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Full Length Research Paper

The efficiency of mitochondrial DNA markers in constructing genetic relationship among *Oryx* species

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To date, only parts of mitochondrial DNA from cytochrome b, 12S rRNA, 16S rRNA and non-coding D-loop had been sequenced for different species of *Oryx*. Discrepancy in the genetic relationship among *Oryx* species was previously revealed when combinations of these sequences were analyzed. In the present study, various combinations of these data sets and different sampling sizes of the closely related tribes of the family Bovidae were manipulated using bioinformatics. These data were used to provide the genetic kinship among different *Oryx* species. The complete cytochrome b gene sequence was also used alone for the same purpose after excluding the third position of its codons. Using Bayesian (BA), maximum-parsimony (MP), maximum-likelihood (ML) and neighbor-joining (NJ) analytical methods, a single relationship was obtained in which the different *Oryx* species were sisters and the Arabian *Oryx* was the oldest. Our results demonstrated that, the molecular markers and the samples size were more robust and efficient in building the relationship than computational methods.

Key words: Conservation, endangered species, *Oryx*, mitochondrial DNA (mtDNA) markers.

INTRODUCTION

Oryx is a desert herbivorous mammal belonging to the family Bovidae. It consists of four species, which are: the Arabian *Oryx* (*Oryx leucoryx*), scimitar-horned *Oryx* (*Oryx dammah*), plains *Oryx* (*Oryx gazella*) and beisa *Oryx* (*Oryx beisa*). The scimitar *Oryx* inhabits the semi-arid deserts of North Africa and the Saharan region. The Arabian *Oryx* is originally found in Syria, Iraq, Palestine, Sinai and Arabian Pininsula. *O. gazella* and *O. beisa* inhabit Eastern and Southern Africa with three sub-species for the former (Nowak and Paradiso, 1983). Considerable controversy exists regarding the taxonomy of the *Oryx* tribe. Although the validity of *O. dammah* and *O. leucoryx* has been widely accepted, whether *O. beisa* should be considered a subspecies of *O. gazella* is still argued. However, due to their distinct ranges and characteristics, they were discussed here as separate species according to Grubb (2005).

Recently, *Oryx* populations have been sharply decreased and their ranges have been reduced, as a consequence of habitat loss, human disturbance, hunting and poaching. Before the 1970s, *Oryx* inhabited the African continent and all the Arabian Peninsula but was extirpated from the wild in the latter after that (Henderson, 1974). In Saudi Arabia, in 1990, the Arabian *Oryx* was first released into a desert area in the west-central region and later reintroduced into a sand dune (Khan et al., 2008a).

Mitochondrial DNA (mtDNA) is considered as an important tool in studying molecular relationships among various taxa and in estimating their time of origin. MtDNA genes are characterized by their conservation, high variability and lack of recombination (Boore, 1999; Kumazawa, 2007). Mutation rate of mitochondrial genome is different among genes and has been used to examine various relationships. As the different genes in the mitochondrial genome retained somewhat high conservation, these genes were used efficiently in addressing the genetic relationship on the familial, generic and specific levels. However, the control region (CR) acquired much faster mutation rate and therefore, it

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Abbreviations: cyt b, Cytochrome b; **ML,** maximum-likelihood; **MP,** maximum-parsimony; **NJ,** neighbor-joining; **BA,** Bayesian; **mtDNA,** mitochondrial DNA.

Table 1. Genbank accession numbers for the mitochondrial genome regions used in this study.

Taxon	cyt b	12S rRNA	16S rRNA
Oryx leucoryx	AF036286	U86971	U87021
Oryx dammah	AJ222685	U86970	U87020
Oryx gazella	AF249973	U86972	U87022
Addax nasomaculatus	AF034722	U86973	U87023
Hippotragus equinus	AF022060	U86975	U87025
Beatragus hunteri	AF034968	U87038	U86988
Connochaetes gnou	AF016637	U87043	U86993
Madoqua kirkii	AF022070	M86495	M86495
Raphicerus sharpie	AF022050	AF249982	AF249976

is considered to be a powerful tool for inferring relationships within and among populations. This study describes the potential of the protein coding gene (cytochrome b gene) and the combination of more data for understanding the molecular relationships of *Oryx* species.

MATERIALS AND METHODS

The sequences of cytochrome b, 12S rRNA and 16S rRNA genes of representative species from the subfamily Hippotraginae including the three species of the genus *Oryx* (*O. leucoryx*, *O. dammah* and *O. gazella*) and the subfamily Alcelaphinae were obtained from GenBank. Two species (*Rhaphicerus sharpie* and *Madaqua kirkii*) from the subfamily Antilopinae were used as outgroups due to the close relationship between the subfamily Antilopinae and the other two subfamilies (Matthee and Davis, 2001). Table 1 shows the details of these sequences, including the GenBank accession numbers.

The obtained sequences were aligned separately using MacClade v.4. The unalignable and gap-containing sites were deleted and the aligned data were then concatenated so that 1716 bp were used in the analyses. The aligned nucleotide sequences could be obtained from author for correspondence upon request. The tree analyses were done by the maximum-parsimony (MP), maximum-likelihood (ML) and neighbor-joining (NJ) methods with PAUP* 4.0b10 (Swofford, 2003). In these analyses, heuristic searches with the tree bisection reconnection (TBR) branch swapping and 10 random taxon additions have been adjusted. We set the bootstrapping replicates to 1000 with simple additions for the three methods. For the ML analysis, the general reversible model (GTR+I+G) and parameters optimized by modeltest 3.0 (Posada and Crandall**,** 1998) were used. Bayesian analysis (BA) was also used to examine the tree topology. For more details in conditions of the BA analysis, see Kumazawa (2007).

The complete sequence of the protein-coding cytochrome b gene was analyzed separately using the same computational methods after excluding the third position from its codons. Therefore, 1135 and 757 bp were left for constructing the genetic relationship among the three *Oryx* species. *Addax nasomaculatus* was used as an outgroup due to close relationship of *Addax* and *Oryx* (Hassanin and Douzery, 1999; Iyengar et al., 2006).

Partial sequences of the control region for the same species of Hippotraginae were obtained from the GenBank and combined to the data of cyt b, 12S rRNA and 16S rRNA genes. These data were used to construct the relationship using both *Addax* and Roan as outgroups. Data of the control region were obtained from the Genbank with their accession numbers (AJ235326, AJ235324, AJ235325, AJ235310 and AF068840).

RESULTS AND DISCUSSION

In the present study, two data sets with different sampling sizes were used separately: 1716 nucleotides represented the cyt b, 12S rRNA and 16S rRNA genes and 1135 nucleotides represented the complete sequence of the cyt b gene. In the analyses of both data sets, 378 characters were excluded as they represent the third codon positions of the cyt b gene. Therefore, 1338 and 757 unambiguous sites were used to construct the ML tree topology of Figures 1 and 2, respectively. The base frequencies of 1338 were A = 32.2%, C = 28.06%, G = 15.3% and T = 24.4%, while those of the 757 were A = 24.8%, C = 25.1%, G = 18.4% and T = 31.7%. Of the 1338 nucleotides, 1158 were constant and 180 were variables. Ninety seven (97) of the variable sites were parsimony-uninformative and 83 were informative under parsimony criterion. Among the 757 nucleotides, 731 were constant and 26 were variables. Twenty two of the variable sites were parsimony-uninformative and 4 were informative under parsimony criterion.

The best-fit model that explained both data sets was GTR+G. Model parameters for the 1338 data set were as follows: Substitution rate matrix R(a) = 1.287; R(b) = 10.43; R(c) = 0.922; R(d) = 0.315; R(e) = 19.28; R(f) = 1; gamma distribution shape parameter = 0.1865 and proportion of invariable sites (I) = 0. A single ML tree was found with a negative log likelihood score –lnL = 3256.1832. For the 757 data set, model parameters were with equal rates for all sites and proportion of invariable sites (I) = 0. A single ML tree was found with a negative log likelihood score –lnL = 1192.9866.

The two ML trees (Figures 1 and 2) obtained from the two data sets of 1716 and 757 bp showed similar topologies regarding the three *Oryx* species with strong statistical supports (Table 2). Both tree topologies discriminated *Addax* from *Oryx*. The two African species (*O. dammah* and *O. gazelle)* were grouped together and the Arabian *Oryx* was the oldest sister to both. The same topology was revealed by Khan et al. (2008b) when they

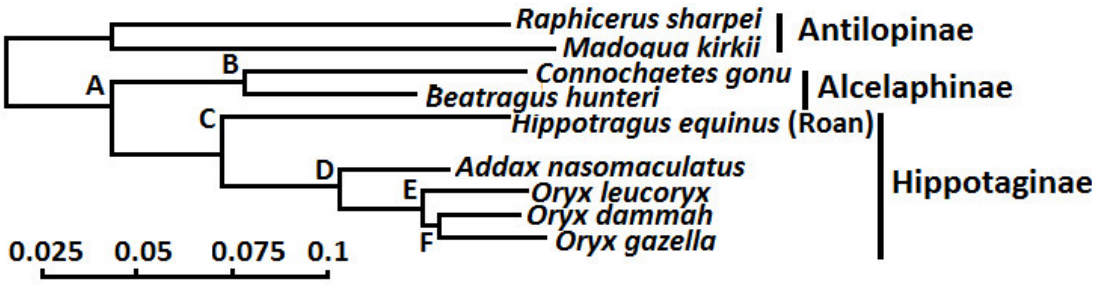

Figure 1. Maximum-likelihood tree constructed from 1338 alignable nucleotide sites of cytb gene, 12 rRNA and 16S rRNA genes. The tree was rooted with the two antilopine species as an outgroup. Bootstrap and posterior probabilities (BP, PP) are shown in Table 2 for each node.

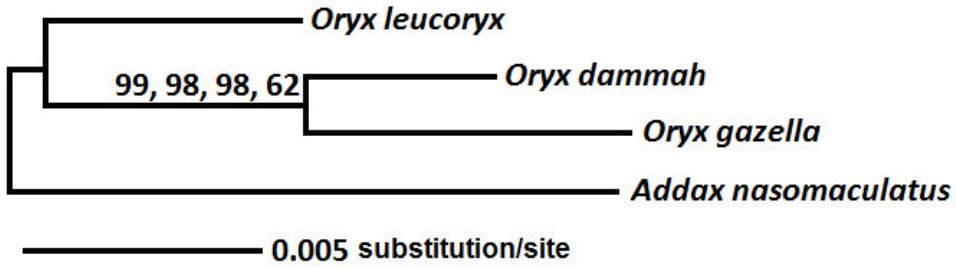

Figure 2. Maximum-likelihood tree constructed from 757 alignable nucleotide sites of cytb gene. Bootstrap and posterior probabilities for maximum parsimony, maximum likelihood, neighbor joining and bayesian analyses are shown at the node (from left to right).

Table 2. Support values in percentage obtained for different computational methods for the data set of cyt b, 12S rRNA and 16S rRNA genes. Number of bootstrap replications and their percentages (BP) at each node were shown for the MP, ML and NJ methods. The Bayesian Posterior probabilities (PP) are also shown at each node.

Method	Bootstrap replications	Node					
		A	B	C	D	E	F
MP-BP	1000	97	87	92	100	74	75
ML-BP	1000	96	96	91	100	100	74
NJ-BP	1000	100	100	73	100	100	62
Bayes-PP	-	100	100	100	100	100	73

used 16S rRNA or cytochrome b genes separately. The appli-cation of both mtDNA markers was appropriate because the results of sequence alignments showed the absence of any indels in these genes.

The sequence of cyt b gene produced a fixed relation-ship, whether we used small or large sample size. The robustness of the relationship was strong probably because the gene is a protein-coding with no gaps. The bootstrap probabilities for MP, ML and NJ were 99, 98 and 98, respectively. Moreover, the cyt b gene is one of the fastest mitochondrial protein genes regarding the mutational rate (Kumazawa et al., 2004) and was expected to yield a number of base changes in the intrageneric analyses. When we used the cyt b gene data set with large sample sizes, the tree topology did not change. A single ML tree similar to that of Figure 1 has been obtained with strong statistical supports (Table 3).

Using the different computational methods, 12S rRNA or 16S rRNA genes (Khan et al., 2008a; Arif et al., 2009) constructed controversial relationships among the three *Oryx* species. The authors did not try to use the combined data and built their conclusion depending mainly on the efficiency of the computational methods without focusing on the sample size.

When the pairwise distance was estimated (Table 4), the results indicated that, the kinship between the two African *Oryx* species was higher than that between any of them and the Arabian *Oryx*. The lowest genetic distance

Table 3. Support values in percentage obtained for maximum-parsimony and maximum likelihood methods for cyt b gene at each node of the tree in Figure 1. Number of bootstrap replications (BP) was shown at each method.

Method	Bootstrap replications	Node					
		A	B	C	D	E	F
MP-BP	1000	86	53	71	92	61	83
ML-BP	1000	93	76	84	92	72	86

Table 4. Pairwise genetic distances from cyt b gene (above) and from the combined 1716 nucleotides (below) for the different studied species.

Species	*Oryx leucoryx*	*Oryx dammah*	*Oryx gazella*
Oryx leucoryx	-		
Oryx dammah	0.048 0.036	-	
Oryx gazella	0.055 0.042	0.044 0.034	-
Addax nasomaculatus	0.069 0.054	0.065 0.053	0.067 0.054

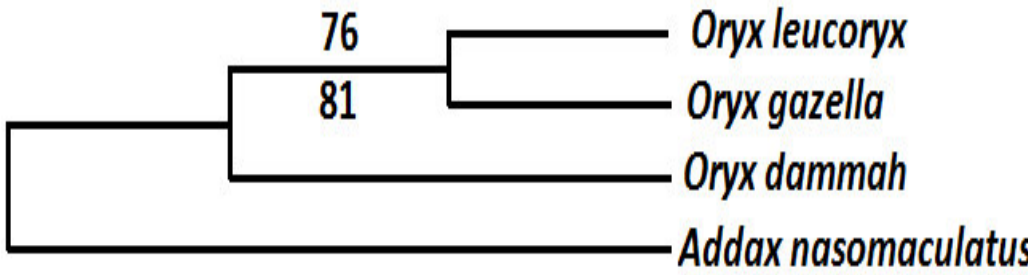

Figure 3. Maximum-likelihood tree constructed from 2466 alignable nucleotide sites of cyt b gene, 12S rRNA, 16S rRNA and control region. The value above the branch is the Bootstrap probability for maximum likelihood analysis.

for both data sets was found between the two African *Oryx* species (0.044 and 0.043). These results are in concordance with the constructed relationships of Figures 1 and 2.

The application of the mtDNA control region by Khan et al. (2008b) seemed to be inappropriate because numerous indels were incorporated for a satisfactory alignment and the samples used were very small in size. So, the obtained tree by the control region in which the Arabian *Oryx* was grouped with *O. dammah* cannot be trusted. We combined the sequence of the control region to that of the two ribosomal RNA genes and cyt b gene and 2466 bp were left to construct the relationship. In spite of the large data set, controversial relationship was obtained by changing the sample size. When we used *Addax* only as an outgroup, the resultant ML tree (Figure 3) was similar to that obtained by Khan et al. (2008b, c). When we added Roan to outgroups, the relationship (Figure 4) retained the same topology as shown in Figure 2. Several investigations approved that, the data from the control region is robust in resolving inter and intra-population relationships and is not efficient for higher rank relationships (Chu et al., 2003; Rodríguez-Monge et al., 2003; Jiang et al., 2005; Eichmann and Parson, 2007; Pan et al., 2007).

The genetic variability within different populations of the *Oryx* species is a matter of interest for conservation strategy's management. With respect to the Arabian *Oryx*, the situation occupies high priority in the light of the recent history of the species, its captive breeding and reintroduction in Saudi Arabia. Several investigations highlighted the importance of genetic diversity in conservation managements of the Arabian *Oryx* (Vassart et al., 1991; Marshall et al., 1999; Arif et al., 2010a**,** b). The present study, therefore, recommends deeper investigations on the population and individual levels in order to build up an appropriate plan for future con-servation.

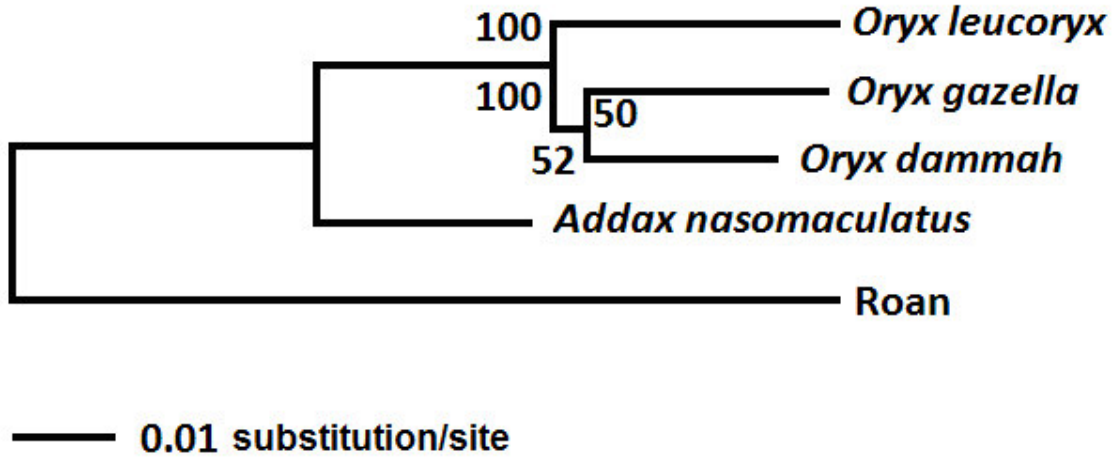

Figure 4. Maximum-likelihood tree constructed from 2292 alignable nucleotide sites of cytb gene, 12S rRNA, 16S rRNA and control region. The values at the branches (complete gene sequence, above; with excluded third position, below) are the bootstrap probabilities for the maximum likelihood analysis.

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