواسمات الثلاث عددا من الأليلات يتسراوح مسن 5-8. وعليه فانه يمكن استخدام هذه الواسمات الثلاثة في اختبار معرفة ألأبوية أو النسب وبالإضافة إلى ذلك يمكن استخدامها في المواقع الجينية المرتبطة بالصفات الإنتاجية الكمية واستخدمها في برامج التربية الحديثة والتي تسمى بالانتخاب بمساعدة الواسمات الوراثية.

أظهرت النتائج أيضا أن السلالتين قيد الدراسة ليستا في حالة انزان بين هاردي- واينبرج. وقد أظهــر اختبار عدم التجانس أن جميع القيم قد تجاوزت 0.5 وهذا يعني ارتفاع نسب الخلط، و كانت نــسبة الخلــط الكاية لسلالة المعز البلدى 0.917 بينما كانت 0.833 لسلالة المعز الزرايبي.

وقد أظهرت النتائج أيضا ارتفاعا ملحوظا في متوسط قيم التنوع الجيني لجميع الواسمات المستخدمة في السلانين قيد الدراسة. كما وجد أن قيمة محتوى معلومات تعدد المظاهر الوراثية أو ما يعرف أيضا بالنتوع الوراثي المتحصل عليها تتراوح من (0.730- 0.779) وهي قيم عالية مما يثبت وجود درجة عاليـة مـن النتوع الوراثي في السلالتين.

وقيما يتعلق بنتائج النربية الداخلية (inbreeding) بين السلالتين دلت الدراسة على عدم وجود نربيــــة داخلية بين او داخل السلالتين تحت الدراسة. وبالإضافة إلى ذلك وجد أن قيمة الهجرة أو النتفق الجينى في سلالتي المعز المحلية كانت عالية بمتوسط يساوى 9.1578. وهو عالى ويدل على زيادة معدل الهجرة والخلط بين سلالتي المعز تحت الدراسة.

أوضحت الدراسة انبثاق السلالاتان من بعضها، وأنهما قد نشئوا من سلف مشترك والمسافة الوراثية بينهما هي 284 عاما.

النتائج بشكل علم هي دليل على أن سلالتى المعز تحت الدراسة و التي أثيرت في مصر تشير إلى أن هاتين السلالتين ليستا نقيان تماما حتى الان وأن بكل سلالة درجة عالية من النتوع الوراثى يمكن استخدامها في برامج التحسين الوراثى مستقبلا.