Molecular investigation of the Arabian horse breeds of racing, productivity and longevity

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ABSTRACT

The Arabian horse Equus caballus is belonging to family Equidae that all of its species are in danger of extinction. We sequenced 1059 nucleotides from three mitochondrial DNA protein coding genes for some breeds of the Arabian and English horses designated for race, productivity and longevity in order to investigate their genetic variation. The sequenced genes were NADH dehydrogenase subunit 2 (ND2), cytochrome C oxidase subunit 1 (CO1) and ATPase subunit 6 (ATP6). ATP6 discriminated among the different breeds where seven substitutions were recorded of which 2 were non-synonymous. The other loci recoded 8 synonymous substitutions of transitions most of which were among the breeds of longevity. The data of CO1 gene were used to construct the relationship of the Arabian horse to the thoroughbreds. Maximum-likelihood (ML) and Neighbor-joining (NJ) analytical methods were used. The two analytical methods showed clustering of the Arabian breeds in one group and the non-sister relationship within the thoroughbreds. One of the thoroughbreds clustered within the Arabian breeds suggesting the possibility of their out breeding. Increasing tendency in the variation of the studied genes was proportional to the utility of these genes in energy production within the mitochondria. The present study is therefore a preliminary step in the conservational efforts of the Arabian horse of equestrian.

Keywords: Arabian Horse; Thoroughbreds; Mitochondrial DNA; Longevity; Race; Productivity; Genetic Diversity

1. INTRODUCTION

Arabian horse (Equus caballus) was believed to be one of the oldest and the most influential horse breeds in the world. Five major breeds of the Arabian horse are known as Keheilan, Seglawi, Abeyan, Hamdani and Hadban with heterogeneous origin [1]. Private farms in Saudi Arabia contain some of the above-mentioned breeds from which some breeds have been hybridized with European racing and performance breeds. Generally, the molecular studies on horse have three aims: the first is to know the genetic relationships between different breeds [1-9], the second is to know their origin [10-16] and the third is to investigate the genes of race, longevity and performance [17-20].

It was believed that the characteristics of race efficiency of the horses are being inherited through the maternal but not the paternal genetics [18]. Genes of mitochondrial DNA play a role in biochemical processes associated with force, speed and performance efficiency [17] and have a role in cellular oxidation reactions required for vital activities. Harrison and Turrión-Gómez [18] studied eight genes of mitochondrial DNA, which have an effective role in the sportive efficiency of horses and proved that this genome is very important and is inherited to generations through the dam. The authors have revealed that different combinations of the genes of sportive efficiency may play a role in the efficiency of racehorses in terms of the race distance and age. A recent study revealed that there are 18 mitochondrial genes influence fitness and performance phenotypes [20]. The author revealed that these genetic data related to exercise suggest that there will be a great interest in studying equine genetics for performance evaluation and sports medicine applications.

The present study aimed to sequence and analyze mitochondrial DNA genes of some available horse breeds from Saudi Arabian private farms in order to investigate their relationship to other breeds such as thoroughbreds. As some molecular studies have supported the assump-
tion that the characteristics of race efficiency are being inherited through the mitochondrial genome, the present study aimed also to discuss the genetic diversity between the breeds of race, productivity and longevity.

2. MATERIALS AND METHODS

We obtained 15 samples from Al-Massara International Equestrian Center. This center is reputed to be one of the largest horse riding centers in the Middle East and the Gulf region. It is located within the esteemed Alwasiliyah district of Taif City. Blood samples were collected from four different breeds, three samples from each one as follow: Arabian horse for Longevity (Saqre, Nijm and Mubarak), English horse for race (Freyal, Ezz, Ahmar), Arabian horse for productivity (Qumrah, Nor Al-Jazeera, Jana) and English horse for productivity (Joy, Zahrah and Tabuk). With respect to the breed for race, samples were collected during rest state and after 15 minutes of exercise.

All blood samples were collected from jugular vein into heparinized tubes, labeled and immediately taken into the lab and stored at −80°C until processing. DNA was extracted from 0.5ml blood samples with QIAGEN spin-column kits (QIAGEN Inc. Valencia, CA, USA) according to the manufacturer’s instruction. Extracted DNA was spectrophotometrically quantified at 260 nm according to the manufacturer’s instruction. Extracted samples were excised after electrophoresis, purified using BioFlux BioSpin Gel Extraction kit (Bioer technology Co., Ltd) and sequenced. Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using an ABI PRISM. BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq-DNA polymerase (FS enzyme) (Applied Biosystems) according to the manufacturer’s protocol. Single-pass sequencing was performed on each template using the above-mentioned PCR-primers. The primers for PCR amplification and sequencing were designed on the basis of published sequences in the GenBank (NC_001640) The positions and sequences of the primers that have been used in both amplifications and sequencing are listed (Table 1).

Nucleotide sequences of the mitochondrial CO1 region (412 bp) were aligned with those obtained from the DDBJ database (4 E. caballus thoroughbred). One additional outgroup taxon (E. asinus) was included in the alignment in order to root the tree. The alignment was carried out by using the DNASIS 3.5 (Hitachi) and MacClade 4.03 (Sinauer Associates, Inc.) with manual adjustments. We conducted the tree analyses by neighbor-joining (NJ) and maximum-likelihood (ML) methods. These analyses were done in PAUP* 4.0b10 [21] by heuristic searches with the TBR branch swapping, 10 random taxon additions and 1000 bootstrap replications for each method.

3. RESULTS AND DISCUSSION

Unambiguous 1059 sites from ND2, CO1 and ATP6 genes for 15 samples of the Arabian breeds of E. caballus for race, longevity and productivity were sequenced in this study. These data were deposited in DDBJ/EMBL Gen-Bank database with their accession numbers (AB663508-AB663552). The sequences were concatenated and the gap-containing sites were deleted so that 916 bp were left for analysis. The data showed base frequencies of $A = 28.3\%$, $C = 29.4\%$, $G = 14.8\%$ and $T = 27.5\%$. Of these nucleotides, 899 were constant and 17 were variables. Six of the variable sites were parsimony-uninformative and 11 were informative under parsimony criterion. The estimated pairwise genetic distance (data not shown) was lower among the different haplotypes ($D$ range = 0.0098 - 0.001) compared to that a these breeds and its con-
generic E. asinus ($D$ = 0.013). The estimated genetic distance showed a tendency to be high between the breeds for race and/or longevity and those for productivity and it was lower between those for race and longevity.

Figure 1 depicts an NJ tree based on the nucleotide se-
quences of 412 unambiguous sites of CO1 gene aligned in 4 specimens of thoroughbred and 7 Arabian Equestrian breeds. An optimal ML tree was also found with similar topology and a negative log likelihood of 816.2.

Table 1. Primers used for PCR amplification and sequencing of horse genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND2</td>
<td>Forward 5' - CCCCGAACCATAGAAGCCTC -3'</td>
<td>320 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse 5' - AGAGCGCTCGGCTCTCTC -3'</td>
<td></td>
</tr>
<tr>
<td>CO1</td>
<td>Forward 5' - GCCCTCTATCCGAGGCTTGA -3'</td>
<td>420 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse 5' - ATTTGGGATAGGGGTGGTGT -3'</td>
<td></td>
</tr>
<tr>
<td>ATP6</td>
<td>Forward 5' - CTATGGGGCCAGGAGGCTATT -3'</td>
<td>320 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse 5' - AAAGGCTTACCAGGAGGAGT -3'</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Neighbor-joining tree constructed from 412 bp of CO1 gene among the different studied breeds. The underlined values at nodes designate the bootstrap values when the maximum-likelihood method was conducted, while the other values represent the NJ bootstrap support.

The overall base compositions of the L-strand as accounted by the ML analysis were A: 26.5.8%; T: 29.2.4%; C: 26.4% and G: 17.9%. The two analytical methods (NJ and ML) showed clustering of the Arabian breeds (those for race, longevity and productivity in one group. This clustering was trustable because of the reasonably strong statistical support (BP = 82). One of the thoroughbreds clustered within the Arabian breeds while the other thoroughbreds showed sister group relationship to the Arabian group suggesting the impurity of these Arabian breeds. The English breeds for productivity were found in a separate cluster within the Arabian group with reasonable statistical support (BP = 82 and 58). A study on the polymorphism of RAPD-PCR and D-loop of the mtDNA supported distant relationships of the Arabian breeds to the thoroughbreds [8].

Nucleotide substitutions are generally considered in terms of changes within the two structural classes of nucleotides (purines and pyrimidines), that is, in terms of transitions and transversions. 320 bp from ND2 gene showed only one synonymous base transition (T to C) in the race samples and in one of the longevity samples (data not shown). For the barcoding gene codes for cytochrome C oxidase, the sequenced 413 bp showed 7 synonymous transitions (A to G and C to T) among the samples from the longevity breeds only (Figure 2). This fragment showed 29 substitutions (11 transversions and 18 transitions) between the studied haplotypes and the thoroughbreds. Among these mutations, 18 were in the third position and 11 were in the first and second positions and therefore, thirteen substitutions were non-synonymous and 16 were synonymous. One of these thoroughbreds showed complete amino acid identities to the present breeds (data not shown).

ATP6 gene showed the greatest variations among the different samples studied (Figure 3) and therefore, it could be considered as an important marker to study the possible polymorphisms in the Arabian E. caballus breeds. The polymorphism of the 320 base pair representing the partial sequence of the ATP6 gene in 15 individuals of Arabian E. caballus was analyzed. Seven polymorphic sites in this fragment defined four haplotypes. These sites included six transitions and one transversion, which accounted for five synonymous and two non-synonymous substitutions. The non-synonymous changes were found in the breeds of productivity and involved a substitution of glycine with arginine at G331 ➝ A331 and a substitution of glutamine with arginine at A392 ➝ G392 (Figure 3). This substitution may be interpretable with that arginine reduces lactic acid production by the inhibition of glycolysis and enhances work capacity and exercise tolerance [22].

The English Thoroughbred is the best known breed of horse in the western world. Thoroughbreds were developed during the seventeenth and eighteenth centuries in England, largely owing to the enthusiasm of English aristocracy for horse racing and betting [12]. Until recently, it was well-known that the paternal origins of these breeds are well documented as being derived from a handful of Middle Eastern stallions, the most influential
of which are Godolphin Arabian, Darley Arabian and Byerley Turk [15]. A further study proved a similar hypothesis that the Arab breed is one of the most influential horse breeds in the World [10]. It is distributed worldwide and has been involved in the formation of many other horse breeds, such as the Thoroughbred [11] or the Lipizzan [13]. An up to date molecular study argued strongly against this claim. Bower et al. [16] stated that there is no evidence to support exclusive Arab maternal origins or a significant importation of Oriental mares (the term used in historic records to refer to Middle East and western Asian breeds including Arab, Akhal-Teke, Barb and Caspian). Instead, they showed that Thoroughbred foundation mares had a cosmopolitan European heritage with a far greater contribution from British and Irish Native mares than previously recognized. As supportive evidence to this hypothesis, Harrison and Turrion-Gomez [18] have stated that The thoroughbred is a composite breed and the various maternal lines have diverse geographical and founder breed origins established prior to the formation of the thoroughbred studbook. Using RAPD-PCR markers, Mahrous et al. [8] revealed a high genetic distance between the Arabian breeds and the Thoroughbred supporting this postulation. The non-sister relationship within the Thoroughbreds in our tree indicated that some of the tested breeds are, perhaps, not purely Arabian, even they are of the best known breeds. This evidence supports the conclusion that many of the Arabian breeds are now mixed with the European thoroughbreds in order to improve their racing performance.

It has been postulated by Harrison and Turrion-Gomez [18] that the mitochondrial gene variation may represent a measurable component contributing to performance variability. The authors have proven this postulation in terms of the existence of D-loop variation in the populations of thoroughbreds. In the present study, increasing tendency in the variation of the studied genes was proportional to the utility of these genes in energy produc-

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**Figure 2.** The aligned nucleotides of the sequenced fragment of CO1 gene in the different breeds as follow: Tabuk (A), Joy (B), Jana (C), Nor Al-Jazeera (D), Qumrah (E), Saqre (F), Nijm (G), Mubarak (H), Freyal (I) and Ahmar (J). The underlined columns are those with polymorphism.
Figure 3. The aligned nucleotides of the sequenced fragment of ATP6 gene in the different breeds as follow: Tabuk (A), Joy (B), Jana (C), Nor Al-Jazeera (D), Qumrah (E), Saqre (F), Nijm (G), Mubarak (H), Freyal (I) and Ahmar (J). The underlined columns are those with polymorphism.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Nucleotides</th>
<th>Breed</th>
<th>Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tabuk (A)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
<td>Joy (B)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
</tr>
<tr>
<td>Jana (C)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
<td>Nor Al-Jazeera (D)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
</tr>
<tr>
<td>Qumrah (E)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
<td>Saqre (F)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
</tr>
<tr>
<td>Nijm (G)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
<td>Mubarak (H)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
</tr>
<tr>
<td>Freyal (I)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
<td>Ahmar (J)</td>
<td>ATGATGGTTT CATGATTTTA TACGGCTTGA</td>
</tr>
</tbody>
</table>

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tion through the oxidative process within the mitochon-
dria. ATP6, therefore showed the highest variability fol-
lowed by CO1 gene. The least variation among the breeds
was shown in ND2 gene.

4. CONCLUSIONS

In conclusion, the present study showed that the genetic
structure of the different Arabian breeds is a preliminary
guide for conservation management of *Equus caballus*. A
wide-range survey to elucidate inter-and intra-breeds ge-
etic diversity is necessary because: these breeds play a
role in the global economy and the greater part of them is
distributing in the developing countries. They are free from
the genes of other foreign breeds, however, many breeds
are found in Saudi Arabia as a result of cross breeding
with European breeds specially, in the private farms.

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REFERENCES


Laughlin, T.F., Bowling, A.T. and Murray, J.D. (1999) A
comparative gene map of the horse (*Equus caballus*).
*Genome Research*, 12, 1239-1249.
doi:10.1101/gr.9.12.1239

ochondrial variability in the D-loop of four equine breeds
shown by PCR-SSCP analysis. *Genetics and Molecular

[4] Georgescu, S.E., Condac, E., Rebedea, M., DumitruTesio,
genotyping using seventeen microsatellites. *Archiva Zootec-
chnica*, 8, 169-175.

etic diversity among horse populations with a special
focus on the Franches-Montagnes breed. *Animal Genetics*,

based on mtDNA analysis. *Genetics Selection Evolution*,

ability in the Spanish Arab Horse assessed via pedigree
doi:10.1016/j.livsci.2007.02.011

etic variations between horse breeds using RAPD markers.

[9] Cieslak, M., Pruvo, M., Benecke, N., Hofreiter, M.,
ning and history of mitochondrial DNA lineages in dom-
doi:10.1371/journal.pone.0015311

resources and their conservation. *The Genetic of the

of domestication. *The Genetic of the Horse*. CAB Inter-
national, Wallingford.

thoroughbred breeding in Newmarket. Cambridge Uni-
doi:10.1016/S0301-6226(02)00079-9

[13] Zechner, P., Sölkner, J., Bode, I., Druml, T., Baumung,
Analysis diversity and population structure in the Lipi-
zzan horse breed based on pedigree information. *Livestock
Production Science*, 77, 137-146.
doi:10.1016/S0301-6226(02)00079-9

[14] Royo L.J., Álvarez, I., Beja-Pereira, A., Molina, A.,
Fernández, I., Jordana, J., Go`mez, E., Gómez, J.P. and
assessed via mitochondrial DNA. *Journal of Heredity*,
96, 663-669. doi:10.1093/jhered/esi116


[16] Bower, M.A., Campana, M.G., Whitten, M., Edwards,
C.J., Jones, H., Barrett, E., Cassidy, R., Nisbet, R.E.R.,
Hill, E.W., Howe, C.J. and Binns, M. (2011) The cos-
mopolitan maternal heritage of the Thoroughbred race-
horse breed shows a significant contribution from British
doi:10.1098/rsbl.2010.0800

[17] Lesage, R., Simoneau, J.A., Jobin, J., Leblanc, J. and
heart rate, blood lactate and aerobic power. *Human

ochondrial DNA: An important female contribution to
thoroughbred racehorse performance. *Mitochondrion*,

ing performance at different racing distances in Tho-
roughbreds and Arab horses. *Czech Journal of Animal
Science*, 51, 523-528.

cise physiology: An overview of the new applications of
molecular biology as positive and negative markers of
42, 561-568. doi:10.1111/j.2042-3306.2010.00299.x

using parsimony (*and other methods). Version 4.0b10,
Sunderland, Sinauer Associates, Inc., Publishers, Sunder-
land.

[22] Burtscher, M., Brunner, F., Faulhaber, M., Barbara Hot-
rub, B. and Likar, R. (2005) The prolonged intake of L-
arginine-L-aspartate reduces blood lactate accumulation
and oxygen consumption during submaximal exercise.