



GENETIC APPRAISALS OF RED BALADI AND SINAI GABALI RABBITS USING MICROSATELLITE MARKERS AND DNA BARCODING.

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ABSTRACT: The selected cytochrome c oxidase subunit I (COX1) gene, and four microsatellite markers were used to investigate the population genetic structure of Red Baladi (RB), and Sinai Gabali (SG) rabbits as the Egyptian native rabbit breeds. Additionally, genetic assorted variety of these breeds was contemplated by utilizing both microsatellite markers and COI gene as DNA standardized identification barcode. A total of 50 does (25 animal for each breed) were used. Live body weight measured at 17 months of age, also the blood samples were collected for molecular analysis. Four microsatellite markers (SAT3, SAT13, SOL33, and SOL44) were used. A total of 18 alleles were discovered across the breeds. the range of alleles per locus discovered over loci and breeds was 3.63 ± 0.26 alleles. All microsatellite markers were polymorphic, and the average polymorphic information content (PIC) was 53%. The mean of H_e values were 0.67 ± 0.06 , and 0.74 ± 0.1 for SG, and RB, respectively. The H_o for different markers averaged 0.7 ± 0.06 and ranged from 0.52 at marker SOL33 to 0.90 at markers SAT4. The overall mean of H_e was 0.574 ± 0.04 , and ranged from 0.45 at SOL33 to 0.69 at SAT4. Among the four loci, the hardy Hardy-Weinberg equilibrium was highly significant ($P \geq 0.001$) for both of RB and SG for SOL44 locus. Although SOL33 locus was highly significant for SG, but it was not significant for RB. The highest reduction in heterozygosity (F_{ST}) values varied from 0.008 (SAT13) to 0.27 (SOL44). Concerning live body weight, there was a highly significant difference in live body weight between RB and SG ($P \geq 0.001$), where the difference in body weight between RB and SG was 419 ± 83.3 , the least square means were 3188 ± 47.1 , and 2768 ± 68.7 for RB and SG breeds, respectively. In the present study, the similarity percentage between sequence reads for RB and SG breeds were 99%. At a starting codon 3, the RB has 227 amino acid, and the SG has 226 amino acid. Moreover, two single nucleotide polymorphisms have detected (SNP: C>A, and G>A) located at 273, and 275bp. There was a significantly association between the enclosed C>A SNP with BW. In addition, the general linear model analysis showed significant association between the live body weight and SAT4 locus ($P \geq 0.05$).

From the present study, both microsatellite and the COI gene as a DNA barcode had proved to be exceedingly successful in distinguishing between breeds under investigation and genetic assorted variety between them.

Keywords: DNA barcode- microsatellite marker-body weight-diversity.

INTRODUCTION

In Egypt, there are three breeds of rabbits, Baladi, Gabali and Giza White. Considering Baladi rabbits, there are three strains; White-, Black- and Red Baladi. Red Baladi was obtained from mating of heavy Giant Baladi does with unadulterated Giant Flander bucks for several generations and selected for its color for many generations until characters and color were set up (Khalil 2002). Sinai Gabali and Desert Gabali are considered as two strains of Gabali rabbits, the two strains appear to be adjusted to the desert conditions (Khalil 1999).

Body weight for Red baladi is varied from 1500 to 2160 g at about four months old, while Gabali rabbits recorded the highest body weight around 3000 g (Iraqi et al., 2010). The vast majority of the Egyptian rabbit genetic resources are imperiled since the total number of reproducing rabbits is around 3000 (FAOSTAT, 2018), most of them scattered in state farms. Several attempts have been conveyed to add more information or edit to available data on rabbit genetic resources (Khalil 1999, 2002; Afifi et al., 2002; Rabie 2012; El Aksher et al., 2016). Genetic diversity among the local breeds showed an important data to comprehend the domestication and development history (Hailu and Getu, 2015). Molecular techniques in rabbit's field turn out to be essential devices for the identification of markers associated with the important economical traits. There are various examinations accomplished on indigenous rabbits using macromolecules procedures such as division protein which known as protein electrophoresis (Rabie, 2012 and El-Sabroun and Aggag 2015) and molecular markers for instance, RAPD (Khalil et al., 2008; Al Saef et al., 2008;

and Badr et al., 2016), Microsatellite markers (El-Sabroun and Aggag 2015; El-Aksher et al., 2016, 2017), lastly single nucleotide polymorphisms (SNPs) (El-Aksher et al., 2017; El-Sabroun and Aggag 2017). In breeding projects, information utility from linked genes can possibly essentially improve the accuracy of selection (Helal et al., 2014). Therefore, utilizing SNP markers in assessing the rabbit genetic assorted variety related genes with the vital traits, for example, PGR, MSTN, and POU1F1 genes (Shevchenko, 2015; Rafayová et al., 2009; Wang et al., 2015), in addition, the GHR gene which identified as associated with growth performance of rabbits (Sahwan et al., 2014). DNA barcoding implies the utilization of target DNA as an apparatus to distinguish animal species, and lately, it has been the new improvement of natural scientific categorization. DNA barcode is a short sequence of institutionalized genomic region and each species has a particular standardized tag "barcode". Therefore, the cytochrome c oxidase subunit I (COX1) gene is the primary mitochondrial molecular marker assuming a vital role in phylogenetic research and is a significant barcode sequence for practically all animals (Herbert et al., 2003).

COI has proved to be exceedingly successful in distinguishing birds (Herbert et al., 2004), fishes (Ward et al., 2005; Hubert et al., 2008), and numerous other animal groups. The COI marker intended to intensify this gene in vertebrates permitted speedy and simple barcode and the analysis of phylogenetic. DNA barcoding can possibly be undermined by the inadvertent intensification of numts (i.e., nuclear duplicates of mitochondrial DNA). Thus,

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reliably identifying species via DNA barcoding requires careful examination of numt contaminations and their effect on the results of barcode analyses (Pietsch, 2011). The purpose of this study was to explore the reliability of COI standardized tags "barcodes" in recognizing of the Egyptian local rabbits. Consequently, variations of selected COI gene and population genetic structure of two native rabbit breeds of Egypt were investigated. Additionally, genetic assorted variety of these breeds was contemplated by utilizing both microsatellite markers and COI gene as DNA standardized identification barcode.

MATERIALS AND METHODS

Collecting data and samples

A total of 50 individual blood samples representing 25 sample from each of Sinai Gabaly, and Red Baladi rabbit's breeds collected randomly from does according to the institutional ethical norms of the Faculty of Agriculture, Suez Canal University, Egypt. About 1 ml of blood from the marginal ear vein was individually collected in a tube treated with K3-EDTA (FL medical, Italy) and stored at 20°C until DNA extraction. Live body weight for each animal at 17 months of age was recorded.

DNA extraction

Genomic DNA extracted by using PureLink Genomic DNA Mini; Microcentrifuge spin-column format (Invitrogen™ K182001, USA) to provide a flexible method that meets the requirements of current demands including: Superior performance and high purity and yield of extracted DNA. The quality of extracted DNA examined by NanoDrop® ND-1000 UV-Vis Spectrophotometer enabling highly

accurate analyses of extremely small samples with remarkable reproducibility.

Selection of markers and genotyping

Four microsatellite markers were selected (Table 1). Criteria for selected primers are the size of product in such a way that all selected markers are polymorphic. Inception, allele-size scoring was measured according to DNA ladder size (50-1000 bp). To facilitate, all markers obtained were first tested on the rabbit's genomic DNA for polymorphism, then the PCR reactions were performed in a final volume of 50 µl reaction mixture composed of 3µl DNA (40 ng/µl), 45µl of PCR SuperMix 1.1x concentration (Invetrogen, USA), 1.5µl of each primer (10 pmol/µl). The amplification conditions on a Genemate B960 gradient thermal cycling platform were as follows: initial denaturation step at 94°C for 4 min, 35 cycles of amplification (40s of denaturation at 94°C, 60s of annealing at 55°C, 58°C or 60°C based on the optimal annealing temperature for the used primer, 60s of extension at 72°C), and followed by final extension at 72°C for 10 min. PCR products were electrophoresed on 1.5% agarose gel containing 0.5 % ethidium bromide which viewed under UV light, and documented using Uvp-BioDoc- It™ system. Therefore, genotyping of the microsatellite markers were analyzed in the automatic multi-capillary electrophoresis QIAxcel system using the QIAxcel DNA Screening Kit. Polymorphism information content (PIC) was calculated according to the formula:

$$PIC = 1 - \sum_{i=1}^j p_i^2 - 2 \sum_{i=j+1}^j \sum_{j=1}^{i-1} p_i^2 p_j^2$$

$$H_e = 1 - \sum_{i=1}^n P_i^2,$$

$$N_e = 1 / \sum_{i=1}^n P_i^2, \text{ and}$$

$$PIC = 1 - \sum_{i=1}^n P_i^2 - \sum_{j=1}^{n-1} \sum_{j=i+1}^n 2P_i^2 P_j^2$$

where P_i and P_j are the frequencies of the i^{th} and j^{th} alleles at a locus with l alleles in a population, respectively and n was the number of alleles (Botstein et al., 1980) by using CERVUS version 3 software (Kalinowski et al., 2007).

DNA Sequencing

To evaluate the genetic relationships between breeds, partial sequences of the mitochondrial COI gene, which can provide information on phylogeny in delineating species were amplified. Universal Primers for COI gene were used to generate around 660bp amplicon for the COI gene using *CoiF* (5'-TTCTCAACTAACCAYAAAGAYATYGG-3') and *CoiR* (5'-TAGACTTCTGGGTGGCCRAARAAY-3') as Ward et al. (2005) indicated. PCR condition described by Kochzius et al. (2010). PCR products were cleaned using The QIAquick system uses a simple bind-wash-elute procedure. DNA fragments purified with the QIAquick system are ready for direct use in sequencing using the forward primer. Therefore, the COI marker was used for PCR amplification. Each 25µl reaction contained 12 µl 2X MasterMix (Qiagen), 40ng of gDNA, 20 pmole of each primer and miliQ water. Master cycler gradient (eppendorf) was used for amplification using the subsequent thermal profile, a pair of minutes at 94°C of initial denaturation step, followed by thirty-five amplification cycles (30s at 94°C, 30s at 52°C, 3 min at 72°C), then one final step for 10 min at

72°C as final extension. electrophoresis, Gel image was captured using gel advanced ver.2 software package and EgGel-analyzer software was applied to check the relative concentration of the band. PCR product was visualized in 1.5% agarose gel documentation system then analyzed and performed using BigDye Terminator v3.1 Cycle Sequencing Kit, for a total volume of 20 µL each reaction contained 8 µL Terminator prepared reaction mix, 3.2 pmol Primer, DNA template (template quantity was calculated according to the PCR product size) and miliQ water. The thermal profile for Cycle Sequencing PCR was 1 min at 60°C. When further step of purification with CENTRI-SEP Columns (PRINCETON SEPARATIONS), DNA sequencing was applied by 3500 Genetic Analyzer, Applied Biosystems. 96°C and 25 Cycles (10s at 96°C, 5s at 50 and 60°C for 4 min.

Statistical analysis

Phenotypic analysis

Shapiro-Wilk normality test as proposed a highly intuitive goodness-of-fit test of normality with nuisance location and scale parameters was performed (Shapiro and wilk, 1965) followed by a Quantile-Quantile Plot (Q-Q plot). This can give an appraisal of "goodness of fit" that is graphical, instead of diminishing to a numerical synopsis. Both of goodness of fit and analysis of variance were implemented by the R packages (R Core Team, 2013), least squares means, and standard errors of body weight was estimated by using "emmeans" package in R according to Lenth (2016), and a compact letter display (cld) method that listings the LS means accompanied by grouping symbols for pairwise contrasts, and P value adjustment with "tukey" method for comparing the breeds of body weight estimates was used by

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“multcompView” package (Graves et al., 2015) which are available from the comprehensive R archive network (<https://cran.r-project.org/>)

Genotypic analysis

From the data observed for codominant markers, genetic diversity was assessed by calculating the observed (N_o) and effective (N_e) number of alleles, the observed (H_o) and the expected (H_e) heterozygosity using the GenAlEx 6.5 program (Peakall and Smouse, 2012). Polymorphism information content (PIC) was assessed using the program Cervus 3.0.7 (Kalinowski et al., 2007). The F_{ST} statistics of pairwise genetic differentiation among the populations (F_{ST}), reduction in heterozygosity due to inbreeding for each locus (F_{IT}) and the diminishment in heterozygosity due to inbreeding within each population (F_{IS}) were calculated. Additionally, deviation from HWE at each locus in each population was tested using GenAlEx 6.5. To minimize the consequences of genotyping errors, those alleles found in only one population in at least two individuals were considered to be private ones. Chi-square test was performed to identify the existence of significant differences between breeds with regard to genotypic frequencies and significant associations between the allelic segregation of the markers. Consequently, the association between microsatellite markers and BW trait was evaluated with the generalized linear model by R as follows:

$$Y_{ijk} = \mu + G_i + B_j + e_{ijk},$$

where Y_{ijk} is the observed value of the ijk^{th} trait; μ is the mean value of the trait; G_i is the effect of the i^{th} genotype; B_j is the effect of the j^{th} breed; and e_{ijk} is the random error effect ($P \geq 0.05$). The statistical model was based on that described by Ma et al

(2014) with amendment that followed by multivariate analysis of variance which was performed using “mvtnorm” package in R for the association between detected alleles and phenotype observations.

Sequence read analysis

The arrangements acquired were broke down utilizing Basic Local Alignment Search Tools BLAST (Zhang and Madden, 1997). For various sequence alignment, Clustal-omega programming has been utilized (Sievers et al., 2011). Therefore, sequences were submitted to GenBank (Acc Nos MK412567 and MK412568 for Red Baladi, and Sinai Gabali, respectively). Investigations were led utilizing the Maximum Composite Likelihood model (Tamura et al., 2004). This examination included two sequence reads. Codon positions included were 1st+2nd+3rd+Noncoding. Every uncertain position was expelled for each sequence pair (pairwise erasure choice). There was an aggregate of 685 positions in the last dataset. Evolutionary investigations were led in MEGA X (Kumar et al., 2018).

RESULTS AND DISCUSSION

Goodness of fit analysis

For small sample sizes the Shapiro-Wilk perform much better than any other normality test. The p-value is larger than $\alpha 0.05$ ($0.06085 > 0.05$), Therefore, the hypothesis that the respective distribution is normal shouldn't be rejected. If the sample size is adequately expansive this test may distinguish even unimportant takeoffs from the null hypothesis, as occasion, despite the fact that there might be some impact of statistically significance, it might be too small to even think about being of any practical significance. As a sample size was 25 per breed, thus, additional investigation of the effect size is typically advisable (Field 2009), and performed as Q-Q plot. The

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results revealed that the data of both RB and SG breeds are valid for further statistical analysis (Figure 1). Where, Q–Q plot is commonly a more dominant way to deal with this than the basic procedure and is generally used to contrast an informational collection with a hypothetical model (Thod 2002). Q–Q plots are additionally used to compare two theoretical distributions with each other (Gibbons and Chakraborti 2003). Therefore, the analysis of variance was implemented.

Body weight

The analysis of variance for body weight data revealed that there was a highly significant difference on live body weight between RB and SG ($P \geq 0.001$), where the pairwise analysis resulted in the difference of body weight between RB and SG was 419 ± 83.3 , the least square means were 3188 ± 47.1 , and 2768 ± 68.7 for RB and SG breeds, respectively.

In the present study, the similarity percentage between sequence reads for RB and SG breeds were 99%. At a starting codon 3, the RB has 227 amino acid, and the SG has 226 amino acid, may be due to both breeds were existing in the same ecosystem for long time. Furthermore, there was a solid association between recombination problem areas and high density of the 7-nucleotide oligomer “CCTCCCT” both in human (Myers et al., 2005) and mouse (Shifman et al., 2006). Consequently, the highly density segment (CCTCCCT) has been blasted against the all obtained sequence reads using BLASTN software (ver. 2.3.1), resulted in there was no verification for similarity or interpretation between them. Moreover, two single nucleotide polymorphisms have detected (SNP: C>A, and G>A) located at 273, and 275 bp (Figure 3). There was a significantly association

between the enclosed C>A SNP with BW. Needless, to report that more advanced analysis should be done on both level laboratory and bioinformatics analysis to get more information and add discovered SNPs to the rabbit’s maps. Also, Fabuel et al., (2004) and Ruane, (2000) emphasized the preservation decisions for animal genetic resources should consider molecular marker-based genetic diversity as well as different factors, for example, productive performance, specific traits, and future financial interest. Nevertheless, the generalized linear model analysis showed significant association between the live body weight and SAT4 locus ($P \geq 0.05$) that revealed feasibly, SAT4 locus linked with the gene(s) that responsible regulator for rabbits’ body weight. Similarly, Xin-Sheng et al. (2008) reported that SOL33 locus linked with the gene D which manipulate wool yield at Wan line Angora rabbits.

Microsatellite marker

A total of eighteen alleles were discovered across the breeds. The typical range of alleles per locus discovered over loci and breeds was 3.63 ± 0.26 alleles. The highest observed number was five alleles and was detected in markers SOL44 and SAT13, however the lowest number was four alleles and was detected in markers SOL33 and SAT4. These results were relatively coordinated with Xin-Sheng et al. (2008) who found that the average number of alleles was 4.5 alleles in Wan line Angora rabbit which ranged from 3 to 6 alleles. Furthermore, Tian-Wen et al. (2010) found that the average number of alleles was 6.63 and varied from 2.86 for SAT8 to 9.92 alleles for SOL44. Interestingly, the average number of alleles for SOL44 was 2.28 and 2.96 for SG and RB, respectively (Tian-Wen et al., 2010). Likewise, El Aksher et al. (2016)

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found that the average number of alleles for Moshtohor line rabbits was 6.75, with the highest values which were 10, 9, 8, and 7 alleles for SOL33, SAT13, SOL44 and SAT4 loci, respectively. Allelic frequencies across microsatellite loci were mostly polymorphic (Figure 4), and this is due to the differences in distribution of the allele frequency for each allele size among the breed. The highest allele frequencies were 0.66 and 0.74 for the markers SOL33 with the allele sizes of 214 bp and SAT13 and 0.62 for the markers SAT13 with the allele sizes of 150 bp in SG and RB rabbits, respectively. These results agreed with Xin-Sheng et al. (2008) who found that the allele frequencies were ranged from 0.049 to 0.48 for marker SAT13, and from 0.98 to 0.412 for marker SOL44.

The observed and expected heterozygosity across breeds

The observed (H_o) and expected (H_e) heterozygosity and the polymorphic information content (PIC) for each marker across the populations are presented in Table 4. The most widely parameters used to measure the genetic diversity across and within the populations is H_e or the gene diversity as defined by Nei (1973). The H_o in all microsatellite markers was higher than H_e at both of SG and RB rabbits. The means of H_e values were 0.67 ± 0.06 and 0.74 ± 0.1 for SG and RB, respectively. The H_o for different markers averaged 0.7 ± 0.06 and ranged from 0.52 at marker SOL33 to 0.90 at markers SAT4. The overall mean of H_e was 0.574 ± 0.04 , and ranged from 0.45 at SOL33 to 0.69 at SAT4. These results in full agreement with Ben Larbi et al. (2014) who realized that H_o ranged from 0.3 to 0.53 across 36 loci used in twelve rabbit populations. The distinguished results might be due to the number of markers and/or the number of populations that used. Similarly, to the

obtained results, Xin-Sheng et al. (2008) found that the highest heterozygosity was 0.721 at locus SOL33, and the lowest level of heterozygosity was 0.63 at SAT4.

The polymorphic information content (PIC) might be used to ascertain the heterozygosity and the alleles' numeral in the population. The PIC average is 0.53 with the values ranging from 0.41 at locus SOL33 to 0.65 at loci SAT4. These values were similar with those of Xin-Shenget al. (2008) who found that the average PIC was 0.642 with the range from 0.559 at locus SAT4 to 0.705 at locus SOL33. Similarly, Schwartz et al. (2007) found the lowest PIC was at locus SOL33 (0.27) and the highest PIC value was at locus SAT16 (0.70). Also, Xin-Sheng et al. (2008) found that the average was 0.642, the highest PIC value was 0.705 at SOL33 locus, and the lowest PIC value was 0.559 at SAT4 locus. Additionally, El-Aksher et al. (2016) found the average of PIC was 0.76 (ranged from 0.60 to 0.86). Therefore, the microsatellite markers that used could propose their effectiveness in the genetic polymorphism studies and linkage mapping programs in rabbits (Schwartz et al., 2007; Xin-Sheng et al., 2008; Hongmei et al., 2008; Tian-Wen et al., 2010).

Hardy-Weinberg Equilibrium and private alleles across the breeds studied

Among the four loci, the Hardy-Weinberg equilibrium was highly significant ($P \geq 0.001$) for both of RB and SG for SOL44 locus. Although, SOL33 locus was a highly significant for SG, it was not significant for RB. Instead, the SAT4 locus was highly significant in RB, it was significant in SG breed (Table 2). Moreover, all the microsatellite loci in this examination were polymorphic, showing that the loci were appropriate for the genetic investigation of lab rabbits in

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Egypt. Private alleles were likewise present in both breeds and were mostly realized in SG. Whereas, the SG had four private allele and RB had three private alleles (Figure 6). regards to SG, the locus SOL33 had two private alleles at 220 and 240 bp with equality of allele frequency (0.08) (freq: 0.08). The locus SAT13 had private alleles for both breeds (158 bp (freq: 0.10), and 167 (freq: 0.08)) in SG and RB, respectively. Although the locus SOL44 had two private alleles at 216, and 228 bp with the equality of allele frequency (0.14) in RB, it had one private allele at 252 bp (0.10) as in SG (Figure 6).

Genetic Variation and breeds diversity

To estimate the genetic variation of the two rabbit breeds, two parameters were evaluated in this study: genetic differentiation (F_{ST}), and genetic distance. The negative F_{IS} values observed for all studied locus (Table 3) as Tian-Wen et al. (2010) when observed negative F_{IS} values. Contradictory, El-Aksher et al. (2016) found positive values for F_{IS} but it was

closed to zero for SAT13, and SOL33 that indicated low inbreeding within the population. In addition, the negative F_{IS} values would reflect random sampling error or the individual had a fewer homozygotes than one would expect by chance at the genome-wide level.

The values of F_{ST} four locus a cross breeds are shown in Table 3. The F_{ST} values varied from 0.008 (SAT13) to 0.27 (SOL44). Other reports indicated that the positively low F_{ST} (0.0137 and 0.099) (Grimal et al., 2012; Tian-Wen et al., 2010). Also, F_{ST} comparisons from totally different elements of the genome will offer insights into the demographic history of populations (Holsinger and Weir, 2009). A previous report prompt that microsatellite markers employed in studies of genetic variation and distance ought to don't have any fewer than four alleles so as to cut back the standard errors of distance estimates (Barker, 1994) and that such microsatellite markers ought to have a H_o of between 0.3 and 0.8 within the population (Takezaki and Nei, 1996).

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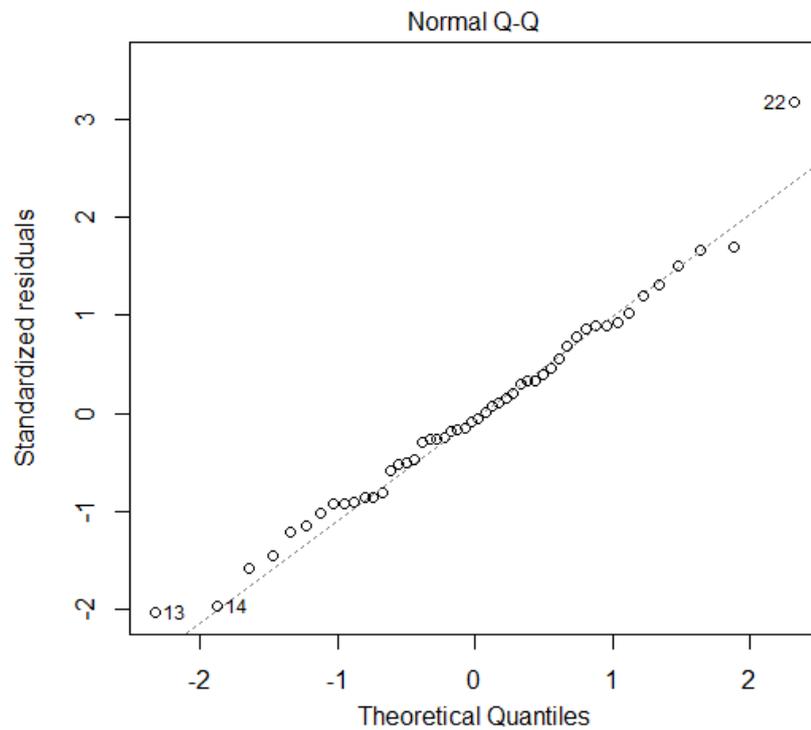


Figure (1): The normal Q–Q plot comparing randomly generated an independent standard normal data on the vertical axis to a standard normal population on the horizontal axis. The linearity of the points suggests that the data are normally distributed.

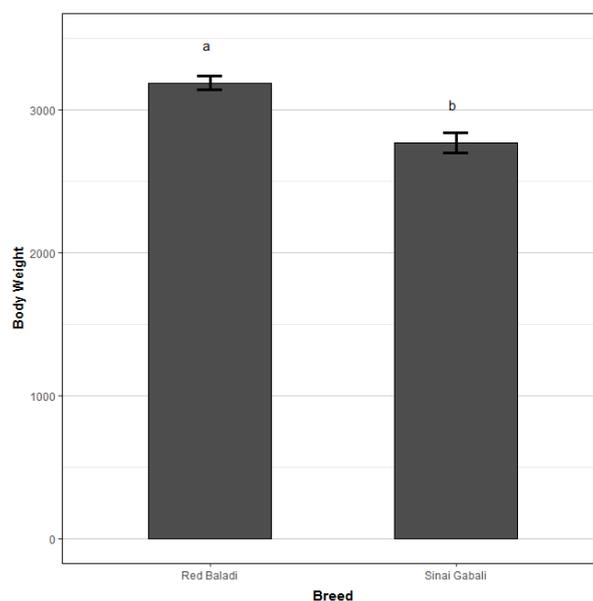


Figure (2): Least square means \pm SEM of individual body weight in Red Baladi and Sinai Gabali rabbits. ^{ab}Means with different superscripts are significantly different ($p \leq 0.05$). The line above each column is standard error of mean (SEM).

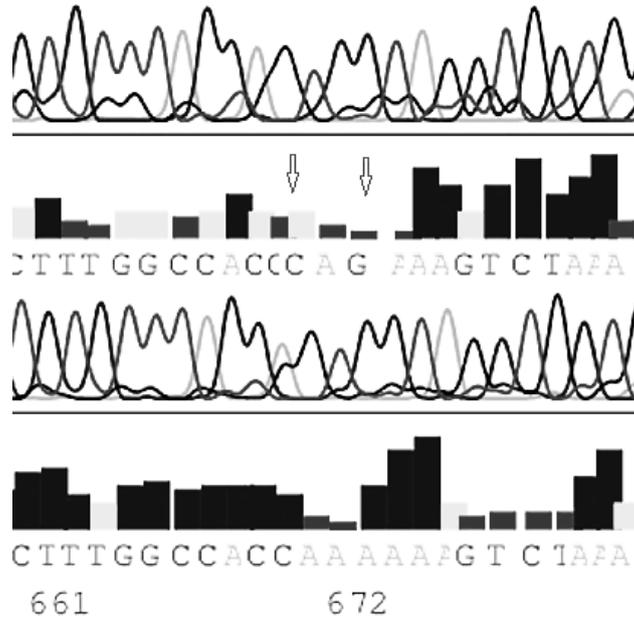


Figure (3) Sequence map of the two genotypes of rabbit COI gene in codon 3 region. The arrow shows the single nucleotide polymorphism (SNP) sites.

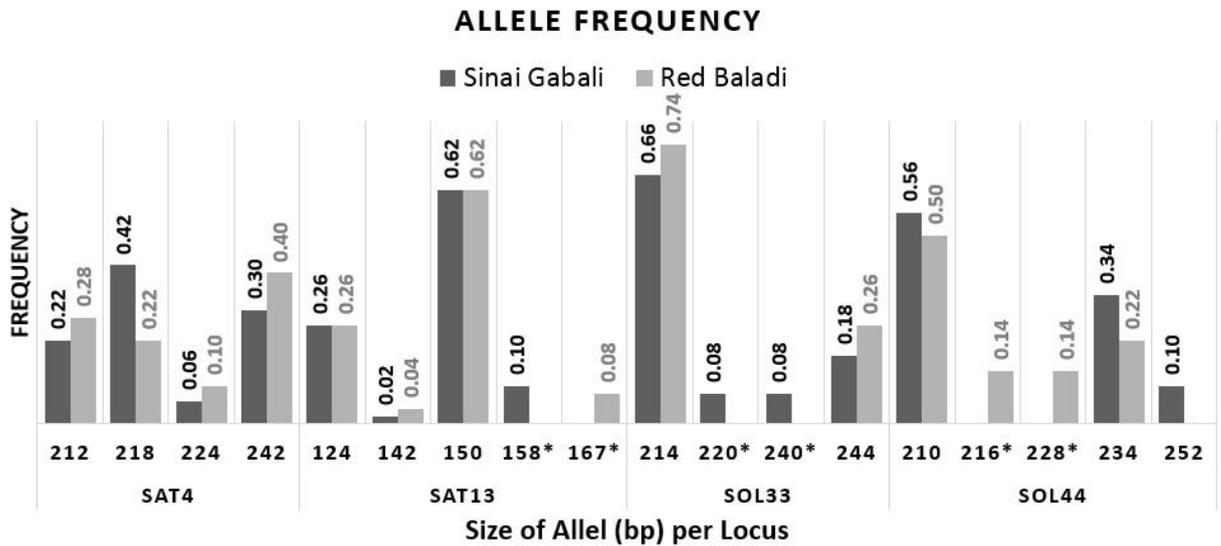


Figure (4): The observed allelic size and frequency in each breed of rabbits per locus. The allele size with asterisk is a private allele for each breed.

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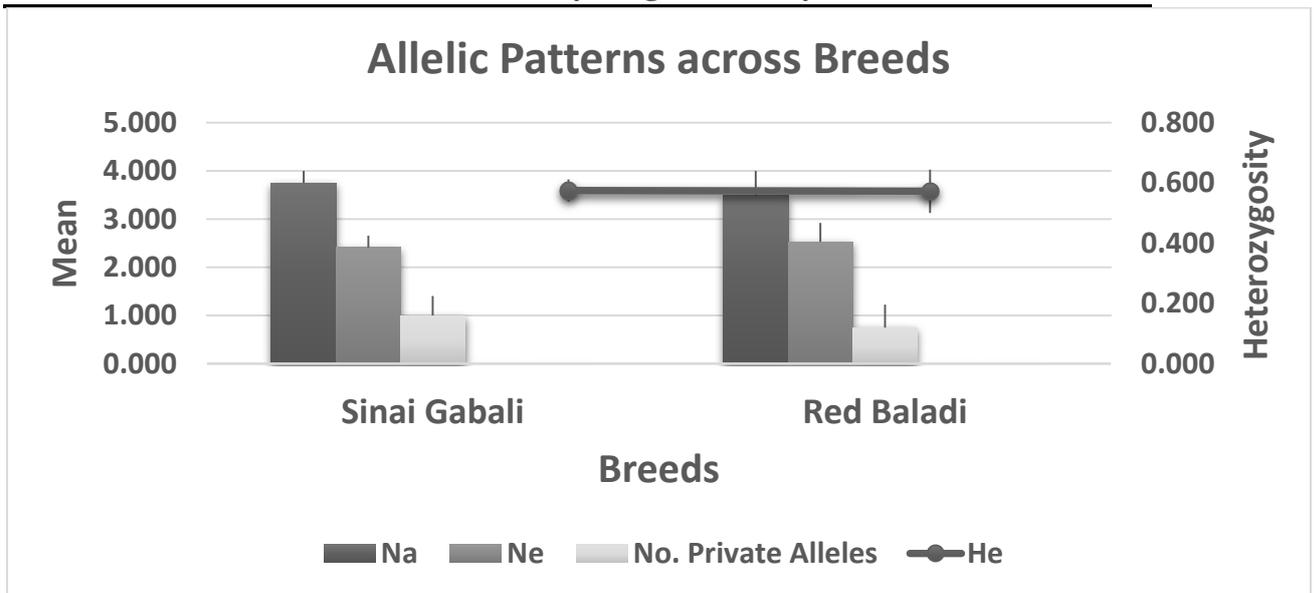


Figure (5): Observed allelic patterns across two breeds studied, Na: number of different alleles, Ne: number of effective alleles, No: number of private alleles, and He: Expected heterozygosity.

Table (1): Molecular characteristics, primer sequences and annealing temperature for four microsatellite loci.

Marker	Sequence of Forward Primer 5'-3'	Sequence of Reverse Primer 5'-3'	Ann.Temp. (°C) ¹
SAT3	GGAGAGTGAATCAGTGGGTG	GAGGGAAAGAGAGAGACAGG	60
SAT4	GGCCAGTGTCTTACATTTGG	TGTTGCAGCGAATTGGGG	60
SOL33	GAAGGCTCTGAGATCTAGAT	GGCCAATAGGTAATGATCCATT	55
SOL44	GGCCCTAGTCTGACTCTGATTG	GGTGGGGCGGCGGGTCTGAAAC	58

¹Annealing temperature (°C).

Table (2):The observed (Ho) and expected (He) heterozygosity and Hardy-Weinberg equilibrium (HWE) for the two studied' breeds.

Breed	Locus	Na	Ne	Ho	He	F	Hardy-Weinberg Equilibrium	
							P-value	HWE
Sinai Gabali	SAT4	4.000	3.141	0.800	0.682	-0.174	0.037	*
	SAT13	4.000	2.163	0.640	0.538	-0.190	0.462	Ns
	SOL33	4.000	2.080	0.520	0.519	-0.002	0.000	***
	SOL44	3.000	2.277	0.720	0.561	-0.284	0.000	***
	Mean± SE	3.750±0.250	2.415± 0.245	0.670±0.060	0.575±0.037	-0.162±0.059		
Red Baladi	SAT4	4.000	3.369	1.000	0.703	-0.422	0.000	***
	SAT13	4.000	2.174	0.680	0.540	-0.259	0.499	Ns
	SOL33	2.000	1.625	0.520	0.385	-0.351	0.079	Ns
	SOL44	4.000	2.962	0.720	0.662	-0.087	0.000	***
	Mean± SE	3.500±0.500	2.533±0.391	0.730±0.100	0.573±0.072	-0.280±0.072		
Over all Mean± SE		3.625±0.263	2.474±0.215	0.700±0.055	0.574±0.037	-0.221±0.049		

Table (3): Characterization of the microsatellite markers used in the present study.

Microsatellite marker (Locus)	Na Mean	Ne Mean	Ho Mean	Hs	Ht	F_{IS}	F_{IT}	F_{ST}	PIC
SAT4	4.000	3.255	0.900	0.692	0.706	-0.300	-0.274	0.020	0.65
SAT13	4.000	2.168	0.660	0.539	0.543	-0.225	-0.215	0.008	0.49
SOL33	3.000	1.853	0.520	0.452	0.458	-0.150	-0.134	0.014	0.41
SOL44	3.500	2.619	0.720	0.612	0.628	-0.177	-0.146	0.027	0.58

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الملخص العربي

التقييمات الوراثية لأرانب البلدي الأحمر والجبلي السيناوي باستخدام المعلمات الوراثية
مايكروستلايت و رمز الباركود للحمض النووي الريبوزي منقوص الأكسجين

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تم استخدام كلا من الجين Cytochrome C Oxidase الأول (cox1) ، وأربعة معلمات وراثية من نوع المايكروستلايت للتحقيق في التركيب الوراثي لسلاطين من الأرانب المحلية ، البلدي الأحمر (RB) ، والجبلي السيناوي (SG). بالإضافة إلى ذلك ، تم دراسة التنوع الوراثي لهذه السلالات من خلال استخدام كل من المعلمات الوراثية وجين COI كالباركود الموحد لتحديد الحمض النووي. تم استخدام مجموعه مكونة من 50 أم (25 أرنب لكل سلالة). تم قياس وزن الجسم الحي عند عمر 17 شهراً، كما تم جمع عينات الدم لمزيد من التحليل. استخدمت أربعة معلمات وراثية (SAT3 ، SAT13 ، SOL33 ، و SOL44). تم اكتشاف إجمالي 18 أليل عبر السلالات. كان عدد الأليلات في الموقع الذي تم اكتشافه عبر المواقع والسلالات 3.63 ± 0.26 أليل. كانت جميع المعلمات المستخدمة متعددة الأشكال وكان متوسط محتوى المعلومات متعدد الأشكال (PIC) 53%. كان متوسط قيم He 0.06 ± 0.07 و 0.1 ± 0.07 لـ RB و SG على التوالي. بلغ متوسط القيمة للعلامات المختلفة 0.7 ± 0.06 وتراوحت من 0.52 عند العلامة SOL33 إلى 0.90 عند العلامات SAT4، بينما كان المتوسط العام لـ 0.04 ± 0.574 وتراوحت بين 0.45 إلى SOL33 إلى 0.69 في SAT4. من بين المواضع الأربعة، كان اتزان هاردي-واينبرغ معنوي جداً ($p < 0.001$) لكل من RB و SG لموضع SOL44. على الرغم من أن موقع SOL33 كان ذا معنوية كبيرة بالنسبة إلى SG، إلا أنه لم يكن ذا معنوية بالنسبة لـ RB. تراوح أعلى انخفاض في قيم اختلاف الزيغوت (F_{ST}) من 0.008 إلى (SAT13) 0.27 (SOL44).

فيما يتعلق بوزن الجسم الحي، كان هناك اختلاف كبير في وزن الجسم الحي بين RB و SG ($P \geq 0.001$) حيث كان الفرق في وزن الجسم بين RB و SG 83.3 ± 0.419 . كان متوسط الأوزان 47.1 ± 2768 و 68.7 لسلاطين RB و SG على التوالي. في هذه الدراسة ، كانت نسبة التشابه بين القراءات لتسلسل القواعد النيتروجينية لسلاطين RB و SG 99%. في 3 codon، يحتوي RB على 227 حمض أميني بينما SG لديها 226 حمض أميني. علاوة على ذلك فقد اكتشف تعدد الأشكال النوكليوتيدية الفردية (C < SNP: A ، و A < G) التي تقع عند 273 و 275 bp. كان هناك ارتباط كبير بين A-C و المرتبطة معنويًا مع وزن الجسم BW. بالإضافة إلى ذلك ، أظهر تحليل النموذج الخطي ارتباطاً كبيراً بين وزن الجسم الحي وموضع SAT4 ($P \geq 0.05$). الخلاصة أنه ثبت من كل المعلمات الوراثية لنوع مايكروستلايت وجين COI كرمز الباركود للحمض النووي DNA نجاحاً فائقاً في التمييز بين السلالات قيد الدراسة والتنوع الوراثي بينها.