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Assessing Inter and Intra Molecular Genetic Variation of Litter Size in Three Egyptian Goat Breeds Using COI and F-AFLP Markers

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Abstract

Most of the livestock breeds in Egypt lack molecular characterization required for establishing adequate utilization of genetic variation in developing livestock breeding programs. Cytochrome oxidase subunit I (COI) gene technique was used in the present study to identify and differentiate the main three Egyptian local goat breeds (i.e. Baladi, Zaraibi and Barki) via DNA barcoding to confirm its species identity and provide valuable DNA sequence source in the nucleotide online database for further studies. Blast (Basic Local Alignment Search Tool) results confirmed samples to be *Capra hircus* (100%) with no variation among the studied breeds. On the other hand, the Fluorescently Amplified Fragment Length Polymorphism (F-AFLP) technique was applied to assess genetic variation among and within the three breeds for litter size character. F-AFLP analysis of triplicates per breed produced 164 polymorphic loci. At the same time fixed and private bands varied among the three breeds; 47, 17 and 14 bands and 9, 19 and 27 bands for Baladi, Zaraibi and Barki, respectively. Analysis of Molecular Variance (AMOVA) showed 3.8% and 96.1% variance among and within breeds, respectively. Population re-allocation showed that all samples of Baladi breed are outliers, Zaraibi breed one outlier and two hybrids and in Barki breed one hybrid, one outlier and one allocates itself. Private bands in excel filter (using virtual inspection in excel) showed fixed bands of 213bp molecular weight at locus 35 in both Baladi and Zaraibi breeds. These bands considered as genetic marker for litter size trait (i.e. high prolific animals).

Keywords: COI sequencing, F-AFLP technique, Egyptian goats, Molecular variance, Litter size

1 Introduction

Goats are considered fundamental animals for rural and urban people due to their better productivity, small size, and ability to withstand and utilize poor quality roughages (Ahlawat et al 2015 and 2016). They are extensively reared at global level, especially in the developing countries and serve as source of milk, meat, fiber and skins (Machugh and Bradley 2001; Qureshi et al 2014). As such, the world is advancing in applying genetic principles to exploit the biological potential of live-

stock. More progress was achieved in developed countries, but less was noticed in developing countries. Generally, the goats sector in Egypt remained behind in applying advanced technology of genetic principles.

There are considerable numbers of goat breeds in Egypt and among them are five indigenous goat breeds namely Zaraibi, Baladi, Barki, Saidi and Sinaoy (Galal et al 2005). Goats in Egypt are mainly reared for meat, milk and skins. However, these breeds are not fully described genetically.

In animals, Cytochrome oxidase subunit I (COI) gene is considered as one of the most conserved mitochondrial protein-coding genes in animal (Muller 2006), and thus display a better phylogenetic signal to discriminate among closely related taxa (Herbert et al 2003). On the other hand, the AFLP-PCR based technique (Amplified fragment length polymorphism) has been applied successfully to study the genetic diversity and relationships in various domestic species such as in cattle (Ajmone-Marsan et al 2001 and 2002), chicken (De Marchi et al 2006; Abdelmoniem 2017) and sheep (Hoda et al 2010). Thus, the current study was used both the COI barcoding system and the amplified fragment length polymorphism technique to generate enough markers for goat breeds in Egypt.

Therefore, this study aims to find out genetic variability among and within three main Egyptian local goat breeds (i.e. Zaraibi, Baladi and Barki) related to litter size trait (i.e. twining rate character), considered as one of the most important economic traits, based on information at the DNA level. The information obtained will help in the selection process for the conservation and improvement of goat breeds in Egypt.

1 Materials and Methods

2.1 Experimental animals

2.1.1 Geographical distribution

In Egypt, goats are distributed across the country, especially dense in the Nile valley and delta and with lower concentration in the north-western coastal region and at oases (Galal et al 2005).

According to their historical background found in literature review, these three goat breeds vary in twining rate character (i.e. litter size), having high rate in both Zaraibi and Baladi breeds and low rate in Barki breed.

2.1.2 Population size and structure and origin of the sampled flock

Experimental animals of Baladi and Zaraibi samples were taken from goats' experimental farm (Ain Shams flock) owned by the Faculty of Agriculture, Ain Shams University, situated about 30 km north of Cairo in Shalakan, Kaliobia Governorate, where breed status is well known and the pedigree information has been maintained over several generations. Zaraibi and Baladi goat herd is composed from 20 adult Zaraibi does and 30 adult Baladi does of different ages with their progeny. Only Barki blood samples were brought from the north-western coastal region of Egypt and were taken randomly from animals of different private herds of different size (ranged from 30 to 50 adult does of different ages) around Alexandria Governorate and Libya borders.

2.1.3 Production environment

Two broad small ruminant production systems exist in Egypt, the first in the Nile valley and Delta and the second in the Northwestern Coast of Egypt and Sinai. In the valley-delta system, the land is intensively cropped in a mixed farming system with a small number (1-5) of small ruminants per household. In the desert and semi-desert system, in which animals are managed in a transhumant system under extensive grazing and opportunistic cropping in rain-fed areas, there are larger mixed sheep and goat flocks (average size >30) that make use of available range during winter and early spring but the contribution of range to the feed budget in these areas is decreasing. In both systems there are feed lot operations for sizable lots, up to 300 heads.

2.1.4 Socio-economics

In the Nile valley goats are usually found in small holdings as mixed flocks with sheep and other farm animals like cattle and buffaloes, while in the north-western Mediterranean coast they are in large herds mixed with sheep.

2.1.5 Number of samples animals/ each breed

A total of 28 animals of both sexes and of different ages (10, 10 and 8 from Zaraibi, Baladi and Barki goat breed, respectively) were used in the present study.

2.1.6 Breeding system

The pedigree information was used to sample animals of the Zaraibi and Baladi goat breeds, which were unrelated. A selection programme was undertaken for each breed where special attention was paid to avoidance of inbreeding. The breeding plan adopted by the authority of the farm was to obtain 3 kidding every two years. The females were mated in May, February and October. A Baladi buck was designed to mate the thirty Baladi does which represent the size of the Baladi herd and a Zaraibi buck was used to mate the twenty Zaraibi does which represent the size of the Zaraibi herd. Before the start of the breeding season, a flushing regime was used to improve the twinning rate in the herd of both breed. Animals of Barki breed came from different unrelated herds.

2.1.7 Feeding system

For Zaraibi and Baladi goats the animals were constantly kept in a closed pen joined with a suitable yard for exercise where they received their daily requirements. They were given concentrate mixture (16 % crude protein and 70 % TDN) at the rate of about 0.55 kg/head/day. Berseem (*Trifolium Alexandrinum*) was added at 12 noon (3–5 kg/head/day). Green maize was replaced the Berseem in summer and autumn. Goat requirements were changed according to their physiological status and production level. Rations were calculated based on NRC allowances (1995) by using the Least Cost Ration program (Alan and Munford, 1988). Water and straw were allowed *ad libitum* to animals all the day and night.

2.1.8 Performance recording

After kidding the litter size per parturition for each doe was recorded and weighed. Litter size (i.e. twining rate), defined as the number of kids born /doe/parturition was the soul measurement considered in the present study to evaluate the three goat breeds.

2.2 Sample collection and DNA extraction

A total of 28 blood samples (10, 10 and 8 from Zaraibi, Baladi and Barki goat breed, respectively) were collected via the jugular vein using 1.5ml vactutainer tubes containing anticoagulant disodium EDTA. All samples were then stored at -20º C until needed, then transferred directly to the Molecular Genetics Laboratory in the Faculty of Agriculture, Ain Shams University department of genetics. Genomic DNA was extracted from samples using Ease Pure® Blood Genomic DNA kit.

2.3 PCR amplification and sequencing

Three blood samples (one sample/breed) chosen randomly for amplification and sequencing using specific forward and revers primer set. The COI gene was amplified using 2x Easy Taq® PCR super mix (Cat.No:AS111) by applying the primer pair FF2D-1 (5′ - TTC_TCC_ACC_AAC_CAC_AAR_GAY_ ATY_GG - ′3) and FR1D-1 (5′ - CAC_CTC_AGG_GTG_TCC_GAA_RAA_ YCA_RAA′ -3); the PCR reaction was set as standard with annealing Tm of 55ºC /30s. Successful PCR products were purified and concentrated using BigDye®Terminator™ purification kit (Pub.No.4337035).

2.4 COI sequence analysis

Assembling of two direction primers sequences were done by using BioEdit (Hall 1998), while sequences were identified, aligned by Blast search against gene bank database of domestic goat.

2.5 AFLP-polymerase chain reaction (PCR)

The protocol follows Vos et al (1995) with primers fluorescently rather than radio actively labeled. Adaptors and primers set were synthesized by Eurofins (Germany) as shown in Table1. Three different selective primer combinations (3Eco+NNN*1Mse+NNN primers) were amplified using the original PCR program.

2.6 AFLP data analysis

Chromatogram were analyzed using peak scanner™ (Applied Bio system, USA), bands scoring and pseudo-gel imaging were performed using RawGeno package on R.FAMD software (Schluder and Harris 2006). It was used to filter rare alleles (0.5% > alleles presence per individual< 95%), and to estimate loci polymorphism, group reallocation, genetic dissimilarity matrix and principle coordinate analysis (PCoA).

Simulation tests, in which likelihood statistics were calculated in order to assess the degree to which (re) allocation of individuals to a set of possible source population were done using the AFLPOP software package (Duchesne and Bernatchez 2002). Analysis of molecular variance (AMOVA) was performed using Arlequin V 3.5 (Excoffier and Lischer 2010) to test the population genetic differentiation and estimated the part of the observed variance that corresponds to the variability within and among the breeds.

3 Results and Discussion

3.1 COI results

The sequence analysis of the three Egyptian goat breeds with a sequence of the domestic goat species (*capra hircus*) in the Gene Bank database showed no variation at sequence nucleotide bases of COI gene. This database reported 100% similarity among the three goat breeds and Monglia Cashmere goat, Black goat of China, San Clemente goat of USA, Pashmina Cashmere goat of India and Black Bengal goat of Bangladesh. Cytochrome oxidase subunit I (COI) results of the present study, thus, showed 100% similarity for the three Egyptian local goat breeds to the species *Capra hircus*. This result varies from what was found by Asif et al (2016) in common goat breeds of Pakistan using COI in DNA barcode to differentiate two breeds (Beetal & Berberi) and cross breed showing 99% maximum homology with the black goat. In a study of 14 breeds of goat and sheep in Philippines, the mean genetic diversity in COI sequences were found to be 62.6% among goat breeds and 32.9% in sheep (Orville et al 2013) using different number and species of breeds, or alternatively, the high relatedness among the Egyptian goat breeds can be the reasons for such contradiction.

3.2 AFLP results

3.2.1 AFLP peaks scoring statistics and DNA polymorphism

The overall samples statistics showed 164 loci with mean bands presences per individual of 66.66±11.86. Peak numbers and scored bands per primer combination are shown in **(Table 2 and Fig 1**).

3.2.2 Polymorphism

Baladi individuals showed nine pure private bands for distinguishing the breed, while Zaraibi individuals showed 19 private bands but mixed in Baladi and Barki bloods. However, the Barki breed is not related to Baladi and it has 27 private alleles mixed with Zaraibi blood as fixed and private bands **(Table 3)**.

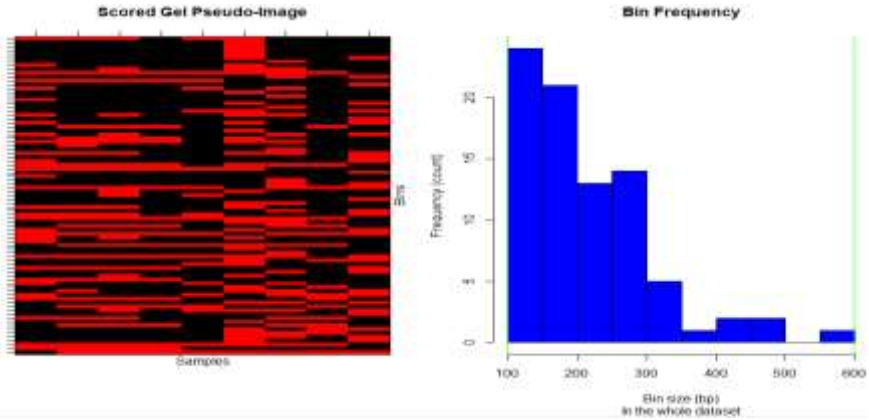
Table 1. Sequences of primers and adaptors used to establish the AFLP-PCR technique

Items	P/A	5′-sequence-3′	P/A	5′-sequence-′3	Paired combined paired
Adaptor	EcoRI-A1	CTCGTAGACTGCGTACC	MseI-A1	GACGATGAGTCCTGAG	-
	EcoRI-A2	AATTGGTACGCAGTC	MseI-A2	TACTCAGGACTCAT	-
Pre-selective PCR	EcoRI-A	GACTGCGTACCAATTC**A**	MseI-C	GATGAGTCCTGAGTA**C**	-
Selective PCR	EcoRI+ ACA	**FAM**GACTGCGTACCAATTC**AA**	MseI-CTC	GATGAGTCCTGAGT**TC**	PP1
	EcoRI+ AGG	**HEX**GACTGCGTACCAATTC**AG**			PP2
	EcoRI+ ATA	**CY3**GACTGCGTACCAATTC**AA**			PP3

Table 2. AFLP scoring statistics and polymorphism for three primer pairs

Primer pair	Dye	No. of samples	Min Peak no.	Max Peak no.	Min Size (bp)	Max Size (bp)	MNP*	% P**
PP1	Blue	9	49	89	101	574	30	100
PP2	Green	9	32	54	50	248	17	100
PP3	Yellow	9	100	117	102	688	21	100

*MNP: Mean number of bands per individual; ** %P: polymorphism percentage



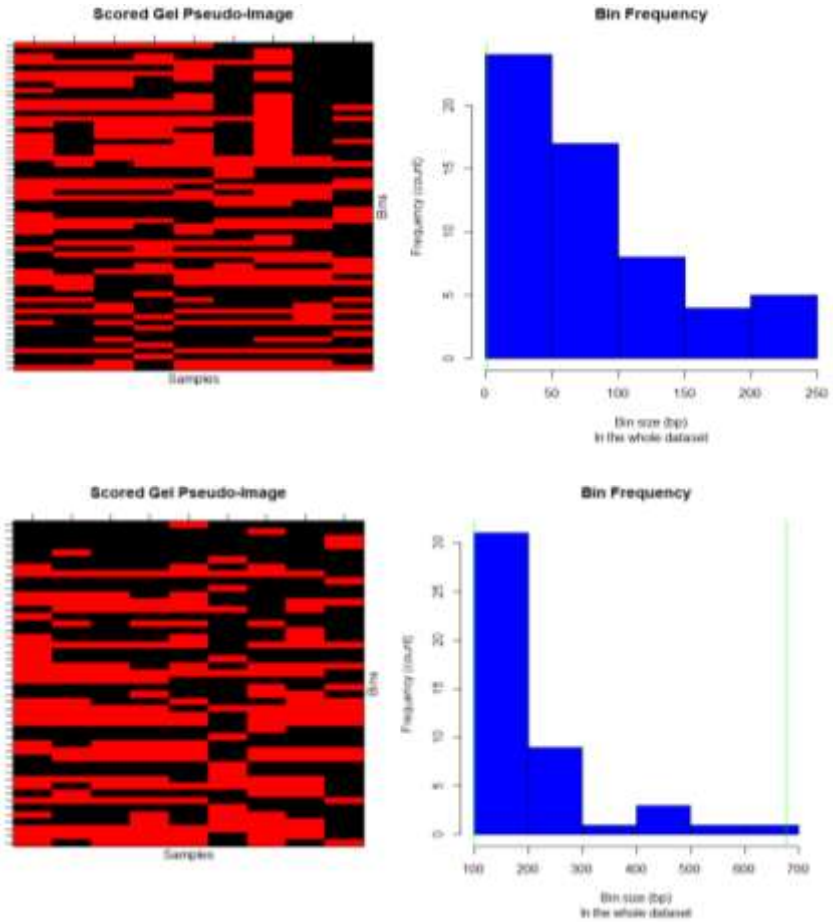


Fig 1. Scored gel pseudo-image and bin frequency of the scored bands for blue dye (above), green dye (middle) and yellow dye (below).

Table 3. Fixed and private bands for the three goat breeds

Breeds	Fixed bands	Private bands
Baladi (B)	47	9
Zaraibi (Z)	17	19
Barki (R)	14	27

3.3 Population analysis

3.3.1 Population re-allocation

By applying the maximum likelihood hybrid index method, the group re-allocation of samples was performed based on all alleles. Using the minimum log-likelihood value criteria, population number (P1) representing Baladi breed, when re-allocated, showed that all samples S1, S2 and S3 were outliers. While, population number (P2) representing the Zaraibi breed showed, in re-allocation, two samples S4 & S5 re-allocated from Baladi breed and seems possibly to be hybrid, but S6 for the Zaraibi breed, which showed outlier re-allocation. The last population number (P3) represents the Barki breed showed S7 outlier while S8 hybrid re-allocated from Zaraibi breed but S9 re-allocated itself within the same breed (Barki; **Table 4**).

3.3.2 AMOVA results

Based on the acquired information, AMOVA analysis was performed among the three populations and within each group. The percentage of variation among the three populations (3 breeds) was low (= 3.8%) compared to 96.1% high genetic differentiation within-group **(Table 5)**.

Table 4. Reallocation of the three goat breed populations

Pop.	S1	S2	S3	S4	S5	S6	S7	S8	S9
B	-41.4	-38.6	-36.9	-31.6	-38.4	-57.5	-49.3	-41.9	-55.2
Z	-43.1	-36.9	-35.8	-44.9	-46.7	-53.4	-46.5	-39.8	-52.1
R	-49.7	-42.1	-45.5	-42.3	-44.8	-52.7	-48.4	-45.9	-49.6
Difference	1.7	1.6	1.3	10.7	6.4	0.648	1.9	2.17	2.5
Group H0	P1	P1	P1	P2	P2	P2	P3	P3	P3
Allocation	None	none	None	P1	P1	None	none	P2	P3

Table 5. Genetic differentiation through the AMOVA test of three goat breeds based on AFLP data

Source of variance	Df*	SS**	Variance components	Percentage of variance (%)
Among	2	0.436	0.007	3.80
Within	6	1.171	0.195	96.19
Total	8	1.607	0.202	-

*The degrees of freedom (df); ** the sum of squares (SS).

3.4 Genetic marker alleles based on group

Based on characterization of the three goat populations according to literature review, both Baladi and Zaraibi are prolific breeds, while Barki is non-prolific. Private bands in excel filter (using virtual inspection in excel) showed fixed bands that found in group one; Baladi and Zaraibi (prolific breeds) at locus 35 which is absent in group two; Barki (non-prolific breed) at the same locus and of the same molecular weight (213bp). These bands considered as genetic markers for litter size trait (i.e. high prolific animals) **(Table 6)**.

The appearance of a clear band with no polymorphism means non-contaminated DNA extracts were used in this study, ensuring that the diversity recorded by F-AFLP marker is reliable and belong to the goat breeds under the study. Anila et al (2012) studied six Albanian goat breeds using the AFLP marker and found that the most variance of AMOVA analysis was high within breed diversity (97%). This almost similar to our results where AMOVA analysis results 96.19% variance within the three Egyptian goat breeds. The present finding AFLP variation analysis also showed high value than those finding of Ajmone-Marsan et al (2001) and Crepaldi et al (2001) who separately reported 75% and 91.2%, respectively, using AFLP marker in assessing the genetic diversity of Italian goat populations.

Table 6. Genetic marker bands for prolificacy in Baladi and Zaraibi goat breeds (S1-S6).

Locus	MWt	S1	S2	S3	S4	S5	S6	S7	S8	S9
35	213	1	1	1	1	1	1	0	0	0

4 Conclusions

Our study revealed that the diversity recorded by F-AFLP marker is reliable and belong to the goat breeds under the study. Private bands in excel filter (using virtual inspection in excel) showed fixed band of 213bp molecular weight at locus 35. This band considered as genetic marker for litter size trait (i.e. high prolific animals).

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تقييم التباين الوراثي الجزيئى بين وداخل السلالات لصفة حجم البطن فى ثلاث سلالات من الماعز المصرية باستخدام الواسمات الجزيئية COI و F-AFLP

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الموجـــــز

تفتقر معظم سلالات الحيوانات الزراعية في مصر إلى التوصيف الجزيئي المطلوب لتحقيق الاستخدام المناسب للإختلاف الوراثى في تطوير برامج التربية فى الإنتاج الحيواني. فى هذه الدراسة تم استخدام التقنية الجينية سيتوكروم أوكسيديز Cytochrome oxidase subunit I (COI) لتحديد وتمييز سلالات الماعز المحلية المصرية الثلاثة الرئيسية (وهى البلدي والزرايبي والبرقي) عن طريق الترميز الشريطي للحمض النووي DNA لتأكيد هويتها من حيث النوع الحيوانى وتوفير مصدر ذو قيمة للتسلسل الجزيئى للحمض النووي في قاعدة بيانات النوكليوتيدات على الإنترنت لإجراء المزيد من الدراسات مستقبلاً. أكدت نتائج أداة تقصى الترتيب الموضعى الأساسى Basic Local Alignment Search Tool) BLAST) أن العينات هي لنوع الماعز المستأنسة *Capra hircus* (بنسبة تشابه للتسلسل النيوكليوتيدى قدره 100٪) مع عدم وجود اختلاف فى تسلسل القواعد النيوكليوتيدية بين السلالات المدروسة. من ناحية أخرى، تم تطبيق تقنية الملصقات ذات العلامات الفلورسينتية F-AFLP Fluorescently Amplified Fragment Length Polymorhism) لتقييم التباين الوراثي بين وداخل السلالات الثلاثة لصفة حجم الخلفة (البطن). أنتج تحليل F-AFLP لثلاث نسخ لكل سلالة 164 موضعًا متعدد الأشكال. في الوقت نفسه ، تباينت الحزم الثابتة والخاصة بين السلالات الثلاثة ؛ حيث بلغت عدد الحزم الثابتة 47، 17، 14 وعدد الحزم الخاصة 9، 19، 27 لسلالة البلدي والزرايبي والبرقي على التوالي. أظهر تحليل التباين الجزيئي (AMOVA) تبايناً قدره 3.8٪ و96.1٪ بين السلالات وداخلها على التوالي. وقد أظهر إعادة توزيع العشائر أن عينات سلالة البلدي كلها متطرفة، وعينات سلالة الزرايبي واحدة متطرفة واثنتان هجينة مع البلدى والبرقى، وفي البرقي عينة واحدة هجينة وواحدة متطرفة والأخيرة مشابها لنفسها. وأخيراً أظهرت معاينة الحزم الخاصة باستخدام برنامج اكسيل (باستخدام الفحص الظاهري) وجود حزمة ثابتة مشتركة بين سلالتى البلدى والزرايبى بوزن جزيئي 213 قاعدة زوجية عند الموقع رقم 35. وتعتبر هذه الحزمة بمثابة واسمة وراثية لصفة حجم البطن (للحيوانات عالية الخصوبة).