Genetic diversity in Egyptian buffalo using microsatellite markers

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ABSTRACT

Three bovine microsatellite markers named: ETH02; CSSM060 and BM1706 were used to study the genetic diversity in six Egyptian buffalo populations. The total number of animals sampled were 92, and collected from Alexandria, Kafr El-Sheikh, Great Cairo, Menofya, Al-Minya and Qina. All markers showed polymorphism with a total number of alleles 12 for each of ETH02, and BM1706 and 13 for CSSM060. Heterozygosity and Wright's F-statistics (F_{IS}, and F_{ST}) were calculated to determine the genetic variation in these populations. High values of observed heterozygosities were noticed in all populations. The lowest heterozygosity was 0.846 observed in Qina for the microsatellite BM1706, in contrast to heterozygosity 1.00 observed in many populations, especially for the microsatellite ETH02. All the F_{IS} values were below zero, indicating the absence of inbreeding within the populations under study. Depending on the genetic distances, a dendrogram showing the genetic relationship among the different populations was prepared. High values of the average gene diversity were noticed, for all markers and all populations, ranging from 0.795 to 0.904 with an overall mean of 0.867. Values of gene flow or migration between populations were high, meaning that migration and admixture could have taken place between these populations. It is concluded that the Egyptian buffaloes belong to one breed.

Key words: *Egyptian buffalo, microsatellite, genetic diversity.*

INTRODUCTION

The karyotype of the Egyptian buffalo was first described by De Hondt and Ghanam (1971), it was later confirmed and fully described by Hassanane (1986), it was found that is consists from 5 meta and submetacentric chromosomes. According to the standard karyotype of buffalo, the biarmed chromosomes correspond to the fused 1/25; 2/23; 8/19; 5/28 and 16/29 chromosomes of cattle (Iannuzzi, 1994). The remaining 20 pairs are acrocentric and include the sex chromosomes. Syntenic conservation between cattle and river buffalo were reported (Othman and El Nahas, 1998).

It is believed that the Egyptian buffalo are of one breed with two vaguely differentiated local types, the Beheri of the delta and the Saidi of Upper Egypt. They vary in color, size and production in accordance with differences in management and environment (El-Itriby, 1974). Many animal breeders classify the Delta buffaloes into Beheri, Menofya, and Baladi (Raghab and Askar, 1968).

In the past, all studies of population genetic structure used allele frequency data at protein coding (mainly allozyme) to study the genetic diversity (Ward *et al*., 1992). Recent revolution of molecular biology and discovery of polymerase chain reaction (PCR) provided new methodologies and new markers for the study of genetic variations at the DNA sequence level (Avise, 1994). Microsatellites are, in the meantime, the best molecular markers since they are highly polymorphic, easy to study and score as well as their inheritance is in a Mendelian fashion (Bruford and Wayne, 1993). Microsatellites have many applications in genetic diversity studies, parentage testing, and forensics, constructing of linkage maps and finally mapping quantitative trait loci, QTLs (Kappes *et al.*, 1997).

Only two reports have considered the typing of some microsatellites in Egyptian buffalo, the first was by Hassanane *et al*. (2000), and aimed to identify the possibility of bovine microsatellites to work with the buffalo genome as well as the study the polymorphism. The second was by Moioli *et al*. (2001), and aimed mainly to identify the genetic diversity between the Egyptian, Greek and Italian buffaloes. None of these studies dealt with the buffalo populations living in Upper Egypt region.

Genetic characterization to assess the existing biodiversity and differences among the different Egyptian buffalo populations is an essential prerequisite to facilitate the breeding programs in an effective and meaningful way.

The aim of the present study is to employ some microsatellite markers to identify the genetic variations within and between six Egyptian buffalo populations raised in Egypt, four of them belong to Delta region, and two belong to Upper Egypt. Moreover, answering the question: are these populations of different breeds or of some of them or of one breed? If they form many breeds, so what are their purities and what are the relationships among them?

MATERIALS AND METHODS

Blood sampling

Blood samples were collected from 92 buffaloes raised in six different regions in Egypt. For each animal, 10 ml of blood were loaded in a sterile tube containing EDTA as an anticoagulant. There was no relationship between animals in the same farm. The samples were collected from 6 regions distributed over Egypt and their numbers were as follows: Great Cairo (16), Menofya (17), Alexandria (13), Kafr El-Sheikh (16), Al-Minya (17) and Qina (13).

DNA isolation

DNA was extracted and purified using a standard saline as described by Miller *et al*. (1988). The stock DNA was kept frozen -20°C and its concentration was adjusted to 50 ng/µl before performing the polymerase chain reaction (PCR).

Microsatellites used

The microsatellite markers used in this study were chosen according to a joint meeting recommendation, between the International Society of Animal Genetics (ISAG) and FAO (1998), for genetic diversity studies.

Table (1): Microsatellites and primers used in this study.

Microsatellite	Allelic Range	Primer Sequence (5′-3′)	Stain	Reference
ETH02	192-231	TACTCGTAGGGCAGGCTGCCTG GAGACCTCAGGGTTGGTGATCAG	Fam (Blue)	Solinas -Toldo *et al*., 1993
CSSM060	85-141	AACATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	Hex (Yellow)	Barker *et al*., 1997
BM1706	211-271	ACAGGACGGTTTCTCCTTATG CTTGCAGTTTCCCATACAAGG	Tet (Green)	Slate *et al*., 1998

PCR conditions

PCR was carried out on 50 ng of genomic DNA in 10µl reaction mixture. The three-microsatellite markers (ETH02, CSSM060 and BM1706) were carried out in TaqMan Universal PCR Master Mix (Applied Biosystems). The PCR cycle was: primary denaturation at 95°C for 5 min, denaturation (95°C for 30 sec.), annealing at 57° C for 1 min, and an extension at 72°C for 1 min. Then a final extension cycle at 72°C for 5 min, and storage at 4°C.

The success of the PCR was detected by running horizontally 5 µl of the PCR product on 1.5% agarose gel electrophoresis and stained by ethidium bromide for viewing the bands on the UV transilluminator. The PCR product size was measured according to a size length DNA marker ØX174/*HAE*III run at the same time with the samples.

In order to determine the allele sizes, the successful PCRs were run further on polyacrylamide vertical electrophoresis under denaturing conditions in an automated DNA sequencer (ABI Prism 377, Applied Biosystems) supported by Gene scan and Genotyper software. The polyacrylamide sequencing gel (Long Ranger® Single Pack®, Cambrex USA) was used for running on the sequencer, and the gel was prepared according to the manufacture instructions.

For allele size measurements, 0.9 µl of PCR products were mixed with 2.1 µl Loading Master Mix [250 µl formamide, 50 µl Gene Scan 350 Tamra internal size standard, 50 µl, and 50 µl loading stain (50 ml/gm loading stain, and 1 ml EDTA 25 mM)]. The resulting mixture was denaturated by incubation at 94°C for 5 min and the samples were kept in cold icebox until injection in polyacrylamide gel electrophoresis. Specific size marker was run on special well for determining the allele sizes.

Statistical analysis

POPGENE software package (Yeh *et al*., 1999) was used to calculate allele frequencies, observed number of alleles, effective number of alleles (Kimura and Crow, 1964), observed (Ho) and expected (He) heterozygosity at each locus in the six populations under study. Polymorphism information content (PIC) value for each locus was calculated by using the method described by Bostein *et al*. (1980). Pair-wise allele sharings were calculated manually from the raw data.

Using the variance-base method of Weir and Cockerham (1984), population differe-ntiation by *F*-statistics was computed using FSTAT version 2.9.3.2 computer program (Goudet, 2002). Mean standard deviations of the *F*-statistics program that are analogue to Wright's (1951, 1978). F_{IS} and F_{ST} were obtained across breeds by the Jackknifing procedure over loci (Weir, 1990). The extent of global inbreeding was further studied with the same software by estimated F_{IS} value.

The effects of migration and gene flow on the genetic structure of the analyzed populations were estimated between pairs of populations according to an island model under neutrality and negligible mutation (Slatkin, 1985). Genetic distances among populations were estimated using (Ds)

standard genetic distance of Nei (1972) and the DA distance of Nei *et al*. (1983).

RESULTS AND DISCUSSION

Microsatellite polymorphism

All the microsatellites studied showed polymorphism in the six populations. The numbers of observed alleles were 12 for ETH02, and BM1706 and 13 for CSSM060. This means that these microsatellites could be used for further studies in mapping quantitative trait loci as well as parentage testing.

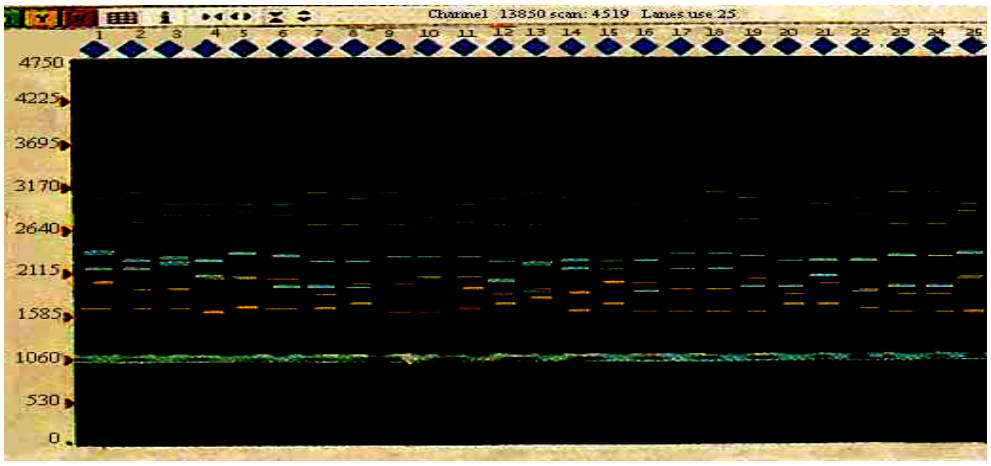

Fig. (1): The separated alleles for the three markers tested on a PCR multiplex, run vertically on polyacrylamide.

Table (2): Observed allele sizes and frequencies for the microsatellite CSSM060 in the different populations under study.

Allele No.	Allele Size (bp)	Cairo	Menofya	Alex.	Al-Minya	Kafr El-Sheikh	Qina	Mean of all populations
1	85	0.000	0.000	0.000	0.118	0.063	0.192	0.124
2	87	0.167	0.177	0.154	0.000	0.125	0.000	0.156
3	89	0.000	0.000	0.115	0.000	0.188	0.115	0.140
4	91	0.100	0.206	0.039	0.088	0.000	0.000	0.110
5	93	0.000	0.000	0.039	0.059	0.031	0.039	0.042
6	95	0.200	0.088	0.192	0.265	0.093	0.115	0.159
7	121	0.067	0.029	0.039	0.029	0.031	0.000	0.039
8	123	0.100	0.118	0.076	0.059	0.125	0.077	0.092
9	125	0.067	0.059	0.039	0.059	0.063	0.077	0.060
10	127	0.066	0.088	0.000	0.029	0.000	0.039	0.056
11	129	0.066	0.147	0.115	0.029	0.125	0.000	0.181
12	131	0.167	0.088	0.192	0.236	0.156	0.346	0.319
13	141	0.000	0.000	0.000	0.029	0.000	0.000	0.029

Details of the observed number, sizes and frequencies of microsatellite alleles are presented in Tables (2, 3, and 4).

In the present study, the microsatellite CSSM060 (Table 2) showed 13 alleles with sizes ranging from 85 to 141 bp, while this microsatellite showed 6 alleles in the Italian buffaloes, 7 alleles in Greek buffaloes, and 7 alleles in a previous genetic diversity of Egyptian buffalo populations (Moioli *et al*., 2001). The difference of allele sizes in Egyptian buffaloes in both studies is expected, since the present study included buffaloes from three different regions (Qina, Al-Minya and Kafr El-Sheikh). On the other hand, microsatellites showed 15 alleles in cattle (Ihara *et al*., 2004). The most frequent allele was number 12 (131bp) and observed with a frequency of 0.346 in Qina population only. In contrast, the lowest allele frequencies were 0.29 for each of the alleles numbered 7, 10, 11, and 13, in Menofya and Al-Minya populations, with sizes of 121, 127, 129 and 141bp, respectively (Table 2).

Table (3): Detected allele sizes and frequencies for the Microsatellite BM1706 in the different populations under study.

Allele No.	Allele Size (bp)	Cairo	Menofya	Alex.	Al-Minya	Kafr El-Sheikh	Qina	Mean of all populations
1	211	0.000	0.000	0.000	0.147	0.031	0.191	0.123
2	217	0.000	0.000	0.077	0.000	0.125	0.000	0.101
3	223	0.133	0.177	0.000	0.177	0.000	0.154	0.160
4	226	0.133	0.029	0.077	0.088	0.063	0.077	0.078
5	229	0.133	0.117	0.192	0.000	0.063	0.000	0.127
6	232	0.100	0.177	0.192	0.118	0.219	0.154	0.160
7	253	0.133	0.088	0.116	0.206	0.155	0.231	0.155
8	256	0.000	0.000	0.192	0.206	0.125	0.154	0.169
9	259	0.200	0.147	0.000	0.029	0.000	0.039	0.104
10	262	0.168	0.265	0.154	0.000	0.125	0.000	0.178
11	265	0.000	0.000	0.000	0.029	0.063	0.000	0.030
12	271	0.000	0.000	0.000	0.000	0.031	0.000	0.031

In the present study, the microsatellite BM1706 (Table 3) showed 12 alleles with sizes ranging from 211 to 271 bp. This microsatellite was shown to be polymorphic in cattle, revealing 10 alleles (Bishop et al., 1994), while it showed 5 polymorphic alleles in Red deer, *Cervus elaphus*, versus no polymorphism in Sika deer, *Cervus Nippon* (Slate *et al*., 1998). The most frequent allele was number 10 (262 bp) with a frequency of 0.265 in Menofya population only. In contrast, the lowest allele frequencies were 0.29 for each of the alleles No. 4, 9 and 11, in Menofya and Al-Minya populations with sizes of 226, 259 and 265bp, respectively (Table 3).

In the present study this microsatellite ETH02 showed 12 alleles ranging in size from 192 to 231 bp (Table 4), while this microsatellite showed 6 alleles in cattle (Solinas-Toldo *et al*., 1993; Ihara *et al*., 2004). The most frequent allele was No.2 (195 bp) with a frequency of 0.385 for the allele in Qina population only. In contrast, the lowest allele frequencies were 0.29 for the alleles No. 3, 5, 8 and 10, in Menofya and Al-Minya populations, with sizes of 219, 198, 204 and 225 bp, respectively; their frequencies were 0.029 only (Table 2).

Table (4): Detected allele sizes and frequencies for the Microsatellite ETH02 in the different populations under study.

Allele No.	Allele Size (bp)	Cairo	Menofya	Alex.	Al-Minya	Kafr El-Sheikh	Qina	Mean of all populations
1	192	0.033	0.177	0.154	0.000	0.000	0.000	0.121
2	195	0.167	0.118	0.039	0.235	0.250	0.385	0.199
3	198	0.000	0.000	0.191	0.029	0.187	0.039	0.112
4	201	0.267	0.147	0.000	0.148	0.000	0.115	0.169
5	204	0.067	0.000	0.039	0.029	0.063	0.000	0.050
6	207	0.000	0.059	0.077	0.059	0.000	0.039	0.059
7	216	0.100	0.117	0.191	0.118	0.125	0.115	0.128
8	219	0.000	0.029	0.077	0.029	0.063	0.038	0.047
9	222	0.133	0.117	0.077	0.000	0.000	0.000	0.110
10	225	0.033	0.000	0.039	0.029	0.000	0.038	0.035
11	228	0.133	0.177	0.077	0.265	0.281	0.231	0.194
12	231	0.067	0.059	0.039	0.059	0.031	0.000	0.051

Observed and expected heterozygosity

The observed average heterozygosity for all populations was higher than the expected heterozygosity (Table 5). This is considered as an indication of heterozygosity excess in all populations and values of (He) and (Ho) were significant ($P<0.01$) overall populations, indicating high genetic differentiation among all populations under study. Statistical significant deviation was shown for all markers (87.255 for ETH02, 86.770 for CSSM060, and 114.73 for BM1706), showing p-values of 0.013, 0.000004, and 0.004, respectively. This indicates the presence of a high level of heterozygosity, which could be due to sorts of specific matings.

The observed value of the heterozigosity is expected, since the blood samples collected were from unrelated animals (FAO regulations for genetic diversity studies).

Heterozygosity is a good parameter to reflect genetic variability within breeds. The high genetic heterozigosity could also be expected due to the presence of mixed generations, mixed populations from different territories, genetic drift, and natural selection (Arranz *et al.*, 2000).

Concerning the measurement of genetic variation within each population (observed and effective number of alleles), observed and expected heterozygosity and polymorphism information content (PIC) are presented in Table (5).

The Polymorphism Information Content is a parameter for indicating the genetic variation; markers with high PIC values are considered highly informative markers (Arora *et al.*, 2004). All markers were highly informative in all the populations studied and showed PIC values ranging from 0.736 to 0.862.

Average gene diversity

The values of average gene diversity ranged from 0.795 to 0.904, with an overall mean of 0.867. Values of gene flow or migration between populations were high, indicating that migration and admixture could have taken place between these populations.

Concerning the gene diversity for the three markers in the six populations studied, the results are presented in Table (6).

Table (5): Microsatellite alleles heterozygosity (Ho, observed; He, expected) and polymorphism information content (PIC) at each locus in the different populations under study.

Populations			ETH02	CSSM060	BM1706	Mean
Cairo	Het.	Ho	0.933	0.933	1.000	0.956
		He	0.874	0.897	0.881	0.884
	PIC		0.827	0.852	0.833	0.837
Menofya	Het.	Ho	1.000	0.941	1.000	0.980
		He	0.893	0.890	0.849	0.877
	PIC		0.852	0.848	0.800	0.833
Alexandria	Het.	Ho	1.000	0.923	0.923	0.949
		He	0.908	0.899	0.874	0.893
	PIC		0.861	0.849	0.820	0.843
Al-Minya	Het.	Ho	1.000	0.941	0.941	0.961
		He	0.854	0.865	0.865	0.858
	PIC		0.808	0.821	0.818	0.816
Kafr El-Sheikh	Het.	Ho	1.000	1.000	1.000	1.000
		He	0.825	0.903	0.895	0.874
	PIC		0.770	0.862	0.853	0.828
Qina	Het.	Ho	0.846	0.923	0.846	0.872
		He	0.797	0.834	0.865	0.832
	PIC		0.736	0.779	0.809	0.775

Het. = Heterozygosity Ho = Observed Heterozygosity He = Expected Heterozygosity

Table (6): Average gene diversity.

Populations	Marker			Mean
	ETH02	CSSM060	BM1706	Estimate
Cairo	0.879	0.898	0.871	0.883
Menofya	0.890	0.888	0.844	0.874
Alexandria	0.904	0.897	0.872	0.891
Al-Minya	0.849	0.862	0.862	0.858
Kafr El-Sheikh	0.819	0.900	0.892	0.870
Qina	0.795	0.830	0.865	0.830
Total the population	5.136	5.275	5.206	5.206

Table (7): Number of shared alleles between the different populations under study.

Populations	Marker		
	CSSM060	BM1706	ETH02
Cairo & Menofya	9	7	7
Cairo & Alex.	8	5	8
Cairo & Al-Minya	8	5	7
Cairo & Kafr El-Sheikh	7	5	5
Cairo & Qina	5	5	5
Menofya & Alex.	8	5	8
Menofya & Al-Minya	8	5	7
Menofya & Kafr El-Sheikh	7	5	5
Menofya & Qina	5	5	6
Alex. & Al-Minya	8	4	9
Alex. & Kafr El-Sheikh	9	7	7
Alex. & Qina	6	4	7
Al-Minya & Kafr El-Sheikh	8	6	7
Al-Minya & Qina	7	7	8
Kafr El-Sheikh & Qina	7	5	5
All the population	4	3	3

In the present study, the highest average gene diversity value was 0.904 in Alexandria, while the lowest average gene diversity value was 0.795 in Qina. The values of average gene diversity obtained in this study agreed with the values reported previously by (Moioli *et al*., 2001) they found average gene diversity of 0.754 in Italian buffalo, 0.812 in Greek buffalo and 0.755 in Egyptian buffalo.

Allele Sharing

The results of allele sharing for each marker under study in the different populations are presented in Table (7).

Exclusive and prevailing alleles

Exclusive alleles refer to unique alleles or breed specific alleles, while prevailing alleles refer to common alleles with highest frequency among the populations.

The results of exclusive and prevailing alleles for each marker under study in the different populations are presented in Table (8).

Table (8): Number of Exclusive and prevailing alleles between the different populations under study.

Marker	prevailing alleles	Exclusive alleles
CSSM060	6 (95) 8 (123) 9 (125) 12 (131)	13 (141) Al-Minya
BM1706	4 (226) 6 (232) 7 (253)	12 (271) Kafr El-Sheikh
ETH02	2 (195) 7 (216) 11 (228)	

Table (9): Inbreeding estimates (F_{IS} = f) within populations under study.

Locus	Populations					
	Cairo	Menofya	Alexandria	Al-Minya	Kafr El- heikh	Qina
ETH02	-0.066	-0.124	-0.106	-0.177	-0.221	-0.065
CSSM060	-0.044	-0.060	-0.059	-0.092	-0.111	-0.112
BM1706	-0.148	-0.185	-0.029	-0.092	-0.121	0.022
Mean Estimates	-0.086	-0.123	-0.065	-0.12	-0.151	-0.052

Inbreeding Measures

The observed data for inbreeding within the different populations (F_{IS}) for the different markers under study are presented in Table (9).

All the values of inbreeding within the populations were negative, indicating the absence of inbreeding within the populations. This means that no breed or strain could be recognized for the different populations under study. This result is confirmed by the high values of heterozygosity observed for all the markers in all the populations. On the other hand, the absence of homozygosity is another indicator for this result.

The observed values for the inbreeding between the different populations (FST) under study are presented in Table (10).

Table (10): F_{ST} (Fisher statistics) estimate (Wright 1951).

Area	Cairo	Menofya	Alexandria	Al-Minya	Kafr El- Sheikh	Qina
Cairo	--------	0.006	0.025	0.026	0.040	0.045
Menofya		--------	0.022	0.047	0.040	0.067
Alexandria			--------	0.038	0.005	0.053
Al-Minya				--------	0.025	-0.011
Kafr El- sheikh					--------	0.020
Qina						--------

Table (11): Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

	Cairo	Menofya	Alexandria	Al-Minya	Kafr El-Sheikh	Qina
Cairo	****	0.957	0.719	0.764	0.650	0.668
Menofya	0.044	****	0.754	0.638	0.663	0.547
Alexandria	0.330	0.282	****	0.667	0.880	0.603
Al-Minya	0.269	0.450	0.405	****	0.781	0.987
Kafr El-Sheikh	0.430	0.412	0.128	0.248	****	0.829
Qina	0.404	0.603	0.506	0.014	0.188	****

Also values of inbreeding between the populations under study were below zero meaning the absence of inbreeding between the populations under study. This is expected because of the animals exchange policy between the different regions over Egypt.

Genetic distance

The Nei's genetic identity and genetic distance between the different populations under study are presented in Table (11).

The lowest genetic distance value was 0.014 and was between the two buffalo populations located in Upper Egypt (Al-Minya and Qina). This is since the two regions are closed to each other. In contrast, the highest genetic distance value was 0.603 between the buffalo of Delta region Menofya and that of Qina. This is also expected due to the long geographical distance between these two regions.

The dendrogram illustrating the relationship between these populations is shown in figure 2. The dendrogram shows that the Egyptian buffaloes come from mixing of two main clusters; the first cluster is composed of only one subcluster including Cairo and Menofya. The second cluster includes two subclusters, one is composed of Al-Minya and Qina (the Saidi region) and the other subcluster is composed of Alexandria and Kafr El-Sheikh.

The phylogeny tree showed that the Egyptian buffalo populations under study are composed of three united subclusters, the first is composed of two adjacent governorates: (Cairo and Menofya), the second is composed of two coastal governorates: (Kafr El-Sheikh and Alexandria) and the third cluster is composed of the two adjacent Saidi governorates: (Al-Minya and Qina). This result is generally expected due to the easy transportation of animals between the adjacent regions.

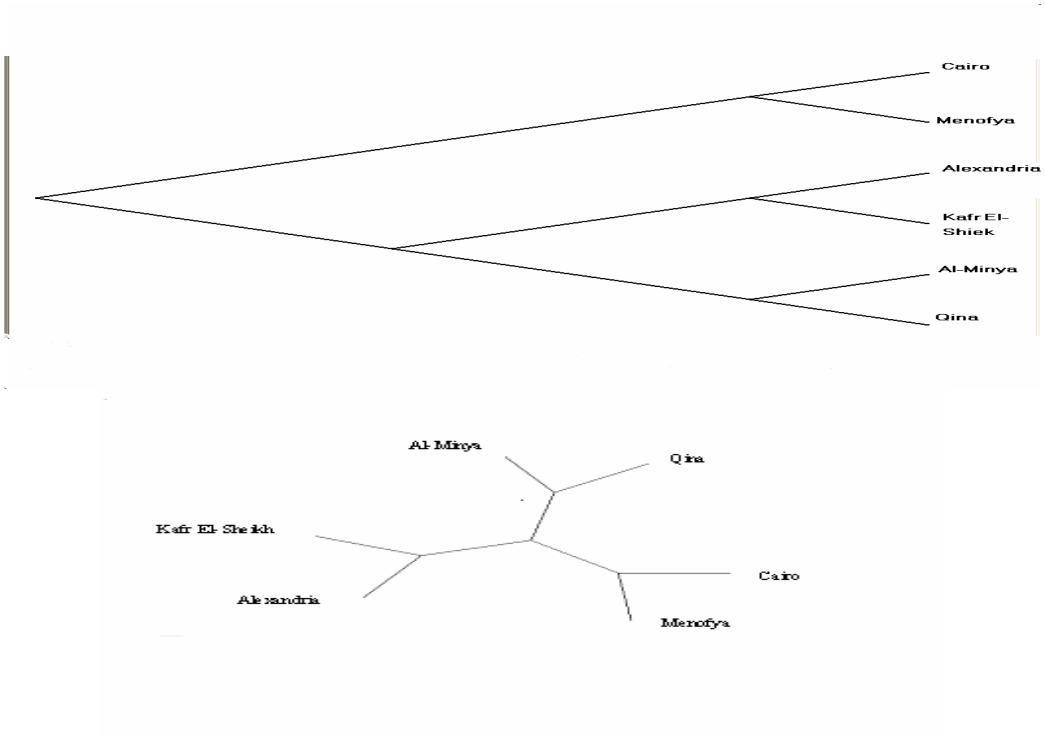

Fig. (2): Dendrogram and phylogeny tree based on Nei's (1978) Genetic distance: Method=UPGMA – Modified from NEIGHBOR procedure of PHYLIP Version 3.5.

Table(12): Chi-square test of significance of pairwise comparisons between two populations homogeneity for the studied 3 loci in the six buffalo populations.

	CSSM060	BM1706	ETH02
	χ^2	χ^2	χ^2
Cairo and Menofya	5.01 ns	2.10 ns	2.91 ns
Cairo and Alexandria	5.62 ns	3.22 ns	3.22 ns
Cairo and Al-Minya	3.18 ns	4.96 ns	5.10 ns
Cairo and Kafr El-Sheikh	5.50 ns	2.33 ns	3.20 ns
Cairo and Qina	3.92 ns	1.99 ns	1.02 ns
Menofya and Alexandria	7.52 ns	7.02 ns	6.41 ns
Menofya and Al-Minya	6.35 ns	9.05 ns	3.21 ns
Menofya and Kafr El-Sheikh	4.31 ns	7.40 ns	6.25 ns
Menofya and Qina	2.95 ns	3.14 ns	9.23 ns
Alexandria and Al-Minya	5.05 ns	7.25 ns	3.11 ns
Alexandria and Kafr El-Sheikh	4.30 ns	6.11 ns	7.56 ns
Alexandria and Qina	1.13 ns	7.28 ns	4.35 ns
Al-Minya and Kafr El-Sheikh	8.52 ns	1.34 ns	6.12 ns
Al-Minya and Qina	10.01 ns	7.57 ns	3.48 ns
Kafr El-Sheikh and Qina	6.37 ns	4.54 ns	5.67 ns

Homogeneity test

Chi-square test, with a null hypothesis Ho: "allelic distribution is identical across studied populations, for all studied loci", in addition to two pairwise comparisons for all studied loci are presented in Table (12) as a test of homogeneity of population. Results indicate that the populations under study are not, in general, genetically distinct from each other at loci under study. Increasing the

number of samples and the markers to insure from these results must support this result.

CONCLUSION

The obtained results confirm that the Egyptian buffalo is of one population, and no breed could be identified. Migration and admixture between populations took place extensively. Our results agree with the study of Moioli *et al*. (2001), concerning the Egyptian buffalo. The differences in the productivity among the different populations may therefore be due to some environmental factors (mainly temperature).

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الملخص العربي

دراسة التباين الوراثي في الجاموس المصري باستخدام التوابع الوراثية الدقيقة

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في هذه الدراسة استخدمت ثلاثة من واسمات التتابعات الوراثية الدقيقة (Microsatellites) من أصل بقرى: (CSSM060, BM1706, ETH02) وذلك لتحديد الاختلافات الوراثية الموجودة في ست من عشائر الجاموس التي ترعى في مصر. وكان عدد الحيوانات المستخدمة 92 حيوان, جمعت من القاهرة الكبرى والمنوفية والإسكندرية وكفر الشيخ والمنيا وقنا. أظهرت جميع الواسمات تحت الدراسة تعدد أليلى بمعدل 12 أليل لكل من ETH02, وBM1706 و13 أليل لـ CSSM060 . تم تقدير متوسط معامل التربية الداخلية داخل وبين المجموعات وذلك عبر جميع المواقع الأليلية (FIS, FST) لتحديد الاختلافات الوراثية داخل المجموعات وبين العشائر. أظهرت النتائج غياب التربية الداخلية داخل العشائر. لوحظ في جميع العشائر ارتفاع متوسط النسبة المشاهدة لقيم الخلط الوراثي heterozygosities , وكان أقل قيمة هي 0.846 وشوهدت في عشيرة الجاموس في قنا وذلك للموقع الواسم BM1706 , على العكس فإن اكتمال النسبة المشاهدة كان في بعض العشائر خاصة للموقع الواسم ETH02 . كانت قيم محتوى التنوع الوراثي Polymorphism Information Content لجميع الواسمات في جميع المناطق عالية وتراوحت بين 0.736 إلى 0.862. القيم العالية لمتوسط الاختلافات الجينية لوحظ لكل الواسمات المستخدمة وكذلك لكل العشائر تحت الدراسة حيث تراوحت بين 0.795 – 0.904 بمتوسط 0.867. قيم التدفق الجينى أو الهجرة كانت عالية مما يعني حدوث الهجرة أو الخلط بين المجموعات تحت الدراسة. تم حساب المسافات الوراثية ومنها تم استنباط شجرة العلاقة بين العشائر المختلفة تحت الدراسة علاوة على حساب عدد الأجيال التي لزمت لتفرد المناطق المختلفة عن بعضها.