J. Agric. Sci. Mansoura Univ., 33 (1): 149 - 166, 2008

GENETIC DIVERSITY IN EGYPTIAN BUFFALOES (*Bubalus bubalis*) USING MICROSATELLITE MARKERS

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ABSTRACT

Sixty four blood samples were collected from unrelated buffaloes from different populations representing three suspected breeds (Menofi, Beheri and Saidi). The first population was the middle Delta region included: Kafr El-Sheikh and Menoufia; the second was Alexandria population included Alexandria only, while the third population was Upper Egypt (from Assuite up to Sohaj). Five bovine microsatellites (HEL013; CSSM066; ILSTS005; INRA035 and HEL001) were analyzed in the three different populations of Egyptian buffalo in order to determine the genetic diversity between and within populations as well as assess the purity or the heterogenecity of each population. The tested microsatellites showed polymorphism in all populations meaning that they could be used further in parentage testing as well as mapping of quantitative trait loci (QTLs) in buffalo. Contrarily, the microsatellite HEL001 was monomorphic in the studied populations. A total of 29 alleles were detected across the microsatellite loci when they were screened in all populations. The mean number of alleles per locus was 7.25 alleles. However, the observed number of polymorphic alleles ranged from five (ILSTS005) to ten (HEL013). Heterozygosity and Wright's F-statistics (FIS, FST, and FIT) were calculated to determine the genetic variation in these populations. High values of observed heterozygosities were noticed in all populations, the lowest heterozygosity value was 0.333 which observed in Upper Egypt for ILSTS005. In contrast, a complete heterozygosity value (1.00) was observed in many populations, since it observed in Upper Egypt for HEL013 and INRA035; in Alexandria for HEL013; CSSM066; INRA035 and also was observed in Delta for CSSM066 and HEL013. All FIS values were below the zero, meaning the absence of inbreeding within each population. High averages of gene diversity were noticed for all markers and all populations, ranging from 0.665 to 0.753 with an overall mean of 0.709. Values of gene flow or migration between populations were high meaning that migration and admixture could have taken place between these populations. Depending on the genetic distances, a dendrogram showing the genetic relationship among the different populations.

Keywords: Egyptian buffaloes, microsatellite, gene diversity, genetic distances.

INTRODUCTION

More than 3,920,000 buffalo animals are raised in Egypt provide 2,300,000 tons of milk and 270,000 tons of meat (FAO, 2005). Buffaloes were unknown in Egypt during the time of the Pharaohs. Movement of buffaloes to Egypt took place when Arabs took these animals from India after the first invasion in the ninth century. Now the largest buffalo population in the Near East and Europe is centered in Egypt (Bhat, 1992). 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). Several animal breeders reporting that the Delta buffalo is classified into some breeds like Beheri, Menoufi, Baladi

De Hondt and Ghanam (1970), described the karyotype of the Egyptian buffalo, that was later confirmed and fully described by Hassanane (1986), who found that it is consists of 5 meta and submetacentric chromosomes. According to the standard karyotype of buffalo (Iannuzzi, 1994), the biarmed chromosomes correspond to the fused 1/25; 2/23; 8/19; 5/28 and 16/29 chromosomes of cattle. The remaining 20 pairs are acrocentric and include the sex chromosomes. Syntenic conservation between cattle and river buffalo has been reported by Othman and El Nahas (1998). O'Brien (1991) classified molecular genetic markers into two types, the first are the markers associated with a gene of known function while the second are the markers associated with anonymous gene segments of one sort or another.

In the past, all the studies of population genetic structure used allele frequency data at protein coding (mainly allozyme) to study the genetic diversity (Ward *et al*., 1992). The recent revolution of molecular biology and the discovering of polymerase chain reaction (PCR), provided new methodologies and new markers for the study of genetic variations at the DNA sequence level (Avise, 1994). Among these markers, simple sequence repeats (microsatellite) loci which have to be common in all eukaryotic genomes. Microsatellite DNA markers are simple sequence repeats (SSR) (Schlotterer *et al.,* 1998 and Karp, 1999) or short tandem repeats (Fong *et al*,. 2002 and Smithson and Macnair,. 2004) consists of tandemly arranged reiterated units of noncoding DNA sequence, typically ranging between 2-5 base pairs (bp) in length (Bradley and Magee, 2006)

In Egyptian buffalo two studies only dealt with the typing of some bovine microsatellites, the first study to identify the possibility of bovine microsatellites to work with the buffalo genome and polymorphism (Hassanane *et al*., 2000) and the second to identify the genetic diversity between the Egyptian, Greek and Italian buffalo (Moioli *et al*., 2001).

The aim of the present study is to use some bovine microsatellite genetic markers to study the genetic diversity between and within buffalo populations kept at different regions of Egypt to answer the question: Is the Egyptian buffaloes belong to one or different breeds?.

MATERIALS AND METHODS

Blood sampling:

Sixty four blood samples were collected from unrelated buffaloes raised in different regions representing three suspected breeds (Menoufi, Beheri and Saidi). The first region was the middle Delta region included: Kafr El-Sheikh and Menoufia, while Alexandria region included Alexandria only, in contrast Upper Egypt region started from Assuite and ended at Sohaj. The details of the taken samples are shown in Table (1):

Table (1): Number of samples collected from several regions.

Region	Suspected breed	Number of samples
Middle Delta	Menoufi	32
Alexandria	Beheri	16
Upper Egypt	Saidi	16

About 10 ml peripheral blood was collected from the jugular vein of each animal into a tube containing 0.5 ml EDTA (0.5 M) as an anticoagulant. The samples were transferred at 4°C and processed for DNA extraction in a period not exceeds 3 days from its arrival to the laboratory.

Isolation and purification of DNA:

DNA was isolated and purified using standard salting out method described by Miller *et al.* (1988). The stock DNA was kept frozen and its concentration was adjusted to 50 ng/µl before performing the polymerase chain reaction (PCR).

Microsatellites used information:

The microsatellites markers which 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. Details of these markers are shown in Table (2):

Table (2): Details of the microsatellite markers.

Marker	Chromosome location	Primer sequence	Reference
HEL001	D15S10	CAACAGCTATTTAACAAGGA AGGCTACAGTCCATGGGATT	**Kaukinen & Varvio (1993)**
HEL013	D11S15	TAAGGACTTGAGATAAGGAG CCATCTACCTCCATCTTAAC	
ILSTS005	D10S25	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	**Brezinsky *et al.* (1993)**
CSSM066	D14S31	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	**Barendse *et al.* (1994)**
INRA035	D16S11	ATCCTTTGCAGCCTCCACATTG TTGTGCTTTATGACACTATCCG	**Vaiman *et al.* (1994)**

The standard PCR run cycle was usually as: Primary denaturation : 95 °C for 3 min. then 35 cycles as : 95 °C for 15 sec ; 55 –60 °C for 30 – 60 sec; 72 °C for 30 sec. Then, final extension as 72 °C for 5 min, then 15 °C forever. For optimization the PCR, both the temperature and time of the annealing temperature were changed.

PCR conditions:

The PCR reaction volume was 20 µl for each sample. The PCR master mix formula contained 50 ng/µl from the DNA template, 10 X PCR buffer that included 1.5 mM MgCl2 and 10 pmol from each forward and reverse primers and 200 µM final concentration from each dNTP.

The initial PCR cycle was at 95 °C for 3 min, 35 cycles at 95 °C for 15 sec, 55-60 °C for 30-60 sec, 72°C for 30 sec, then final extension at 72 °C for 5 min and storage at 15 °C.

The PCR products were tested for success on 2% agarose in TAE buffer in a horizontal electrophoresis chamber and stained with ethidium bromide. The successful runs were subjected to the vertical electrophoresis run on 12% polyacrylamide. The polyacrylamide gels were stained with ethidium bromide and the images were captured using gel documentation system. Allelic sizes were determined using free software namely Lab. Image V2.7 (Proland Co,Germany).
http://www.labimaging.com/servlet/engine/ home/ start.html).

Preparations and staining of the polyacrylamide gels were done using the protocol described by Sambrook *et al*. (1989).

Statistical analysis:

Allele frequency was estimated using POPGENE Version 1.31 (Yeh *et al*., 1999), while the observed and expected number of alleles or heterozygosity, average gene diversity, genetic identity and genetic distance was estimated according to Nei's (1978). The genotype deviation from Hardy-Weinberg was determined by using chi square test, while phylogenic analysis, average gene diversity was calculated according to Nei's (1987) for each breed and for all breeds together. Polymorphism Information Content (PIC) value for each locus was calculated according to Botstein *et al*. (1980). F-statistics, FIS, FIT and FST were calculated using updated version from FSTAT software, version 2.9.3.2 (Goudet, 1995). The Tree View 32 software (Page, 1996) was employed to draw the dendrogram showing the genetic distances and relationships between breeds under study. The Dendrogram is based on Nei's (1972) using Genetic distance: Method = UPGMA (computer software), modified from NEIGHBOR procedure of PHYLIP Version 3.5.

RESULTS AND DISCUSSION

Microsatellite polymorphism:

Data in Table (3) show that the tested microsatellites showed polymorphism in all populations studied, except the microsatellite HEL001 which was monomorphic. Such trend means that they could be used further in parentage testing as well as mapping of quantitative trait loci (QTLs) in buffalo. A total of 29 alleles were detected across the microsatellite loci when they were screened in all populations. The mean number of alleles per locus (MNA) was 7.25 alleles. However, the observed number of polymorphic alleles ranged from 5 (ILSTS005) to 10 (HEL013).

Table (3): Number and size (bp) of alleles detected in buffalo populations.

Micro-satellite	N	Allele size (bp)									
		1	2	3	4	5	6	7	8	9	10
ILSTS005	5	173	175	177	179	183					
CSSM066	6	168	172	176	180	186	200				
INRA035	8	100	102	108	110	114	118	120	122		
HEL013	10	165	168	171	174	177	180	183	186	189	191

N: Number of alleles

Soysal *et al.* (2005) reported that the ILSTS005 microsatellite loci were found to be polymorphic in both Turkish Tarai and Anatolian water buffalo (*Bubalus bubalis*) populations. This contrasted the present study, wheras the lowest polymorphic microsatellite was with ILSTS005 (5 alleles). However in similar trend with the present results, a lower polymorphism for this microsatellite was observed in Turkish buffalo (Soysal *et al.,* 2005), being only 3 alleles in Anatolian water buffalo populations. Also, Moioli *et al.* (2001) found that the number of alleles per locus for ILSTS005 was only two alleles for Greek, Italian and Egyptian buffalo populations using 13 polymorphic microsatellite loci. In Southwestern European Bovine breeds, Beja-Pereira *et al.* (2003) noticed that mean number of alleles per locus was 6.5 for ILSTS005.

It is of interest to note that the microsatellite alleles were found to vary in their numbers and sizes among populations. Some alleles were common in all populations while others were not. Details of the microsatellite alleles for the different microsatellites in different populations are shown in Table (4).

Table (4): Number of alleles for each microsatellite in different buffalo populations and mean number of alleles in each population for all microsatelites.

Population	Microsatellite				N	Mean No./ population
	CSSM066	HEL013	INRA035	ILSTS005		
Upper Egypt	3	4	5	3	15	3.75
Alexandria	6	4	4	4	18	4.5
Delta	2	7	6	4	19	4.75

N: Total no. of alleles

Generally, the overall mean number of alleles for all microsatellites for all populations was 4.33 (Table 4). In this respect, Barker *et al.* (1997) found that the mean number of alleles for all river buffalo populations was 3.8 while it was 4.8 for all Swamp buffalo populations. This number is nearly similar to that obtained in our populations (4.33). On the other hand, Moioli *et al*. (2001) found the higher overall mean number of alleles for all the microsatellites, being (6) for Egyptian buffalo and 5.7 for all Italian, Greek and Egyptian buffalo populations. The obtained lower mean number of alleles in this study (4.33) may be due to the great number of microsatellite (13 microsalelites) and/or the higher number of animals studied by authors. In addition, Van Hooft *et al*. (2000) recorded high mean number of alleles (7.1) for all wild African buffalo (*Syncerus caffer*) populations, which means more genetic diversity in these populations. The complete absence of selection could be the main factor affects the allele numbers (Boa, 1993). In contrast Grobler *et al.,* (1996) found that the mean number in another area for African buffalo (*Syncerus caffer*) populations was (9.1). This variation may be related to the number of animals and microsatellite used. The reported higher mean number of alleles than that obtained in our study may be due to the absence of selection in wild animals.

Size and frequency of alleles for different microsatalites:

Microsatellite ILSTS005:

The present results showed that number of alleles of ILSTS005 was 5, ranging in size from 173 to 183 bp. The same results were obtained by Navani *et al*. (2002). Also, Ritz *et al*. (2000) found that ILSTS005 gave 5 alleles ranged in sizes from 173 to 186 bp in buffaloes (*Bubalus bubalis*). However, Moioli *et al.* (2001) found in both Italian and Greek buffalo that the number of the detected alleles for ILSTS005 was two alleles. Also, Arora *et al.* (2004) found that this microsatellite gave two alleles in Bhadwary buffalo and three alleles in Tarai buffalo with allele size ranging from 177 to 183 bp in both populations. However, the microsatellite ILSTS005 was polymorphic in both Anatolian and Tarai Turkish buffalo giving 3 alleles (Soysal *et al.,* 2005).

On the other hand, Van Hooft *et al.* (1999) found that ILSTS005 was polymorphic and gave 7 alleles in African buffalo (Syncerus caffer) with size ranging between 173 and 195 bp.

The present results in Table (5) revealed that A_4 (179 bp) was the most frequent (0.75) in Upper Egypt. In contrast, A_5 (183 bp) showed the least frequency (0.10) in Delta. However, alleles A_3 and A_4 were prevailing alleles, since they found in all populations. However, A_5 was found only in Delta.

Table (5): Size (bp) and frequency of alleles for microsatellite ILSTS005

Allele number	Allele Size (bp)	Allele frequency			
		Upper Egypt	Alexandria	Delta	All populations
1 (A_1)	173	0	0.333	0.100	0.147
2 (A_2)	175	0.083	0.083	0	0.059
3 (A_3)	177	0.167	0.333	0.300	0.265
4 (A_4)	179	0.750	0.250	0.500	0.500
5 (A_5)	183	0	0	0.100	0.029

In accordance with the present results, Soysal *et al.* (2005) found that ILSTS005 was polymorphic in both Turkish Tarai and Anatolian water buffalo (*Bubalus bubalis*). In comparison with the present results, allelic size ranged from 173 to 183 bp (Navani *et al.,* 2002) in riverine buffalo (*Bubalus bubalis*), 177 to 183 bp (Arora *et al*., 2004) in the Northern Indian buffalo (*Bubalus bubalis*), 173 to 195 bp (Van Hooft *et al.,* 1999) in African buffalo (*Syncerus caffe*r), 182 to198 bp (Mukesh *et al.,* 2004), in Indian zebu cattle and 181 to 193 bp (MacHugh *et al.,* 1997) in Taurine and Zebu cattle. The greater number of alleles may be due to the absence of selection since they are wild animals.

Results in Table (5) show that alleles were distributed at a minimum frequency of 0.083 for A_2 in Upper Egypt and Alexandria to a maximum frequency of 0.750 for A_4 in Upper Egypt. In the same time, both of A_3 and A_4 (177 and 179 bp) were found in all, being prevailing alleles, while A_4 (the most frequent allele) had the highest average frequency all over the alleles (0.5000). However, A_5 was an exclusive allele because it was found only in Delta. In Upper Egypt, both A_1 and A_5 were absent, while A_5 was absent in both Upper Egypt and Alexandria and was specific exclusive allele in Delta

population. In the case of A_4, it was found in all populations, showing the highest frequency in Upper Egypt comparing with other populations.

Upper Egypt population gave 3 alleles upon the recorded 5 alleles, while Delta and Alexandria gave 4 alleles for each. Allele A_5 (183 bp) was found only in Alexandria having frequency of 0.1. In the same trend, Moioli *et al.* (2001) found frequency distribution of 34.4 and 38.6% for the first allele of Italian and Greek, respectively. While the second allele showed 65.6 and 71.4% for Italian and Greek, respectively.

Microsatellite CSSM066:

As shown in Table (6) the total number of alleles for CSSM066 was 6 ranged from 168 to 200 bp in size. Soysal *et al.* (2005) found that CSSM066 was polymorphic in Indigenous Anatolian Water Buffaloes. The observed number of alleles were 8. Also, Armstrong *et al.,* (2006) found that CSSM066 was polymorphic and highly informative in Uruguayan Creole bulls (*Bos taurus*). Number of alleles for this marker was 7 displaying 180-200 bp in size. Also, Navani *et al.* (2002) showed that CSSM066 was polymorphic and gave 7 alleles ranged in size from 168 to 202 bp in riverine buffaloes (*Bubalus bubalis*).

For this microsatelite the allelic size was reported to be 168-202 bp (Navani *et al*., 2002) in the riverine buffaloes (*Bubalus bubalis*); 174-186 bp (Arora *et al.,* 2004) in buffaloes of northern India; 168-196 bp (Karthickeyan *et al*., 2006) for Krishna Valley breed of cattle (*Bos indicus*); 177-209 bp (Mukesh *et al.,* 2004) in Indian zebu cattle; 180-200 bp (Armstrong *et al.,* 2006) for Uruguayan Creole bulls (*Bos taurus*), 184-194 bp (Kim *et al.,* 2004) for Korean Goral (*Nemorhaedus caudatus*).

Table (6): Size (bp) and frequency of alleles for the microsatellite CSSM066.

Allele number	Allele size	Allele frequency			
		Upper Egypt	Alexandria	Delta	All populations
1 (A_1)	168	0.417	0	0.167	0.194
2 (A_2)	172	0.167	0	0.083	0.083
3 (A_3)	176	0	0.500	0.250	0.250
4 (A_4)	180	0.417	0	0.083	0.167
5 (A_5)	186	0	0	0.167	0.056
6 (A_6)	200	0	0.500	0.250	0.250

Results in Table (6) showed that alleles distributed in Upper Egypt population at a minimum frequency of 0.167 for A_2, while it distributed at a maximum frequency of 0.417 for both A1 and A4. On the other hand, A3, A5 and A6 were absent. Whereas, in Alexandria population, the maximum frequency was (0.50) for both A3 and A6, while the others were absent. However, in Delta population, the maximum frequency was (0.250) for both A3 and A6 while the minimum was (0.083) for both A2 and A4. Its worthy noting that the observed 6 alleles were found in Delta population. Further more, A5 was exclusively found in Delta population. Noticeably, the most frequent alleles in all populations were A3 and A6.

Microsatellite INRA035:

The total number of alleles for microsatellite INRA035 was 8 in all populations. Allele size ranged from 100 to 122 bp (Table 7). For microsatellite INRA035, Navani *et al.* (2002) found that it was monomorphic with allele size of 135 bp in riverine buffalo (*Bubalus bubalis*). In nearly similarity, Mukesh *et al.* (2004) found that the allelic size ranged from 102 to 114 bp in Indian zebu cattle.

Results in Table (7) showed that the most frequent allele in all populations was A1 (0.367) while the least frequent alleles were A3, A4, A5 and A7. However, A1 and A2 undergoing the same trend through the studied populations. Additionally, A3, A4 and A5 were exclusive alleles in Alexandria population, where as, A7 was exclusive in Upper Egypt. Its worthy noting that A1 is a prevailing allele since it distributed in all populations with a highest frequency (0.500) particularly in Delta population.

Table (7): Size (bp) and frequency of alleles for microsatellite INRA035.

Allele number	Allele Size (bp)	Allele frequency			
		Upper Egypt	Alexandria	Delta	All populations
1 (A_1)	100	0.375	0.200	0.500	0.367
2 (A_2)	102	0.125	0.100	0.167	0.133
3 (A_3)	108	0	0.100	0	0.033
4 (A_4)	110	0	0.100	0	0.033
5 (A_5)	114	0	0.100	0	0.033
6 (A_6)	118	0.250	0	0.083	0.100
7 (A_7)	120	0.125	0	0	0.033
8 (A_8)	122	0.125	0.400	0.250	0.267

For microsatellite INRA035, Mukesh *et al.* (2004) found that it was informative and polymorphic exhibiting different allele sizes ranged from 102-114 bp in Indian native cattle. The observed number of alleles was 7, 8 and 8 for Sahiwal, Hariana and Deoni cattle breeds, respectively. Also, Pandey *et al.* (2006) reported that it was polymorphic and gave seven alleles in Kherigarh breed of Indian zebu cattle (*Bos indicus*). Mean number of alleles per locus was 6.24. Moreover, Karthickeyan *et al.* (2006) found that it was polymorphic and highly informative, giving 7 alleles with high PIC (0.798) in Krishna Valley breed of cattle (*Bos indicus*) in South India.

Microsatellite HEL013:

The total number of alleles which observed for microsatellite HEL013 was 10 alleles ranging from 165 to 191 bp in zise (Table 8). The same range of size was recorded by Navani *et al*. (2002) in riverine buffaloes. However, the alleles sizes ranged from 188 to 198 bp for Korean Goral (*Nemorhaedus caudatus*, Kim *et al.,* 2004) and from 177 to 197 bp in lowland European bison (*Bison bonasus bonasus*, Gralak *et al.,* 2004).

For all populations, the highest frequency was observed for alleles A_2 and A_9 (168 and 189 bp), being the highest (0.50) in Delta and Upper Egypt. It is of interest to note that alleles A_1, A_5, A_6 and A_8 showed lower frequency for all populations and were found only in Alexandria (A_1, A_5 and A_6) and

Delta (A_8). However, alleles A_3 and A_9 showed the same trend and were found only in Upper Egypt. Finally, A_{10} was absent in Upper Egypt and showed lower frequency (0.083) in Alexandria and Delta (Table 8).

Table (8): Size (bp) and frequency of alleles for microsatellite HEL013

Allele number	Allele size (bp)	Allele frequency			
		Upper Egypt	Alexandria	Delta	All populations
1 (A_1)	165	0	0.167	0	0.056
2 (A_2)	168	0.333	0.083	0.500	0.306
3 (A_3)	171	0.083	0	0	0.028
4 (A_4)	174	0.083	0.167	0	0.083
5 (A_5)	177	0	0.250	0	0.083
6 (A_6)	180	0	0.083	0	0.028
7 (A_7)	183	0	0.167	0.250	0.139
8 (A_8)	186	0	0	0.167	0.056
9 (A_9)	189	0.500	0	0	0.167
10 (A_{10})	191	0	0.083	0.083	0.056

Based on these results, A_2 is a prevailing allele which found in all population, while A_1, A_3, A_5, A_6, A_8 and A_9 were exclusive alleles.

By studying a set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (*Bubalus bubalis*), Navani *et al.* (2002) found that microsatellite HEL013 was polymorphic giving 5 alleles ranging in size from 165 to 191 bp. Also, Kim *et al.* (2004) showed that this microsatellite was polymorphic in Korean Goral (*Nemorhaedus caudatus*) displaying 3 alleles ranging size of 188-189 bp. Armstrong *et al.* (2006) found in Uruguayan Creole bulls (*Bos taurus*) that microsatellite HEL013 was polymorphic and number of alleles was 3 displaying sizes from 184 to 192 bp.

On the other hand, microsatellite HEL013 was non polymorphic in comparing three breeds of Bovidae family, European bison (Gralak *et al,* (2004), American bison (Mommens *et al.,* 1998) and three Polish cattle breeds (Lubieniecka *et al.,* 2001).

Prevailing and exclusive alleles:

Prevailing alleles refer to common alleles with the highest frequency among the populations while, exclusive alleles refer to unique alleles or breed specific allele. The exclusive alleles always have extreme value for minimum and maximum frequency values (Arranz *et al.,* 2001).

Results in Table (9) revealed that CSSM066 showed prevailing of A_1 and A_4 in Upper Egypt, while A_3 and A_6 were prevailing in Delta and Alexandria. In contrast, A_5 was exclusively specific to the Delta. However, HEL013 exhibited three prevailing alleles, A_{10} in Upper Egypt, A_2 in Delta and A_5 in Alexandria, whereas A_1, A_3, A_5, A_6, A_8 and A_{10} were exclusive alleles in their correspondent populations.

Microsatellite INRA035 displayed the prevailing of A_1 in Upper Egypt and Delta, while A_8 was prevailing in Alexandria. However, A_7 was exclusive in Upper Egypt, but A_3, A_4, and A_5 were exclusive in Alexandria. The microsatellite ILSTS005 showed four prevailing alleles, A_4 in Upper Egypt and Alexandria , both A_1 and A_3 in Delta, whereas A_5 was exclusively specific in Alexandria (Table 9).

Table (9): Prevailing and exclusive allele numbers (preceding the brackets) and their frequency (between brackets) for each microsatelite in different populations.

Locus	Prevailing alleles			Exclusive alleles		
	Upper Egypt	Alexandria	Delta	Upper Egypt	Alexandria	Delta
CSSM066	1-4 (0.417)	3-6 (0. 50)	3-6(0.250)	-	-	5 (0.167)
HEL013	10 (0.5)	5 (0.250)	2 (0.5)	3 (0.083) 10 (0.5)	1 (0.167) 5 (0.250) 6 (0.083)	8 (0.167)
INRA035	1 (0.375)	8 (0.400)	1 (0.5)	7(0.125)	3-4-5 (0.1)	-
ILSTS05	4 (0.750)	4 (0.500)	1-3(0.333)	-	5 (0.1)	-

Observed and expected heteroxygosity:

Average observed heterozygosity for all populations were higher than the expected. This can be considered as an indication of heterozygosity excess in all populations. Excluding the monomorphic microsatellite HEL001, all microsatellites studied were heterozygous polymorphic. No homozygous alleles for any microsatellite were observed in any population.

Results in Table (10) show that HEL013 had polymorphic pattern in all populations. However, the mean number of alleles for HEL013 in all populations was 5, which was higher than 4.5 alleles (Navani *et al*., 2002) and higher than 2.6 alleles (Kim *et al*., 2004). Meanwhile, the polymorphism information content (PIC) was 0.64, being higher than 0.27 (Armstrong *et al*., 2006). However, the present mean heterozygosity typed by HEL013 for all populations was one, being higher than 0.66 (Navani *et al*., 2002 and Kumar *et al*., 2003); 0.55 (MacHugh *et al*., 1997); 0.50 (Kim *et al*., 2004); 0.33 (Armstrong *et al*., 2006) and 0.65 (Eveline *et al*., 2004).

On the other hand, Gralak *et al*. (2004) reported that HEL013 failed to amplify or produce trace signal when it used with Bison (*Bison bonasus bonasus*).

Microsatellite ILSTS005 displayed a polymorphic pattern in all populations. Its mean number of alleles in all populations was 3.67, being higher than 2 (Moioli *et al*., 2001) and lower than 4.5 (Navani *et al*., 2002), 4.8 (MacHugh *et al*., 1997) and 10.16 (Arranz *et al*., 1996). However, the mean heterozygosity exhibited by ILSTS005 for all populations was 0.60. The same result was reported by Kantanen *et al*. (2000). However the present mean heterozygosity was higher than 0.16 (Moioli *et al*., 2001); 0.56 (Barker *et al*., 1997); 0.51 (Arrora *et al*., 2004) and 0.55 (Soysal *et al*., 2005), while it was lower than 0.62 (Navani *et al*., 2002), 0.68 (Pandy *et al*., 2006), 0.74 (Karthickeyan *et al*.,2006), 0.64 (Mukish *et al*., 2004), 0.66 (Beja-Perera *et al*., 2003 and Kumar *et al*., 2003) and 0.67 (Kim *et al*., 2004).

Microsatellite ILSTS005 was informative since the mean PIC was 0.53 in all populations, being higher than 0.42 (Soysal *et al*., 2005), 0.40 (Arora *et al*., 2004) and lower than 0.67 (Pandy *et al*., 2006) and 0.70 (Karthickeyan *et al*., 2006).

Microsatellite CSSM066 showed a polymorphic pattern in all populations. Its mean number of alleles in all populations was 3.67, being

lower than 4.5 (Navani *et al*., 2002), 4.7 (Arora *et al*., 2004), 5.8 (Mukesh *et al*., 2004), 6.75 (Soysal *et al*., 2005), 6.5 (Beja-Perera *et al*., 2003), 5.45 (Kumar *et al*., 2003) and 4.72 (Karthickeyan *et al*., 2006).

The mean heterozygosity displayed by microsatellite CSSM066 for all populations was 0.94, being higher than 0.53 (Moore et at., 1995), 0.69 (Karthickeyan *et al*., 2006); 0.78 (Soysal *et al*., 2005), 0.64 (Arora *et al*., 2004), 0.84 (Navani *et al*., 2002), 0.65 (Mukesh *et al*., 2004), 0.60 (Kantanen *et al*., 2000), 0.66 (Beja-Perera *et al*., 2003), 0.66 (Kumar *et al*., 2003) and 0.67 (Kim *et al*., 2004). While Armstrong *et al*. (2006) reported higher mean heterozygosity, being one.

Microsatellite CSSM066 was informative since the mean PIC in all populations was 0.58, being higher than 0.49 (Soysal *et al*., 2005) and 0.24 (Gralak *et al*., 2004), nearly similar to 0.56 (Arora *et al*., 2004) and lower than 0.66 (Karthickeyan *et al*., 2006) and 0.79 (Armstrong *et al*., 2006).

Microsatellite INRA035 exhibited a polymorphic pattern in all populations, showing 8 alleles versus 7 alleles (Pandey *et al*., 2006 and Mukesh *et al*., 2004) and 4.72 alleles (Karthickeyan *et al*., 2006). Its mean heterozygosity in all populations was 0.89 versus 0.72 (Pandey *et al*., 2006); 0.70 (Mukesh *et al*., 2004); 0.60 (Kantanen *et al*., 2000); 0.66 (Beja-Perera *et al*., 2003 and Kumar *et al*., 2003) and 0.82 (Karthickeyan *et al*., 2006).

Table (10): Microsatellite alleles (No, observed number of alleles; Ne, effective number of alleles), heterozygosity (Ho, observed; He, expected) and polymorphism information content (PIC) at each locus in the different populations under study.

Population	Locus		HEL013	ILSTS005	CSSM066	INRA035	Mean
Upper Egypt	Alleles	No	4	3	3	5	3.750
		Ne	2.667	1.674	2.667	4.000	2.752
	Het.	Ho	1.000	0.333	0.833	1.000	0.792
		He	0.682	0.439	0.682	0.875	0.665
	PIC		0.611	0.278	0.602	0.698	0.548
Alexandria	Alleles	No	7	4	2	6	4.75
		Ne	2.880	3.429	5.143	2.880	3.583
	Het.	Ho	1.000	0.800	1.000	1.000	0.950
		He	0.909	0.711	0.546	0.844	0.753
	PIC		0.571	0.693	0.691	0.571	0.632
Delta	Alleles	No	4	4	6	4	4.50
		Ne	6.000	2.778	2.000	4.167	3.736
	Het.	Ho	1.000	0.667	1.000	0.667	0.833
		He	0.712	0.773	0.879	0.712	0.769
	PIC		0.738	0.611	0.438	0.745	0.633

Average gene diversity:

Average gene diversity for all microsatellites in all populations (Table 11) for all loci was 0.720, ranging between 0.439 for ILSTS005 in Upper Egypt and 0.910 for HEL013 in Delta. As expected, the microsatellite loci showed high genetic diversity for each microsatellite in all populations ranging from 0.62 for ILSTS005 to 0.800 for INRA035. The lowest gene diversity for ILSTS005 was mainly related to the lower number of alleles, being 5 as compared to 10 alleles for HEL013. Moioli *et al*. (2001) noticed

similar trend during studying the genetic diversity between the Egyptian, Italian and Greek buffalo using microsatellite markers.

Table (11): Average gene diversity for all microsatellites in all populations studied.

Area	Microsatellite marker			
	HEL013	**ILSTS005**	**CSSM066**	**INRA035**
Upper Egypt	0.682	0.439	0.682	0.857
Alexandria	0.712	0.733	0.879	0.712
Delta	0.910	0.711	0.545	0.844
Mean	0.760	0.620	0.700	0.800

The present results are similar to previously reported by Loftus *et al*. (2002), being 0.78 during their study concerning the identification of zebu alleles in some cattle breeds including Egyptian cattle.

Inbreeding Measures:

To assess genetic differentiation within each population, Fis is a measure of the within population heterozygote defect (inbreeding). Table (12) shows estimates of within the populations inbreeding (Fixation index statistics, FIS = *f*).

Table (12): Inbreeding or Fixation index (Fis = *f*) within population heterozygosity deficit for each of the studied loci in the populations.

Locus	Population		
	Upper Egypt	**Alexandria**	**Delta**
CSSM66	-0.333	-0.241	-1.000
HEL013	-0.600	-0.532	-0.200
INRA05	-0.333	-0.021	-0.316
ILSTS05	-0.172	-0.059	-0.250
Mean	-0.274	-0.184	-.441

It could be noticed that all the observed values were below the zero and they were approximately near to zero which means the complete absence of inbreeding with each population (Table 12). This result is expected since the materials and methods of the present study are following the regulations of FAO for domestic animals diversity (DAD) which advise by taking samples from unrelated animals. Similar results were observed in Egyptian buffalo populations on genetic diversity between and within Egyptian, Italian and Greek buffalo by Moioli *et al*. (2001).

Gene flow:

Gene flow was estimated according to Nei's (1987) indicating the ratio of the migrants exchanged from one generation to another. The lowest value of genetic differentiation between populations is supported by high level of gene flow between each two breeds (Table13). The highest value of the gene

flow was observed between Alexandria and Delta populations which also have the lowest value of F_{ST}, as a genetic differentiation. That could be explained by the geographical proximity of the two breeds.

Table (13): Estimated pair-wise F_{st} as a measure of the between populations differentiation (above diagonal) and gene flow (below diagonal) between pairs of the studied groups.

Populations	Upper Egypt	Alexandria.	Delta
Upper Egypt	-------	0.102	0.139
Alexandria.	2.192	------	0.069
Delta	1.547	3.399	------

The obtained results are expected as a result of absence of inbreeding between the different populations. No doubt, this is expected since the regions included in this study are far from each other and the possibility of exchanging animals is relatively low.

Genetic distance and Identity:

Genetic distance is a measure based on shared alleles between breeds (Table 14). Genetic distance shows compatible result with the genetic differentiation measure F_{ST}. Genetic distance showed low values between all populations which reflect genetic similarity among the studied populations (Table 14).

Table (14): Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Populations	Upper Egypt	Alexandria	Delta
Upper Egypt	-------	0.579	0.423
Alexandria	0.546	-------	0.667
Delta	0.856	0.405	--------

The lowest genetic distance was between Alexandria and Delta populations (0.405) agreeing with the highest genetic identity (0.667) and highest gene flow between them (3.399). In contrast, the highest genetic distance was observed between Upper Egypt and Delta (0.856) agreeing with the lowest genetic identity (0.429) and lowest gene flow between them (1.547). Genetic distance shows compatible result with the genetic differentiation measure F_{ST}.

Genetic divergence:

Genetic divergence is another measure of how breeds differ from each other in terms of time. **Nei (1978)** calculated genetic divergence (D_s) as

$$D_s = 2\alpha t, \quad i.e. \quad t = D_s * (2\alpha)^{-1}$$

Where α is microsatellite mutation rate and t is the number of generations. The average mutation rate of human loci ($1.2 * 10^{-3}$) as reported by Weber and Wong (1993) and a generation interval of 4.35 years were used in the present study to calculate number of years separating any two populations.

Genetic divergence analysis shows that the highest divergence time was 1209 years between Upper Egypt and Delta (Table 15), while the lowest was 771 years between Alexandria and Delta.

Table (15): Estimated divergence time of the breeds under study on the basis of the 4 microsatellite loci studied.

Population	NSGD	Mutation rate (α)	Divergence time	
			Generations	Years
Upper Egypt & Alexandria	0.579	$1.2 X10^{-3}$	241	1048
Upper Egypt & Delta	0.667		278	1209
Alexandria & Delta	0.425		177	771

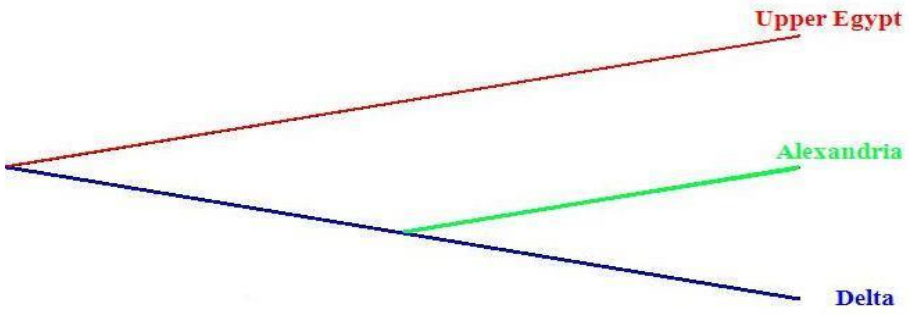
NSGD: Nei's standard genetic distance (Ds)

The results of this table are confirming the phylogeny dendrogram number (1) obtained using UPGMA method.

Relationship dendogram:

The dendogram (1) illustrated below shows separation between Delta, Alexandria and Upper Egypt. The first two were separated together in one sub cluster while Upper Egypt was located in the other sub cluster. It indicates the expected phylogeny tree for the divergence of the different populations from each other.

The populations of Alexandria and Delta showed the lowest divergence, while the greatest differentiation was detected between Delta and Upper Egypt populations. The dendogram confirms the results obtained in Table (15).



Dendogram (1): Phylogenetic tree representing relationship between the three buffalo populations using genetic distance based on four microsatellite loci.

Conclusion:

1 - Bovine markers used successfully in buffalo.

2 - Most of the markers displaying polymorphic pattern indicating that all the buffalo populations represented only one breed and the phenotypic differences are due to the environmental factors.

3 – More bovine microsatellites must be used in the coming researches.

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التنوع الوراثى في الجاموس المصرى بإستخدام واسمات التوابع الو راثية الدقيقة

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لدراسة التباين الو راثي في الجاموس المصرى، تم تحديد التراكيب الجينية لأربعه وستون رأسا من الجاموس كممثلة لثلاثة عشائر هي: منطقة وسط الدلتا ومنطقة الإسكندريه ومناطق الصعيد إبتداء من اسيوط حتى سوهاج وذلك باستخدام خمسة من واسمات التتابعات الو راثية الدقيقة وهى: ILSTS005 HEL001, CSSM066, HEL013, INRA035. تم التعرف على عدد الاليلات الموجودة لكل واسم وقد كانت: 1,8,6,10,5 على التوالي. تم التعرف على قيم الخلط أو الاختلافات الأليلية heterozygosity. كما تم استخدام قوانين العالم رايت في حساب الاختلافات الو راثية بين العشائر علاوة على الاختلافات بداخل العشائر نفسها. أظهرت النتائج وجود نسبة عالية من الاختلافات الوراثية بين وبداخل العشائر تحت الدراسة. وجد أن جميع قيم التربية الداخلية كانت أقل من الصفر مما يعنى غياب التربية الداخلية تماما في جميع العشائر تحت الدراسة.. قيم التباين الوراثى كانت عالية وتراوحت من 0.665 إلى 0.753 بمتوسط عام 0.709 وهى بذلك دالة على وجود هجرة داخلية بين العشائر. من حسابات التدفق الجينى gene flow والمسافات الوراثية تم إعداد دندوجرام يوضح العلاقة المحتملة بين العشائر تحت الدراسة.