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ESTIMATION OF GENETIC DIVERSITY OF ARABIAN ORYX (*ORYX LEUCORYX*) IN WADI RUM AREA OF JORDAN BY MICROSATELLITE MARKERS

A. Eljarah[1]*, M. Ababneh[2], K. Jawasreh[3], Z. Bani Ismail[1], A. Alhalah[4] and S.M. El-Bahr[5,6]*

[1]Department of Veterinary Clinical Sciences, [2]Department of Basic Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan; [3]Department of Animal Production, Faculty of Agriculture, Jordan University of Science and Technology, Irbid 22110, Jordan; [4]Royal Society for the Conservation of Nature, Amman, Jordan; [5]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, King Faisal University, Saudi Arabia; [6]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Egypt.

*Corresponding author'semail: eljarah@just.edu.jo; sabryelbahr@hotmail.com

ABSTRACT

Data regarding genetic diversity of Arabian oryx(*Oryx leucoryx*) inWadi Rum area of Jordan is not available so far. Therefore, the current study aimed to evaluate the genetic composition and structure of the Arabian oryx(*Oryx leucoryx*) herd in Wadi Rum area of Jordan using microsatellite marker analysis.Forty-nine Arabian oryx individuals were captured using a dart gun. Blood samples were obtained via jugular vein puncture into EDTA-blood collection tubes. Then, DNA was extracted and processed using StockMarks® Animal Genotyping System containing 11 microsatellite markers namely, TGLA 227, BM2213, TGLA53, ETH10, SPS115, TGLA 126, TGLA 122, INRA 23, ETH3,ETH225 and BM1824. Then, the samples were analyzed using ABI 310 Genetic Analyzer. Results obtained were used to estimate the level of heterozygosity and allelic diversity and calculation of allelic frequencies of all microsatellites markers, the mean observed and expected heterozygosity, the fixation index, gene flow and Shannon information index using the software GeneAlEx. In the current study the observed Heterozygosity, Expected and Unbiased Expected Heterozygosity were 0.535, 0.431 and 0.435, respectively. These numbers along with the Shannon's Information Index (0.751) indicates high level of within population genetic diversity. High level of genetic variation among Arabianoryx population of the current study ensures genetic diversity in this population which implies that the Arabianoryx population of the current study is almost likely a naturally living wild population surviving on its own.

Key words: Oryx, genetic, diversity, microsatellite, markers, wadi Rum.

INTRODUCTION

Maintaining genetic diversity is a major issue in conservation biology (Hedrick and Kalinowski, 2000). Loss of genetic diversity can lower the potential of populations to respond to environmental variables, both short term and long term (Lacy, 1997; Miller, 1994). Captive breeding programs can be used to maintain the genetic diversity of populations in addition to restocking wild populations with individuals (Lande and Barrowclough, 1987). However, captive populations are considered as closed populations and after several generations, excessive inbreeding can lead to reduction of genetic diversity (Ralls *et al.,*1988),which is the case of the Arabian oryx (Spalton, 1999). In this study, we demonstrated the genetic diversity levels in captive individuals of the Arabian oryx (*oryx Leucoryx*). In Wadi Rum protected area, Jordan, 49 individuals of Arabian oryx animals are kept in a semi wild environment. This population originates from three different Arabian oryx herds from two countries: The original population comprised individuals from Shaumari wildlife reserve in Jordan and the other two populations were brought from United Arab Emirates (UAE). The two populations that came from UAE were added to the Original herd from Shaumari at a three year interval. Nowadays, the existing oryx population constitutes one population living in Wadi Rum protected area. Despite the fact that this population is kept in a semi-wild-area, it is still considered a captive bred herd. The genetic composition and structure of Arabian Oryx have not been identified so far. Therefore, the current study aimed to determine the genetic composition and structure of the Oryx herd using microsatellite marker analysis, evaluate the genetic variation between individuals of the oryx herd, determine the degree of relatedness between the individuals of the herd and finally to evaluate the effect of addition of new individuals to the herd on the genetic pool of the whole population. The results of the four objectives will help in implementing management strategies to increase the genetic diversity among the Arabian oryx herd by the decision of adding/deletion of new individuals to the herd, the utilization of modern reproductive technologies to assess the breeding potential of males and their selection like semen collection and evaluation and implementing assisted reproductive technologies such as artificial insemination**.**Management strategies will be

initiated based on the genetic diversity of the herd where protective actions or measures should be taken to minimize the harmful effects of low genetic diversity such as vaccination against harmful diseases as well as food supplementation with minerals and vitamins and other management plans to be taken especially during harsh weather.

MATERIALS AND METHODS

Animals and samples for study: A total number of 49 Arabian oryx individuals were used in this study. All Arabian oryxindividuals were captured either using a dart gun or physically restrained using light sedation while passing the animals through crates. Whole blood was obtained via jugular vein puncture into EDTA-blood collection tubes. Blood was transported refrigerated to the laboratory.

DNA Extraction and processing: The obtained blood samples were centrifuged at 4000 rpm for 10 min to isolate buffy coat. Then, DNA was extracted from white blood cells from buffy (Wizard Genomic DNA extraction Kit, Promega, USA). In brief, a total of 100µl of buffy coat added to 900µl of cell lysis solution, mixed by inversion, incubated for 10 minutes at room temperature. The mixture centrifuged at 13,500 rpm for 1 minute, discarding the supernatant and the pellet vortexed. A total of 300µl of nuclei lysis solution added and mixed by inversion, 100 µl of protein precipitating solution added and vortexed for 20 seconds. Afterwards, tubes centrifuged at 13,500 rpm for 3 mins and the supernatant transferred to a new tube containing 300 µl isopropanol. After mixing and centrifuging at 13,500 rpm for 1 min the supernatant discarded and 300 µl of 70% ethyl alcohol added and centrifuged at 13,500 rpm for 1 min. The ethanol aspirated and the tubes allowed air-drying for 15 mins. Finally, 100 µl of DNA rehydration solution added to the tube and incubated at 65 ºC for 1 hour. The resulting DNA extracts stored frozen at -20 ºC. Extracted DNA processed using StockMarks® Animal Genotyping System containing 11microsatellite markers namely, TGLA 227, BM2213, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225 and BM1824 (Arif*et al.,*2010a,Arif*et al.,*2010b; Khidhir*et al.,*2011). Then, the samples were analyzed using ABI310 Genetic Analyzer (Xin, 2008; Peakll and Smouse,2012; Goudet, 2001). Results obtained were used to estimate the level of heterozygosity and allelic diversity and calculation of allelic frequencies of all microsatellites markers, the mean observed and expected heterozygosity, the fixation index, gene flow and Shannon information index using the software GeneAlEx (Peaklland Smouse,2012).

RESULTS

In the current study, 9 microsatellite genes have been amplified. The other two genes (TGLA126 and ETH3) were not amplified because the information gained from amplification of the 9 genes was quiet enough to give an idea about gene diversity of Arabian oryxpopulation. Genes amplified by individuals ID are presented in Table 1-5. Each Table indicated the amplification of 9 genes for 10 ID oryx individuals except for the Table 5 which indicated the amplification of 9 genes for 9 ID oryx individuals as the total number of examined oryx was 49.Analyses of the data presented in Tables 1-5 are shown in Table 6. In Table 6, number of Different Alleles (Na), Number of Effective Alleles (Ne), Observed Heterozygosity (Ho), Expected Heterozygosity (He), Unbiased Expected Heterozygosity (uHe), Shannon's Information Index (I) and Fixation Index (F) are presented. Observed Heterozygosity (Ho) was 0.535, Expected Heterozygosity (He) was 0.431, Unbiased Expected Heterozygosity (uHe) was 0.435, Shannon's Information Index (I) was 0.751 and Fixation Index (F) was -0.164.

Table 1.Microsatellite amplification for 9 genes in the first 10 ID Arabian Oryx individuals.

Sample ID-Locus	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA122	INRA23	ETH225	BM1824
172	60.5 bp	127.6 bp	135.3bp	199.1 bp	243.7 bp	137.3 bp	199.1 bp	137.3 bp	171.7 bp
		129.2 bp	137.3 bp			172.1 bp	244.1 bp	148.1 bp	
17	60.5 bp	127.6 bp	135.3 bp	199.1 bp	244.2 bp	137.2 bp	199.1 bp	137.2 bp	172.5 bp
		129.2 bp	137.2 bp		250.1 bp	172.0 bp	244.2 bp	148.0 bp	
169	60.6 bp	127.6 bp	137.4 bp	199.1 bp	244.2 bp	137.4 bp	199.1 bp	137.4 bp	172.6 bp
		129.3 bp			250.1 bp	172.1 bp	244.2 bp	148.1 bp	
160F	60.4 bp	127.6 bp	137.3 bp	199.1 bp	244.1 bp	137.3 bp	199.1 bp	137.3 bp	172.4 bp
		129.3 bp			250.2 bp	172.0 bp	244.2 bp	148.1 bp	
125C	60.4 bp	127.7 bp	137.3 bp	199.1 bp	244.2 bp	137.2 bp	199.1 bp	137.3 bp	172.4 bp

		129.3 bp				172.0 bp	244.2 bp	148.2 bp	
175	60.4 bp	127.7 bp	135.4 bp	199.3 bp	244.1 bp	137.3 bp	199.1 bp	137.4 bp	172.6 bp
		129.3 bp	137.4 bp			172.1 bp	244.1 bp	148.1 bp	
271	60.7 bp	127.8 bp	135.4 bp	199.1 bp	244.1 bp	137.4 bp	199.1 bp	137.4 bp	172.6 bp
			137.4 bp		250.3 bp	172.2 bp	244.1 bp	148.1 bp	
119	60.5 bp	129.2 bp	135.3bp	199.1 bp	244.2 bp	137.3 bp	199.1 bp	137.3 bp	171.7 bp
			137.3 bp		250.2 bp	172.1 bp	244.4 bp	148.1 bp	
160	60.7 bp	129.4 bp	133.4 bp	199.3 bp	245.2 bp	137.5 bp	199.3 bp	137.4 bp	171.9 bp
			135.3 bp			172.3 bp	245.3 bp	148.4 bp	
033	60.6 bp	125.7 bp	137.5 bp	199.2 bp	244.4 bp	137.5 bp	199.3 bp	137.5 bp	171.8 bp
		127.7 bp			250.3 bp	172.3 bp	244.4 bp	148.1 bp	

Table 2.Microsatellite amplification for 9 genes in the second 10 ID Arabian Oryx individuals.

Sample ID-Locus	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA122	INRA23	ETH225	BM1824
386	60.5 bp	127.6 bp	137.4 bp	199.1 bp	250.5 bp	137.4 bp	199.2 bp	137.4 bp	171.6 bp
		129.3 bp				172.1 bp	250.3 bp	148.1 bp	
108	60.4 bp	125.7 bp	137.4 bp	199.1 bp	250.8 bp	137.4 bp	199.1 bp	137.4 bp	172.0 bp
		129.3 bp				172.2 bp	250.1 bp	148.2 bp	
159	60.5 bp	129.1 bp	135.1 bp	199.1 bp	245.2 bp	137.3 bp	199.1 bp	137.3 bp	172.2 bp
			137.3 bp		250.2 bp	172.1 bp	245.2 bp	148.1 bp	
89	60.3 bp	125.8 bp	137.4 bp	199.2 bp	244.3 bp	137.4 bp	199.1 bp	137.3 bp	172.2 bp
					250.1 bp	172.1 bp	250.1 bp	148.1 bp	
170	60.5 bp	125.7 bp	137.4 bp	199.2 bp	250.2 bp	137.4 bp	199.2 bp	137.4 bp	171.7 bp
		127.6 bp		216.9 bp		172.2 bp	250.3 bp	148.1 bp	
155	60.3 bp	129.3 bp	135.3 bp	199.1 bp	250.3 bp	137.4 bp	199.3 bp	137.4 bp	171.7 bp
			137.3 bp			172.2 bp	250.3 bp	148.2 bp	
B	60.5 bp	127.7 bp	135.4 bp	199.1 bp	250.3 bp	137.3 bp	199.3 bp	137.4 bp	171.9 bp
		129.7 bp				172.1 bp	245.3 bp	148.4 bp	
502	60.6 bp	129.7 bp	135.4 bp	199.1 bp	244.2 bp	137.4 bp	199.2 bp	137.4 bp	171.8 bp
			137.4 bp		250.2 bp	172.1 bp	245.1 bp	148.3 bp	
286	60.5 bp	127.7 bp	135.4 bp	199.1 bp	250.3 bp	137.1 bp	199.3 bp	137.1 bp	171.9 bp
		129.7 bp	137.4 bp			172.2 bp	244.3 bp	148.2 bp	
003	60.5 bp	125.7 bp	135.4 bp	199.1 bp	244.1 bp	137.3 bp	199.3 bp	137.4 bp	172.0 bp
		127.7 bp	137.4 bp			172.3 bp	244.1 bp	148.4 bp	

Table 3.Microsatellite amplification for 9 genes in the third 10 ID Arabian Oryx individuals.

Sample ID-Locus	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA122	INRA23	ETH225	BM1824
110	60.6 bp	127.6 bp	135.4 bp	199.2 bp	250.3 bp	137.4 bp	199.2 bp	137.4 bp	171.7 bp
		129.3 bp	137.4 bp			172.1 bp	250.3 bp	148.4 bp	
120	60.6 bp	125.7 bp	137.4 bp	199.0 bp	250.5 bp	137.5 bp	199.2 bp	137.4 bp	172.8 bp
		127.6 bp		216.9 bp		172.1 bp	250.8 bp	148.1 bp	
154	60.5 bp	127.6 bp	135.4 bp	199.2 bp	244.3 bp	137.4 bp	199.2 bp	137.4 bp	172.6 bp
		129.3 bp			250.3 bp	172.2 bp	244.3 bp	148.2 bp	
167	60.5 bp	127.7 bp	135.4 bp	199.3 bp	244.9 bp	137.4 bp	199.3 bp	137.4 bp	171.9 bp
		129.3 bp	137.4 bp	212.1 bp		172.3 bp	244.3 bp	148.1 bp	
173	60.5 bp	125.6 bp	135.4 bp	199.1 bp	244.3 bp	137.3 bp	199.2 bp	137.4 bp	172.2 bp
					250.3 bp			148.1 bp	
351	60.6 bp	125.7 bp	135.4 bp	199.3 bp	245.2 bp	137.4 bp	199.3 bp	137.4 bp	171.8 bp

		127.6 bp	137.4 bp			172.2 bp	245.3 bp	148.1 bp	
124	60.60 bp	124.8 bp	135.3 bp	199.2 bp	245.0 bp	137.6 bp	199.2 bp	137.4 bp	171.6 bp
		129.4 bp	137.4 bp		250.3 bp	172.1 bp	244.2 bp	148.1 bp	
267	60.6 bp	125.7 bp	137.4 bp	199.2 bp	250.7 bp	137.4 bp	199.2 bp	137.3 bp	171.6 bp
		127.6 bp				172.3 bp	250.2 bp	148.3 bp	
151	60.7 bp	125.6 bp	137.4 bp	199.3 bp	244.1 bp	137.4 bp	199.2 bp	137.4 bp	172.2 bp
		127.6 bp			250.2 bp	172.3 bp	244.3 bp	148.1 bp	
166	60.5 bp	127.7 bp	137.4 bp	199.4 bp	244.7 bp	137.4 bp	199.1 bp	137.4 bp	171.8 bp
		129.3 bp			250.1 bp	172.2 bp	244.3 bp	148.1 bp	

Table 4.Microsatellite amplification for 9 genes in the fourth 10 ID Arabian Oryx individuals.

Sample ID-Locus	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA122	INRA23	ETH225	BM1824
105	60.4 bp	127.6 bp	135.3 bp	199.2 bp	244.3 bp	137.4 bp	199.2 bp	137.4 bp	171.8 bp
		129.2 bp	137.3 bp		250.3 bp	172.2 bp	244.4 bp	148.1 bp	
111	60.7 bp	127.6 bp	135.3 bp	199.3 bp	250.3 bp	137.4 bp	199.3 bp	137.4 bp	171.8 bp
		129.3 bp				172.3 bp	250.3 bp	148.1 bp	
164	60.6 bp	129.3 bp	135.3 bp	199.3 bp	244.5 bp	137.4 bp	199.3 bp	137.4 bp	171.8 bp
			137.3 bp			172.3 bp	244.5 bp	147.7 bp	
165	60.5 bp	129.3 bp	133.5 bp	199.2 bp	245.2 bp	137.4 bp	199.1 bp	137.4 bp	171.8 bp
			135.4 bp			172.2 bp	245.2 bp	148.1 bp	
168	60.5 bp	125.7 bp	135.4 bp	199.1 bp	244.2 bp	137.4 bp	199.1 bp	137.4 bp	172.1 bp
		127.6 bp			250.1 bp		244.1 bp	148.1 bp	
A	60.5 bp	127.3 bp	135.3 bp	199.3 bp	244.3 bp	137.3 bp	199.2 bp	137.4 bp	171.7 bp
		129.3 bp			250.4 bp	172.1 bp	244.3 bp	148.1 bp	
127	60.5 bp	127.6 bp	135.3 bp	199.0 bp	244.2 bp	137.4 bp	199.2 bp	137.4 bp	171.2 bp
		129.2 bp	137.4 bp		250.1 bp	172.2 bp	244.2 bp	148.0 bp	
409	60.5 bp	125.7 bp	135.3 bp	199.0 bp	244.3 bp	137.4 bp	199.2 bp	137.4 bp	171.6 bp
		127.6 bp			250.2 bp	172.1 bp	244.2 bp	148.0 bp	
82	60.4 bp	125.6 bp	135.3 bp	199.0 bp	244.2 bp	137.1 bp	199.1 bp	137.3 bp	171.6 bp
		127.5 bp	137.3 bp		250.2 bp	172.2 bp	244.2 bp	148.2 bp	
005	60.6 bp	129.1 bp	135.5 bp	199.2 bp	244.3 bp	137.5 bp	199.2 bp	137.3 bp	171.6 bp
			137.3 bp		250.3 bp	172.2 bp	244.3 bp	148.1 bp	

Table 5.Microsatellite amplification for 9 genes in the last 9 ID Arabian Oryx individuals.

Sample ID-Locus	TGLA227	BM2113	TGLA53	ETH10	SPS115	TGLA122	INRA23	ETH225	BM1824
010	60.5 bp	127.6 bp	137.5 bp	199.3 bp	244.4 bp	137.6 bp	199.3 bp	137.4 bp	171.7 bp
		129.3 bp			250.3 bp	172.3 bp	244.4 bp	148.1 bp	
163	60.5 bp	127.6 bp	135.4 bp	199.3 bp	244.4 bp	137.4 bp	199.3 bp	137.4 bp	171.9 bp
		129.3 bp	137.3 bp		250.3 bp	172.3 bp	244.3 bp	148.0 bp	
153	60.5 bp	127.6 bp	135.4 bp	199.2 bp	244.3 bp	137.4 bp	199.2 bp	137.4 bp	171.8 bp
		129.3 bp	137.4 bp			172.1 bp	244.3 bp	148.1 bp	
156	60.6 bp	129.3 bp	135.3 bp	199.2 bp	244.9 bp	137.4 bp	199.2 bp	137.4 bp	171.7 bp
			137.3 bp		250.8 bp	172.2 bp	244.0 bp	148.1 bp	
157	60.4 bp	125.8 bp	137.4 bp	199.2 bp	244.2 bp	137.4 bp	199.2 bp	137.4 bp	172.0 bp
		127.7 bp			250.8 bp	172.2 bp		148.1 bp	
158	60.5 bp	127.7 bp	135.4 bp	199.3 bp	244.3 bp	137.2 bp	199.1 bp	137.4 bp	171.7 bp
		129.3 bp	137.4 bp			172.3 bp		148.1 bp	
1612z	60.6 bp	129.3 bp	133.3 bp	199.2 bp	244.1 bp	137.3 bp	199.2 bp	137.4 bp	171.6 bp

			135.4 bp		250.2 bp	172.2 bp	244.2 bp	148.0 bp	
162 G	60.5 bp	129.3 bp	135.4 bp	199.2 bp	245.1 bp	137.5 bp	199.2 bp	137.4 bp	171.9 bp
			137.3 bp		251.2 bp	172.3 bp	244.9 bp	148.2 bp	
171	60.7 bp	125.6 bp	135.4 bp	199.2 bp	244.3 bp	137.4 bp	199.2 bp	137.4 bp	171.7 bp
		127.5 bp	137.4 bp		250.3 bp	172.3 bp	244.3 bp	148.3 bp	

Table 6.Summary of microsatellites data analysis: Calculation of observed Heterozygosity (Ho), Expected Heterozygosity (He), Unbiased Expected Heterozygosity (uHe), Shannon's Information Index(I) and Fixation Index (F).

Pop	Locus (n=9)	N	Na	Ne	I	Ho	He	uHe	F
Pop1	TGLA227	49	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	BM2113	49	6.000	3.055	1.260	0.714	0.673	0.680	-0.062
	TGLA53	49	6.000	2.340	1.015	0.571	0.573	0.579	0.002
	ETH10	49	3.000	1.064	0.156	0.061	0.060	0.060	-0.024
	SPS115	49	4.000	3.267	1.268	0.571	0.694	0.701	0.176
	TGLA122	49	2.000	1.997	0.692	0.959	0.499	0.504	-0.922
	INRA23	49	5.000	2.614	1.152	0.939	0.617	0.624	-0.520
	ETH225	49	3.000	2.041	0.743	1.000	0.510	0.515	-0.961
	BM1824	49	3.000	1.333	0.469	0.000	0.250	0.252	1.000
	Mean	49	3.667	2.079	0.751	0.535	0.431	0.435	-0.164
	SEM	49	0.577	0.276	0.156	0.139	0.087	0.088	0.213

N: number of animals; Na: number of different Alleles; Ne: number of effective alleles = 1 / (Sum pi^2); I: Shannon's Information Index = -1* Sum (pi * Ln (pi)); Ho: observed Heterozygosity = No. of Hets / N; He = Expected Heterozygosity = 1 - Sum pi^2; uHe: Unbiased Expected Heterozygosity = (2N / (2N-1)) * He; F: Fixation Index = (He - Ho) / He = 1 - (Ho / He).

DISCUSSION

The detected heterozygosity, expected and unbiased expected heterozygosity and fixation index are the main criteria that describe the genetic diversity of any population. In the current study, observed heterozygosity, expected and unbiased expected heterozygosityalong with the Shannon's Information Index (0.535, 0.431, 0.435 and 0.751, respectively) indicatedhigh level of genetic diversity within population. Therefore, high level of genetic variation among examined Arabian oryx population ensures genetic diversity in this population. This implies that, the examined Arabian oryx population is almost likely a naturally living wild population surviving on its own capable of disease resistance and fighting, capable of sustaining harsh environmental changes that could face and capable of breeding and living in his environment. This implies also that, management actions like vaccination against diseases should not be taken unless an outbreak of disease appears and necessitates vaccinating the animals against. In addition, extra breeding efforts should not be applied like artificial insemination programs and other assisted reproductive techniques which ensure genetic diversity and dissemination of the genetic pool. However, expecting the life span of the Arabian oryx is between 15 to 20 years, one would expect from the results of the current study that the genetic diversity will decrease and theproblems of low genetic variation could arise like inbreeding after approximately 7 to 10 years. Then, management actions should be considered like disease vaccination and addition and/or removal of individuals from the herd. The current study recommended that, no action is required at the meantime just keeping the herd on the management applied is required. After 7 to 10 years from now, the herd has to be re-evaluated again and requires at that time the addition of new genes to it by exchange of animals with other Oryx keeping facilities. It is advisable at this time to start a cooperation plans with neighboring countries for exchange of animals with those countries having Arabian Oryx individuals and would be great if this occurs at a 5 year interval which helps to maintain the genetic diversity in the herd. Keeping good records and monitoring the herd health and checking for signs of inbreeding such as dwarf animals, breeding/fertility problems (Pregnancy rate and calving rate), young or newborn mortality rates and/orphenotype abnormalities. The current study concluded that, high level of genetic variation among Arabian oryx population of the current study ensures genetic diversity in this population which implies that the Arabian oryx population of the current study is almost likely a naturally living wild population surviving on its own.

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