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ESTIMATION OF GENETIC DIFFERENCES BETWEEN RABBIT BREEDS USING MICROSATELLITE LOCI ON CHROMOSOMES 5, 7 AND 19

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ABSTRACT: The objective of this study was to differentiate between five rabbit breeds at four microsatellite loci on autosomal chromosomes 5, 7 and 19, and to assess the association between detected microsatellite alleles and some economical traits. The breeds were Baladi White (BWH) and Baladi Red (BR) as native Egyptian breeds, and New Zealand White (NZW), American Rex (AR) and Chinchilla (CH) as exogenous breeds. Genomic DNA was extracted from nine individuals/breed, and screened by four pairs of microsatellite markers (Sat5, D7Utr4A, D7Utr4B and D19Utr4B). A total of 26 alleles was detected, with an average of 6.5 alleles per locus. The within-breed genomic variability was in general high, and was not significantly differed between breeds. The expected heterozygosity was also high and ranged from 0.769 in NZW to 0.826 in CH, with no significant differences between the breeds. The average polymorphism information content (PIC) varied from 0.286 in BWH to 0.593 in CH. The genetic distance indices revealed close genetic relationship between BWH and CH and averaged 2.629, however the genetic distance between AR and NZW averaged 3.355. Many alleles were found to associate many growth and reproduction traits. D7utr4A`4 showed significant association with body weight and chest circumference at 8 weeks of age and body length at 8 and 10 weeks of age. The microsatellite locus D7Utr4B on chromosome 7 showed significant association with parity.

Keywords: genetic distance - microsatellite loci – polymorphism - rabbit breeds.

INTRODUCTION

Production of food sufficient to meet the tremendous increase in the human population is becoming a great challenge in the world, especially in the developing regions where people suffer from malnutrition. The modernization of agriculture systems in the developing countries, based on promoting sustainable agriculture polices, is expected to raise the degree of food security of the strategic food commodities such as animal protein. Therefore, the production of animal protein should move from the massive production to sustainable production systems. Because rabbits are fast growing with short generation interval and great prolificacy, they can be used to secure food and nutrition in the developing regions. The report of the Food and Agriculture Organization (FAO, 2011) denoted to the significant contribution of rabbits in increasing the agricultural income in the developing regions, with a great opportunity to be an alternative source of high quality animal protein.

It is well documented that accurate determination of the genetic variations within and between breeds is a fundamental step toward animal breeding (Anderson and Georges, 2004; El-Gendy *et al*., 2005). Elamin and Yousif (2011) estimated high coefficients of variation for many economical traits in Baladi Black, Baladi Red, New Zealand White, California and V line rabbits, indicating great variability within the populations. Characterization of local and indigenous breeds, evaluating genetic diversity and estimating the extent of genetic variation within and among breeds is needed for the conservation of genetic resources, which in turn secures the future breeding for animal production (Notter 1999; and Bruford *et al.* 2003), and allows for sustainable development.

The microsatellite markers have been used to differentiate between rabbit genetic groups and revealed the presence of specific alleles for each of Baladi White, Baladi Red and Chinchilla (El-Gendy *et al.,* 2014). It was reported that the genetic uniqueness of the local rabbit breeds may contribute to the survival of the climate in Egypt. A number of microsatellite markers has been developed in rabbits from chromosome-specific library and were incorporated into the genetic map. Korstanje *et al*. (2001; 2003) developed microsatellite markers from chromosomes 3-, 5-, 6-, 7-, 12-specific libraries. Some of these markers have been used for linkage studies. Three markers from the Chromosome 5 library (D5Utr2, D5Utr3 and D5Utr4) were linked to linkage group VI (LG VI), and the physical mapping of metallothionein 1 (MT1) gene to chromosome 5 revealed the chromosomal assignment (Korstanje *et al.,* 2003). It was also reported that five markers from the chromosome 7 library (D7Utr2, D7Utr3, D7Utr4, D7Utr5 and D7Utr6) could be linked to the physically mapped D7Utr1. The physical and genetic location of the marker could determine the orientation of the linkage group on the chromosome.

This study aims at the evaluation of genetic differentiation among native Egyptian and exotic rabbit breeds at microsatellite loci on chromosomes 7 and 19, and the association between detected microsatellite alleles and the economical traits of rabbits.

MATERIALS AND METHODS

Animals and blood sampling

Five rabbit breeds were used, and they were Baladi White (BWH) and Baladi Red (BR) as native Egyptian breeds, and New Zealand White (NZW), American Rex (AR) and Chinchilla (CH) as exotic breeds. Five males and four females represented each breed. Blood samples were collected from the ear vein of each individual in sterilized tubes containing EDTA as an anticoagulant.

DNA extraction and microsatellite-PCR analysis

DNA extraction was performed using column GeneJET Genomic DNA Purification Kit (Cat #: K0722, Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturing procedures using 200µl blood of each individual. Upon the completion of extraction, DNA samples were visualized on 1% agarose gel. DNA concentration and purity were measured using spectrophotometer (PG instruments, UK) at 260 and 280 ηm wavelengths according to Sambrook *et al.* (1989). DNA was then stored at -20°C until use.

The PCR procedure was applied to all DNA samples for screening the microsatellite loci using four pairs of microsatellite markers (table 1). PCR reaction was performed on a total volume of 25 µl (3 µl Genomic DNA, 1.5 µl forward primer, 1.5 µl reverse primer, 8 µl PCR master mix and 11 µl PCR grade water). PCR program started with initial denaturation at 94°C for 5 min. the number PCR cycles was set to 35 cycles and consisted of denaturation (94°C for 40 Sec), annealing (45 Sec at 56°C for Sat5 and 60°C for the other primers) and elongation (72°C for 40 Sec for Sat5 and for 120 Sec for the other primers). The final extension was at 72°C for 10 min and the final hold was at 10°C. The PCR products were then separated and visualized, the DNA images were analyzed for DNA band detection, volume and length (bp) using the TotalLab software (TotalLab Ltd, Newcastle, UK).

Molecular Parameters and Statistical Analysis

The allele frequency at microsatellite loci were estimated, and were then used to estimate the genetic variability (V_G) within breeds and the genetic distance indices (GD) between breeds according to Kuhnlein *et al. (*1989), the expected heterozygosity (*He*) within breeds according to Ott (1992) and polymorphic information content (PIC) within breeds according to Tian-wen *et al.* (2010). The analysis of molecular variance was performed, using GENALEX V6.5 (Peakall and Smouse 2012), to examine the distribution of variation and differential connectivity among populations (PhiPT). The neighbor joining clustering method was used to draw the phylogenetic relationship between different breeds. The association analysis was performed between the growth performance and genomic data to assess the linkage with economical traits, using SAS procedures (SAS, 2000).

RESULTS AND DISCUSSION

Genomic variability, expected heterozygosity and polymorphism

The numbers of detected alleles in different rabbit breeds are presented in table (1). A total of 26 alleles were detected in different rabbit breeds, with

an average of 6.5 alleles per locus. The microsatellite primer D7Utr4A recognized 9 alleles and both of D7Utr4B and Sat5 recognized 7 alleles, whereas D19Utr4B recognized only 3 alleles. The total number of alleles detected in each breed ranged 12-14 with an average of 3.0-3.5 alleles. The alleles detected by the marker D7Utr4A were demonstrated as D7utr4A`1 - D7utr4A`9. The alleles detected at D7Utr4B locus were D7utr4B`1 - D7utr4B`7. Three alleles were detected at D19Utr4B locus and were D19utr4B`1 - D19utr4B`3. The alleles detected at Sat5 locus were Sat5`1 - Sat5`7. Tian-wen *et al*. (2010) reported effective number of alleles averaging 6.625 in 7 breeds of rabbits and indicated that the gene polymorphisms and genetic diversity were abundant. Mohamed (2014) reported that recognition of the microsatellite loci in local rabbit populations (Baladi Red and Baladi White) has revealed the genetic uniqueness of each breed that could enable it to survive certain environmental conditions.

The genetic variability within breeds were in general high and averaged 0.715, 0.717, 0.710, 0.734 and 0.702 in Baladi White, Baladi Red, American Rex, Chinchilla and New Zealand White, respectively (Table 2). The statistical analysis showed no significant differences between breeds in the genomic variability. Based on RAPD analysis, Galal *et al*. (2013) reported low genetic variation within each of three local Egyptian breeds and New Zealand White.

The Expected heterozygosity estimates within rabbit breeds are presented in table (2). The expected heterozygosity within breeds was in general high, and was the highest (0.826) in Chinchilla and was the lowest in New Zealand White (0.769), with no significant differences between the breeds. The high variability and heterozygosity within breeds reflect the multi-allelic nature of the loci and also indicate that the populations are maintained in large size. Tian-wen *et al*. (2010) found that American Rex showed the highest expected heterozygosty of 0.889, compared to six other indigenous and exogenous breeds in China. The average *He* of all loci ranged from 0.675 in Fujian Black rabbits to 0.820 in American Rex rabbits.

The average polymorphic information content (PIC) estimates within breeds over the studied loci varied from 0.286 in Baladi White to 0.593 in Chinchilla (Table 2). The low PIC reported in Baladi White may reflect the relatively small size population. Tian-wen *et al*. (2010) reported mean PIC in 7 rabbit breeds ranging from 0.625 to 0.796 and indicated high genetic diversity.

The pairwise genetic differentiation based on F-statistics (F_{ST}) were calculated at 95% confidence (Table 3). The overall F_{ST} estimate was found to be 0.276, indicating moderate level of genetic differentiation in different breeds. Pairwise Fst estimates were generally low as expected, and ranged from 0.024 (between AR and CH) to 0.404 (between BWH and BR). Whitlock and McCauley (1990) reported that smaller F_{ST} is obtained when the genetic variation within population is increased. Hartl *et al.* (1997) stated that F_{ST} is a result of the diversity within-population and the differentiation among different populations. Accordingly, low F_{ST} values may be obtained in larger populations rather than in smaller populations. In addition, gene flow (Nm) values were calculated (Table 3) and ranged from 1.37

between AR and BWH to 0.37 between NZW and BWH.

The genetic distance

The recognized alleles at the four microsatellite loci were used to estimate the genetic distance indices between rabbit breeds (Table 4). The shortest distance was between BWH and CH and averaged 2.629, reflecting minimum mutation rate between both breeds. However, the longest genetic distance was between AR and NZW and averaged 3.355. The genetic distance between BWH and BR was in general intermediate and averaged 3.058. El-Gendy *et al.* (2014) denoted to the significance of the genetic distance estimates between different genotypes in the genetic improvement. RAPD analysis of Egyptian rabbit breeds (APRI line, Baladi Black and Gabali) as well as New Zealand White (Galal *et al*., 2013), showed that the genetic distance between Baladi Black and Gabali rabbits was small reflecting close genetic relationship between both breeds, however the genetic distance index between any of them and New Zealand White was greater.

The genetic distance indices have been also expressed in the phylogenetic tree (Figure 1), which shows that both New Zealand White and Chinchilla were derived from same origin. American Rex was the closest to the local breeds in Egypt. Baladi White and Baladi Red were close to each other and share same origin.

Association analysis

The association analyses were performed to assess the linkage between using the performance (Table 5) and genotypic data. Many microsatellite alleles were found to be associated with many economical traits in rabbits (Table 6). The microsatellite allele D7utr4A`4 showed significant association with 8-week body weight, 8-week body length, 8-week chest circumference (CC) and 10-week body length (BL), and was highly significant associated with 8-week thigh circumference (TC). The allele D7utr4A`5 showed a significant association with 8-week body weight and 10-week body weight and high significant association with 8-week thigh circumference. The allele D7utr4A`6 showed significant association with 6-week chest circumference. The allele D7utr4A`8 showed significant contribution to 6-week thigh circumference, 8-week chest circumference and 8-week thigh circumference. The microsatellite locus D7utr4B seemed to be linked to a QTL influencing parity, since alleles D7utr4B`5 and D7utr4B`6 associated parity (P <0.441). The allele D7utr4B`7 associated chest circumference at 8 and 12 weeks of age ($P$ <0.326). The allele D19Utr4B`1 seemed to associate parity (P <0.0441). The allele D19utr4B`2 was associated ($P$ <0.0430) with body weight and body length at 6 weeks of age. The allele Sat5`7 showed significant association (P <0.0222) with 12-week body weight. Keliang *et al.* (2008) correlated the genomic-RAPD analysis with the reproductive performance traits in American Rex.

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Table (1): Number of alleles detected at microsatellite loci in rabbit breeds

Marker	Sequence	Total alleles	BWH	BR	AR	Ch	NZW
D7Utr4A	F:TGCTAATGTGCCCAGAAAGGTA R: GGCATCCCAAAAGGCAGTAT3	9	4	2	3	4	6
D7Utr4B	F:TAGGCATTTAGGGAGTGAAC R: GGAGGGGGATGGTAGAG	7	4	5	3	4	2
D19Utr4B	TGTATGTGGGTGTGGGTGTAGAG R: ACTGTTGCTTGCTGGGATTTTTA	3	2	3	2	2	2
Sat5	F:GCTTCTGGCTTCAACCTGAC R:CTTAGGGTGCAGAATTATAAGAG	7	3	3	4	3	4
Total		26	13	13	12	13	14
Average		6.5	3.25	3.3	3	3.3	3.5

Baladi White (BWH), Baladi Red (BR), American Rex (AR), Chinchilla (CH) and New Zealand White (NZW)

Table (2): Genomic variability, expected heterozygosity and polymorphic information content (PIC) detected at the microsatellite loci in different rabbit populations

Genetic Variability (GV)					
Microsatellite	**BWH**	**BR**	**AR**	**Ch**	**NZW**
D7Utr4A	0.877	0.889	0.938	0.716	0.716
D7Utr4B	0.841	0.762	0.841	0.714	0.889
D19Utr4B	0.333	0.407	0.481	0.630	0.333
Sat5	0.810	0.810	0.571	0.875	0.873
Mean	0.715	0.717	0.710	0.734	0.702
SD	0.256	0.213	0.217	0.102	0.259
Expected heterozygosity (He)					
D7Utr4A	0.959	0.938	0.985	0.798	0.848
D7Utr4B	0.947	0.892	0.926	0.767	0.935
D19Utr4B	0.333	0.531	0.597	0.786	0.333
Sat5	0.891	0.912	0.656	0.952	0.961
Mean	0.783	0.818	0.791	0.826	0.769
SD	0.301	0.192	0.193	0.085	0.294
Polymorphic information content (PIC)					
D7Utr4A	0.391	0.173	0.084	1.326	1.912
D7Utr4B	0.391	0.850	0.233	0.541	0.069
D19Utr4B	0.000	0.381	0.239	0.209	0.000
Sat5	0.360	0.463	1.306	0.296	0.243
Mean	0.286	0.467	0.466	0.593	0.556
SD	0.191	0.283	0.565	0.508	0.910

Baladi White (BWH), Baladi Red (BR), American Rex (AR), Chinchilla (CH) and New Zealand White (NZW)

Table (3): Pairwise Population Fst Values (blow diagonal) and pairwise population Nm values based on Fst values (above diagonal)

	BWH	**BR**	**AR**	**Ch**	**NZW**
BWH		0.750	1.371	0.488	0.368
BR	0.404		0.737	0.918	0.942
AR	0.210	0.339		0.492	1.305
Ch	0.161	0.214	0.024		0.424
NZW	0.371	0.337	0.253	0.250	

Baladi White (BWH), Baladi Red (BR), American Rex (AR), Chinchilla (CH) and New Zealand White (NZW)

Table (4): The genetic distance indices between different rabbit breeds

locus	**BWH-BR**	**BWH-AR**	**BWH-Ch**	**BWH-NZW**	**BR-AR**	**BR-Ch**	**BR-NZW**	**AR – Ch**	**AR-NZW**	**Ch-NZW**
D7Utr4A	3.906	4.742	4.300	4.039	3.708	3.461	3.955	4.106	4.685	3.418
D7Utr4B	4.154	3.751	3.465	3.861	4.363	4.145	4.414	2.674	3.396	3.28
D19Utr4B	1.606	1.283	1.749	1.693	1.844	2.241	2.381	1.904	1.283	1.749
Sat5	2.565	2.490	1.000	1.000	2.551	1.000	1.000	4.401	4.055	3.844
Mean	3.058	3.067	2.629	2.648	3.117	2.712	2.938	3.271	3.355	3.073

Baladi White (BWH), Baladi Red (BR), American Rex (AR), Chinchilla (CH) and New Zealand White (NZW)

Table (5): Performance data (mean± SD) used for association analyses

Age (wk)	**body weight (g)**	**body length (cm)**	**thigh circumference (cm)**	**chest circumference (cm)**
6	560±-0.04	20.22±0.16	8.79±0.08	11.03±0.89
8	760±1.32	23.46±0.16	9.59±0.08	11.34±0.83
10	960±0.96	25.73±0.18	10.37±0.09	12.2±0.83
12	1170±1.96	27.98±0.18	11.16±0.11	13.52±0.11

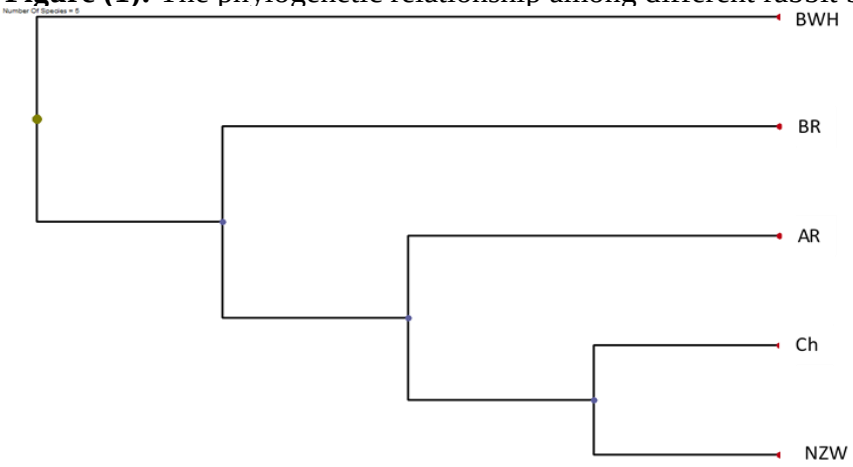
Table (6): The significant contribution of different microsatellite alleles to the economical traits

Microsatellite	Allele	Economical Trait	P<
D7utr4A	D7utr4A`4	8-wk BW	0.0248 *
		8-wk BL	0.0233 *
		8-wk CC	0.0280 *
		8-wk TC	0.0062 **
		10-wk BL	0.0390 *
	D7utr4A`5	8-wk BW	0.0176 *
		8-wk TC	0.0096 **
		10-wk BW	0.0469 *
	D7utr4A`6	6-wk CC	0.0363 *
	D7utr4A`8	6-wk TC	0.0369 *
		8-wk CC	0.0306 *
		8-wk TC	0.0176 *
D7utr4B	D7utr4B`5	Parity	0.0105 *
	D7utr4B`6	Parity	0.0441 *
	D7utr4B`7	8-wk CC	0.0506 *
		12-wk CC	0.0326 *
D19utr4B	D19utr4B`1	Parity	0.0441 *
	D19utr4B`2	6-wk BW	0.0430 *
		6-wk BL	0.0234 *
Sat5	Sat5 7	12-wk BW	0.0222*

* indicates significant effect. ** indicates highly significant effect.

Traits are body weight (BW), chest circumference (CC), body length (BL), and thigh circumference (TC).

Figure (1): The phylogenetic relationship among different rabbit breeds.



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الملخص العربى

تقدير الاختلافات الوراثية بين سلالات الارانب باستخدام مرقمات المايكروساتلابت علي الكروموسومات 5 و 7 و 19

عصام الجندي و مصطفي هلال

قسم الإنتاج الحيواني – كلية الزراعة -جامعة القاهرة -الجيزة – مصر

كان الهدف من هذه الدراسة هو التمييز بين خمس من سلالات الأرانب في مصر باستخدام 4 من مرقمات المايكروساتلايت الموجودة على الكروموسومات الجسمية 5 و 7 و 19 ، وكذلك تقييم الارتباط بين ألاليلات المكتشفة وبعض الصفات الاقتصادية. وكانت السلالات البلدي الأبيض (BWH) والبلدي الاحمر (BR)، والنيوزيلندي الأبيض (NZW) ، الركس الامريكي (AR) والشينشيلا (CH) ، تم استخلاص الحمض النووي وفحصها باستخدام أربعة أزواج من مرقمات المايكروساتلايت وهي Sat5 ، D7Utr4A، D7Utr4Bو D19Utr4B، وتم الحصول علي 26 أليل ، بمعدل 6.5 أليل في كل موقع وراثي. كان التباين الوراثي داخل السلالة مرتفعًا بشكل عام ، ولم يكن هناك اختلاف كبير بين السلالات. كانت نسبة الخليط المتوقعة مرتفعة أيضًا وتراوحت من 0.769 في النيوزيلندي إلى 0.826 في الشنشيلا ، مع عدم وجود فروق معنوية بين السلالات. تراوح متوسط محتوى تعدد الأشكال (PIC) من 0.286 في البلدي الابيض إلى 0.593 في الشنشيلا. كشفت مؤشرات المسافة الوراثية عن وجود علاقة وراثية وثيقة بين البلدي الابيض و الشنشيلا وبلغ متوسطها 2.629 ، ولكن متوسط المسافة الوراثية بين الركس الامريكي والنيوزلندي بلغ 3.355. وتم العثور على العديد من ألاليلات المرتبطة بالعديد من صفات النمو والتكاثر. أظهرالاليل D7utr4A`4 ارتباطًا كبيرًا مع وزن الجسم ومحيط الصدر في عمر 8 أسابيع من العمر وطول الجسم في 8 و 10 أسابيع من العمر. وكذلك أظهر الاليل D7Utr4B الموجود على الكروموسوم 7 ارتباطًا كبيرًا بعدد مرات الولادة.

الكلمات المفتاحية: المسافة الوراثية ، اليلات المايكروساتلايت ، تعدد الأشكال ، سلالات الأرانب.