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Genetic polymorphism of two Egyptian buffalo breeds in comparison with Italian buffalo using two ovine and bovine-derived microsatellite multiplexes

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ABSTRACT

The study was carried out to investigate genetic diversity in Nile-Delta and Southern Egypt buffalo populations in comparison with the Italian buffalo using 15 polymorphic microsatellite loci, in two multiplexes. Animals used were 28, 38 and 38 representing the Delta, Southern-Egypt and Italian buffalo, respectively. Studied microsatellites were CSSM38, CSSM70, CYP21, CSSM42, CSSM60, MAF65, BM0922, CSSM19, INRA006, D5S2, BM1706, BMC1013, CSSM47, INRA026 and RM4. All microsatellites showed allelic polymorphism. Pair-wise Chi-square test for Delta and Soutrhern-Egypt showed significant differences in allelic distribution at CSSM70, CSSM38, BM0922, ETH02 and BM1706. Testing HW equilibrium by population over all loci showed significant deviations for both Italian and Delta-populations. F_{IS} estimates over all loci indicated that Italian buffalo is relatively the most inbred population while Southern-Egypt is the only outbred population. High level of genetic differentiation (F_{ST}) between the Italian group and each of the Delta and Southern-Egypt groups (0.083 and 0.076, respectively) were observed while Southern-Egypt group showed a lower level of genetic differentiation with the Delta group (0.014). Italian buffalo had the highest distance values with the two Egyptian groups (0.25 and 0.23) while much lower values between the Southern-Egypt and the Delta group (0.06) was observed. In conclusion, there was a reasonable genetic variation between the Italian and the Egyptian buffalos and a lower level of genetic variation between Southern-Egypt and Nile-Delta buffalo. Southern-Egypt buffalo could be considered as population distinct from the Nile-Delta buffalo. Southern-Egypt population was the only one showing heterozygosity excess implying a wider gene pool and possibly higher expected genetic improvement gain from selection.

Introduction

FAO, 2003 stated that there are five indigenous buffalo (*Bos bubalus bubalis*) breeds in Egypt distributed over in different regions of Egypt , Baladi (Native), Beheri, Monoufi, Masri, and Saiedi (Southern-Egypt). All indigenous breeds are locally adapted to Egyptian environment. Minor phenotypic differences are reported concerning size, color and production that have not been updated or verified since 1992 (FAO-DAD-IS, 2006). These differences are not well defined to be relied on as taxonomic classification for those populations.

A worldwide interest in indigenous breed characterization and identification has risen for the purpose of biodiversity studies, taxonomic classification, derivative origin, and setting policies for management, maintenance and the improvement of these breeds' role in development. Limited number of researches has been conducted exploring the genetic diversity on molecular genetic basis in buffalo all over the world in comparison with other farm animals. Only very few studies were conducted on the Egyptian buffalo (Moioli et al., 2001a and b). More studies are still needed for the exploration of biodiversity of local buffalo genetic resources.

The present study aimed at studying genetic diversity, on molecular bases, in two main Egyptian buffalo populations (Nile-Delta and Southern-Egypt) in comparison with the Italian and investigating the distinction between the Delta an Southern-Egypt groups .

Materials and Methods

*Sampling of animals:*A total number of 104 buffaloes classified into three groups were sampled , i. 28 Nile-Delta buffalos belonging to Animal Production Research Institute (APRI) where selected

animals were genealogically traced to be unrelated (neither paternally nor maternally) as far as three generations back. ii. 38 Southern-Egypt buffaloes representing 23 small-scale animal holdings scattered in Sohag Governorate (being the most isolated agricultural Governorate in Southern Egypt region) where selected animals belonged to various holdings and different generations (age class) to minimize coancestry. iii. 38 Italian buffaloes belonging to various farms in middle and southern Italy. Blood and hair samples were collected from the Egyptian animals for DNA extraction while DNA samples of the Italian animals were supplied by the Laboratory of Genetic Service (LGS), Cremona, Italy.

*DNA extraction:*DNA from the Egyptian samples was extracted using either the salting out protocol (Miller et al, 1988) or selective adhesion to silica membrane depending on the lab protocol available at the time of sample collection. DNA of Italian animals samples was extracted in LGS using CHELEX/Protinase-K procedure for DNA release from blood and forensic specism using Chelex® 100 (BioRad) resin according to Cano et al.(1993).

Utilized microsatellite markers: Fifteen bovine and ovine-derived microsatellite markers ,recommended by LGS, were used.

Polymerase Chain Reaction (PCR) and Allelic Fragmentation: Studied microsatellites were amplified with PCR in two multiplexes, for which PCR cocktail is shown in Table 1.

Table 1. PCR multiplex cocktail for the two performed multiplexes

Reagent	Concentration	Quantity, ul	
		First multiplex	Second multiplex
Template DNA	300 ng/µl	1	1
H_20	-	1.6	2.95
Buffer	10X	1	1
$MgCl_2$	25 mM	1.2	1.2
dNTP's	10 mM	0.4	0.2
Taq	5U/ µl	0.4	0.4
Primers Multiplex			
MAF65	10 µM	0.4 + 0.4	-
INRA006	10 µM	0.2 + 0.2	-
CSSM47	10 µM	0.4 + 0.4	-
CSSM19	10 µM	0.1 + 0.1	-
RM4	10 µM	0.4 + 0.4	-
CSSM42	10 µM	0.1 + 0.1	-
CYP21	10 µM	0.2 + 0.2	-
ETH02	10 µM	0.2 + 0.2	-
BMC1013	10 µM	0.2 + 0.2	-
CSSM70	10 µM	-	0.15 + 0.15
CSSM60	10 µM	-	0.5 + 0.5
INRA026	10 µM	-	0.5 + 0.5
BM0922	10 µM	-	0.075 + 0.075
BM1706	10 µM	-	0.4 + 0.4
CSSM38	10 µM	-	0.5 + 0.5
Final reaction volume, ul		10	11

Thermal profile was a pre-run at 95°C for 10 min., followed by 30 tri-phasic cycles of denaturation at 95°C for 15 sec., annealing at 57°C for a minute and extention at 72°C for a minute, and final extention for 10 minutes.

PCR product was then denatured in a denaturation mix composed of 0.9 µl PCR product + 2.1 µl loading master mix. The denaturation mix was incubated at 95°C for 4 minutes and immediately chilled in ice to prevent re-annealing. A volume of 1.5 µl of such mix was loaded to the Applied Biosystems DNA sequencer 377. GeneScan v. 3.1 software was utilized by the equipment for fragmentation of various alleles by comparison against the GENESCAN-350 standard.

Quantitative measures were classified into three main categories; a. microsatellite polymorphism in the studied groups of buffalo to assess the appropriateness of utilizing studied microsatellite markers and the comparison of polymorphism between studied groups. Number of alleles detected in every

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studied group as a polymorphism measures was estimated using FSTAT software, version 2.9.3.2 (Feb. 2002), updated from Goudet (1995).

b. Genetic variability measures as: i. Allele frequency indicating genetic constitution of each group and thereafter, ii. chi-square test with a null hypothesis H_0: "allelic distribution is identical across studied groups, for all studied loci". iii. Observed and expected heterozygosity for each studied group all over studied loci and mean heterozygosity (Nei, 1978). All genetic variability measures were estimated using FSTAT.

c. Genetic differentiation measures which includes i. Wright fixation indices (Wright, 1943 and 1978), F-statistics. Two levels of F-statistics were estimated, "F_{IS}", called the inbreeding coefficient (within group heterozygosity deficit), that assesses global variation in individuals, relative to the variation in their groups. The second, "F_{ST}", assesses the variation in the group relative to that in the total groups (the fixation index calculated for each pair of groups) as a measure of the between populations heterozygosity deficit or population differentiation. Wright fixation indices were computed using permutation tests (Goudet, 2002) with 0.05 level of significance and number of permutation test of genotypes within the total population (F_{ST}), and alleles within populations (F_{IS}), and was adjusted with the sequential Bonferroni procedure (Hochberg, 1988) applied over loci. The two levels of fixation indices were estimated using FSTAT. ii. Deviation from HW equilibrium of population over all loci was tested as the null hypothesis H_0:"random union of gametes within each population" and testing the strength of factors affecting allele frequency, e.g. mainly selection, and genetic drift, migration and mutation.as well. The test was performed using the unbiased estimates of HW exact P values according to (Raymond and Rousset, 1995b) using Markov Chain Monte Carlo method. Test was performed using the GenePop software, Version 3.3, March 2001 updated from Raymond and Rousset (1995a). iii. Genetic distance, D, (Nei, 1987) as a measure of the number of detectable genetic substitutions that have accumulated in two populations since they diverged (and therefore a measure of genetic difference).

Results and Discussion

Microsatellite polymorphism. All microsatellites showed polymorphism in all studied groups. Number of polymorphic alleles ranged between four (CSSM38, CSSM70 and CYP21) up to eleven (RM4) as shown in Table 2. Different studied groups (Delta, Southern-Egypt, and Italian) showed some variation in the number of detected polymorphic alleles. Table 2 illustrates that the highest polymorphism in detected alleles over all loci was shown by the Delta group followed by the Southen-Egypt group being 86 and 84, respectively, whilst the lowest polymorphic alleles were exhibited by the Italian buffalo (73 alleles).

Lower degree of polymorphism shown by the Italian population may possibly be explained by the stronger forces the selection applied to the Italian buffalo population, as compared to Egyptian groups, which could have eroded polymorphism through successive generations of selection, assuming the existence of degree of linkage disequilibrium between some of the studied loci and the QTL or functional segments affecting milk production.

Homogeneity test of allele frequency across all groups. Significant heterogeneity ($P<0.01$) shown by Chi-square test (in 14 loci) indicate that various groups are generally genetically distinct from each other at studied loci. CYP21 is the only locus that did not show significant heterogeneity distribution of allele frequency among studied groups. In pairwise comparisons, Delta vs. Southen-Egypt, only five loci showed significant heterogeneity (being CSSM38, CSSM70, BM0922, ETH02, and BM1706) while Egyptian vs. Italian group showed significant heterogeneity in 14 loci, CYP21 being the only non-significant. In a comparable study by Barker et al. (1997), non significant difference between observed and expected number of alleles for loci CSSM019, CSSM038 and CSSM060 was reported, while locus CSSM047 showed significant difference ($P<0.01$).

*Observed and expected heterozygosity.*Average expected heterozygosity for both Delta and Italian groups was higher than the observed while the opposite situation was observed for Southern-Egypt group. Average observed and expected heterozygosity was estimated as 0.66 vs. 0.68; 0.71 vs. 0.69; and 0.65 vs. 0.68 for the Delta, Southern-Egypt and Italian populations, respectively. This can be

considered as an indication of heterozygosity deficit in the Delta and Italian groups. Italian buffalo showed the lowest percentage of observed heterozygotes (0.65), which may be partially explained by the reason stated before.In a comparable study of water buffalo for some similar loci, Barker et al., 1997, studying Asian water buffalo, reported observed heterozygosity range between 0.531 and 0.613 and expected heterozygosity range between 0.564 and 0.607. It can be concluded that in the present study, a general slightly higher heterozygosity estimates than those reported for the Asian water buffalo (either observed or expected) are recognized.

Table 2. Observed number of alleles in each of the three studied buffalo groups/populations

Locus	Delta buffalo	Southern Egypt buffalo	Italian buffalo	All	Locus	Delta buffalo	Southern Egypt buffalo	Italian buffalo	All
CSSM38	4	3	3	4	INRA006	5	6	7	7
CSSM70	3	4	3	4	ETH02	5	5	6	8
CYP21	4	4	4	4	BM1706	9	8	5	9
CSSM42	3	5	5	6	BMC1013	8	7	5	9
CSSM60	6	4	3	6	CSSM47	9	5	5	9
MAF65	5	4	5	6	INRA026	8	8	6	10
BM0922	5	7	6	7	RM4	6	8	6	11
CSSM19	6	6	4	7					
Total						86	84	73	107

Deviation from Hardy-Weinberg equilibrium. Significant deviation was shown for both the Delta and Italian buffalo groups having a P value of 0.0016 and 0.0064, respectively. Exact P value was 0.1028 for the Southern-Egypt population recording a non-significant deviation, which indicates a higher level of heterozygosity due to a sort of random mating (no specific genetic improvement plan is reported to be applied within such group). The wide gene pool and heterozygosity available in such population could be an indication of higher variability, thus allowing the possibility of genetic improvement with a wide base population gene pool.

Inbreeding Measures: Averaging F_{IS} over all loci resulted estimates values of 0.052, -0.005 and 0.059 for the Delta, Southern-Egypt and Italian buffalo groups, respectively indicating that the Italian buffalo is relatively the most inbred population while the Southen-Egypt is the only outbred population. Obtained results are compatible with heterozygosity results

Population differentiation measures, F_{ST}, Table 3. shows the higher level of genetic differentiation between the Italian group and the two Egyptian groups and the lower level of genetic differentiation between the two Egyptian groups. All pairwise comparisons showed highly significant differences ($P<0.01$) after the Bonferroni correction.

Genetic distance: Table 3. shows that Italian buffalo had the highest distance values with the two Egyptian groups and as expectedly, a lower distance estimates was showen between the two Egyptian groups.

Table 3. Estimated pair-wise F_{ST} (above diagonal) and genetic distance (below diagonal) between pairs of studied buffalo groups/populations

Group	Delta	Italian	Southen-Egypt
Delta		0.0827**	0.0142**
Italian	0.2525		0.0762**
Southen-Egypt	0.0574	0.2295	

** P<0.01; P value had been obtained after 1000 permutation after standard Bonferroni correction

In Conclusion, there was a reasonable molecular genetic variation between Italian and the Egyptian buffalo and a lower level of genetic variation between Southen-Egypt and Delta buffalo, expressed in 5 loci. Therefore, Southen-Egypt buffalo could be considered as distinct population from the

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Delta buffalo. Comparatively, lower degree of polymorphism was shown by the Italian population. The Southen-Egypt group was the only group with non-significant deviation from HW equilibrium which implies the weak or the absence of forces that could change gene frequency, e.g. selection, mutation and random drift.

References

Barker, J.S.F., S.G.Tan, O.S. Selvaraj, and T.K. Mukherjee. 1997a. Genetic variation within and relationships among populations of Asian water buffalo (Bubalus bbubalis). Anim. Genet. 28:1-13

Cano R.J., H.N. Poinar. 1993. Rapid isolation of DNA from fossil and museum specimens suitable for PCR. Biotechniques, 15(3):432-436.

FAO. 2006. Domestic Animal Diversity Information System (DAD-IS). http//www/fao.org.dad-is. Access 11 Jan., 2006.

FAO. 2003. The State of Food and Agriculture. First Report on the State of Animal Genetic Resources in the Arab Republic of Egypt, pp 59 (Arabic).

Goudet J. 1995. FSTAT (vers. 1.2): a computer program to calculate F- statistics. J. Hered. 86: 485-486.

Goudet, J. 2002. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). URL:http//www2.unil.ch/popgen/softwares/fstat.htm. date of access

Hochberg, Y. 1988. A sharper Bonferroni procedure for multiple tests of significance. Biometrika, 75, 800-802.

Miller SA, D. D. Dykes and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16: 1215-????.

Moioli, B., A. Georgoudis, F. Napolitano, G. Catillo, S. Lucioli, Ch. Ligda, and J. Boyazoglu. 2001a. Genetic diversity between Italian and Greek buffalo populations. Agric. 29:31-40

Moioli, B., A. Georgoudis, F. Napolitano, G. Catillo, E. Giubilei, Ch. Ligda and M. Hassanane. 2001b. Genetic diversity between Italian, Greek and Egyptian buffalo populations. Livestock Prod. Sci. 70:203-211

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genet. 89:583-587.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, 512 pp.

Raymond, M. and F. Rousset. 1995a. Genepop (Version 12): Population genetics software for exact tests and ecumenism. J. Hered. 86:248-249.

Raymond, M and F. Rousset. 1995b. An exact test for population differentiation. Evol. 49:1280-1283.

Wright, S. 1943. Isolation by distance. Genetics, 28:114-138

Wright, S. 1978. Evolution and the Genetics of Populations, Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago, USA.