Genetic Analysis between and within Three Egyptian Water Buffalo Populations Using RAPD-PCR

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Abstract: The water buffalo represents an important part of animal production in Egypt It is economically a very important farm animal, genetic improvement of these animals is of economic importance, especially in reproductive performance and quantity of meat and milk. Genetic similarity and polymorphisms among the three Egyptian water buffalo populations (El-Delta, Upper and Lower Egypt) were studied using random amplified polymorphic DNA (RAPD) technique. Out of fifteen primers screened using DNA samples of the three populations, thirteen primers generated reproducible and distinct to amplify DNA fragments in these three populations. RAPD patterns with a level of polymorphism were detected among populations. The results showed that a total of 126 loci were amplified and 106 polymorphic bands (84.13%) were produced. The genetic diversity had the highest value (0.2654) in El-Delta and the lowest value (0.2590) in Upper Egypt. This result confirms the closer between the three Egyptian population buffaloes. The dendrogram of genetic relationship based on overall RAPD primers confirmed the movement of Egyptian buffaloes between El-Delta and Upper, Lower Egypt. The results confirms that the Egyptian buffaloes are belongs to one breed. [Journal of American Science 2010;6(6):217-226]. (ISSN: 1545-1003).

Keywords: water buffalo, RAPD-PCR, genetic diversity, Egypt.

1. Introduction

The water buffalo represents an important part of animal production in Egypt. The estimated herd number exceeds 3.6 million heads (FAO, 2002). It is economically a very important farm animal and genetic improvement of these animals is of economic importance, especially in reproductive performance and quantity of meat and milk as well as diseases and parasite resistance (El-Nahas et al., 1998).

Based on the phenotypic characters, Egyptian water buffaloes were classified to three populations: Beheiry, Minoufy and Saidy (El-Barbary and Abdel-Latif, 1985). To identify the genetic relationship among these three populations, random amplified polymorphic DNA (RAPD) technique was used in this study.

Genetic diversity may be measured through genetic markers. These have been used to estimating the genetic diversity of species, breeds and populations, as well as decisions related to selection of breeds/populations to be conserved (Zhang et al., 2006). However, breeders tend to concentrate on specific genotypes for determination of genetic diversity which combined traits of interest and may be used as progenitors in several breeding programs in order to introduce agronomical important traits (Rahman et al., 2006).

Random amplified polymorphic DNA (RAPD) developed by Welsh and McCelland (1990) and Williams et al., (1990), the methodology proved to be a powerful tool in different genetic analyses. This approach detects DNA polymorphisms based on amplification using a single primer of arbitrary nucleotide sequence of genomic DNA fragments. RAPD markers are attractive because they are specific and quick, nanograms of DNA are required, automation is feasible, and there is no requirement for previous DNA sequence information Williams *et al., (*1990), modest coast and ability to detect relatively small amounts of genetic variation (Ragot and Hoisington, 1993).

RAPD markers have been used successfully in estimating genetic relatedness among various breeds and populations of sheep, cattle, goat and chicken (Mahfouz et al., 2008; Hassen et al., 2007; Rahman et al., 2006; Okumus and Kaya, 2005) respectively.

The RAPD methodology proved to be efficient method in assessing genetic diversity, it is used extensively in genetic diversity studies for cattle in many countries including Uruguay (Rincón *et al.,* 2000), South Korea (Yeo et al., 2000), Turkey (GÜNEREN et al., 2010), Ethiopia (Hassen et al., 2007).

The objective of this study was to use the RAPD technique to evaluate genetic diversity and relatedness within and between three buffalo populations: Information from this work provides basic genetic knowledge that is critical for conservation and breeding programs.

2. Material and Methods

Animals: Fifteen water buffaloes representing three Egyptian populations (El-Delta and Upper, Lower Egypt) were carefully selected from three different regions in Egypt. For DNA extraction, five animals from each flock (north, middle and South) were chosen from Upper Egypt, El-Delta and Lower Egypt, respectively. The samples were taken from animals not related to each other.

DNA Extraction: For DNA extraction, blood samples from El-Delta and Upper, Lower Egypt (Beheiry and Saidy) buffaloes were collected on EDTA as a coagulant matter, blood samples were stored at -20°C until DNA extraction.. DNA extraction was carried out by method of Sharma *et al.,* (2000) as fellows: to an aliquot of 100 µl blood (after thawing), 700 µl of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 µg of proteinase K (20 mg/ml) were added. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted by equal volumes of phenol-chloroform- isoamylalcohol (25:24:1) then chloroform-isoamylalcohol (24:1). DNA was precipitated by adding two equal volumes of pre-chilled ethanol in the presence of a high concentration of salts (10% from 3 M sodium acetate). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume of TE buffer according to Pfeiffer et al., (2004).

PCR and Gel Electrophoresis: RAPD-PCR was carried out with the pooled and the individual genomic DNA samples. A total of 15 random decamer primers of arbitrary sequences with 60-70 GC% content were used as listed in table (1). The amplification conditions and PCR mixture were set according to Williams et al., (1990) and Kuske et al., (1998) , PCR was performed in reaction volume of 25 µl using 25 ng of genomic DNA from pooled samples, 25 pmol of each primer, 10X PCR buffer, 0.75 unit from Taq DNA polymerase buffer including MgCl , 25 pmol dNTPs and 0.8 U *Taq* DNA polymerase (Finnzyme). Thermal cycling (ABI 9700) was carried out by initial denaturation at 94°C for 2-5 min, followed by 34-45 cycles each at 94°C for 30-60s, annealing temperature at 28-54 for 30-60s, polymerization temperature at 72°C for 30-60s and final extension at 72°C for 10 min. The samples were cooled at 4°C. The amplified DNA fragments were separated on 3% agarose gel, stained with ethidium bromide, visualized on a UV transilluminator and photographed by Gel Documentation system Gel Pro (version 3.1 for window 3).

Scoring and Data Analysis of RAPDs: The sizes of amplified bands were determined using Gel-Pro analyzer and RAPD banding patterns were scored for the presence (1) and the absence (0) of bands for each sample. The scores were then pooled for constructing a single data matrix. The statistical analysis of the data was performed using the free software "Popgene version 1.31" computer program (Yeh et al., 1999) including the calculation of allele frequencies according to Nei (1987).

The genetic distances are designed to express the genetic differences between two populations as a single number. If there is no difference, the distances could be set to zero, whereas if the populations have no allele in common at any locus, the distance may be set equal to maximum value, i.e., the genetic identity is expressed as the genetic similarity between individuals of same or different populations. The genetic distances (D) and genetic identity (I) were calculated by POPGENE software (Yeh et al., 1999) using standard genetic distance and identity equations (Nei, 1972)

This program estimated the number and percentage of polymorphic loci and the genetic diversity according to Nei (1973). UPGMA dendrogram showed the genetic distance among breeds and was constructed according to Nei (1972).

3. Results and Discussion

RAPD amplification of polymorphic DNA-PCR is a powerful molecular genetic technique for detection of genetic variability in the different breeds/populations of the livestock (Cushwa et al., 1996). The use of RAPD markers is not limited to genetic diversity studies but it is also extended to other studies such as estimation of breeding coefficient in cattle (Bhattacharya et al., 2003).

Out of 15 primers screened using DNA samples of 3 buffalo populations, only 13 primers generated reproducible and distinct RAPD profile. All the primers detected polymorphic bands among three populations. In this study, from the thirteen primers produced 126 loci were amplified, 106 of them were polymorphic (84.13 %). The number of bands varied from 4 to 15 with molecular size of the amplification was in the range of 64 to 1675 bp length. The maximum numbers of bands were observed in OPB12 primer (15), while minimum number of bands was recorded with OPB09 primer (4) in all populations (Table 2). It has been suggested that the sequence of OPB12 primer may occur frequently in all populations and scored maximum number of bands, whereas primer OP09 was found less polymorphic within and between the populations. Sharma *et al.,* (2001) found that RAPD technique detects sufficient polymorphism within and between

populations. In the present study, an individual primer failed to produce any specific population or population specific marker in any of the three populations studied. Similarly, Kumar et al., (2004; 2008) also did not find any breed specific RAPD marker in Indian breeds of sheep.

Five of the 106 polymorphic bands had significantly different frequency distributions across breeds ($P \leq 0.05$, Table 3). These bands could be investigated further by first cloning and conversion to sequence characterized regions (Gu et al., 1995).

The percentage of the polymorphic loci represented in Table (4) which ranged from 59.52% in upper buffalo population to 63.49 % in lower Egypt buffalo population. According to Nei (1987), similar results were obtained; Abdel-Rahman and Elsayed (2007) found a high genetic similarity among three Egyptian water buffalo populations using RAPD-PCR technique. Also, the same results found in another Egyptian Native breeds, The use of RAPDS methodology in Egypt for assessing genetic diversity is not limited to buffalo but it is also extended to other farm animals species, Ali (2003) showed closer proximity in Egyptian Native sheep breeds Barki to Rahmani and Baladi (95.7 and 91.3%), respectively that was detected by random amplified polymorphic DNA markers.

The present and earlier studies (Wei, et al., 1994; Bailey and Lear, 1994; Smith et al., 1996 and Egito et al., 2007) indicate that RAPD analysis requires screening of a large number of random primers in order to detect polymorphism, because the amplification from the arbitrary primers depends on the presence or absence of the corresponding primer binding sites in the genome. Hence, comparatively large numbers of random primers are required to detect sufficient polymorphism to be utilized for genetic analysis.

The characteristics of amplification profiles generated by primer OPB14, OPC15, OPD05, OPD01 in three populations of buffalo are presented in Figs. (1, 2, 3 and 4). Table (3) showed that chi-square test of each studied primer

The genetic identity (I) and genetic distance (D) between the three populations were calculated using Nei (1972) equations through POPGENE software (table 5) in the present study the genetic identity ranged from 0.0713 to 0.1461 and 0.0849 to 0.9186, respectively (Table 5). These two measures of genetic relatedness revealed similar trend of relationship among three populations of buffaloes. Similar ranges of genetic identity and genetic distance have also been obtained by other workers (Atta ,et al., 2009;. Abdel-Rahman and **Elsayed** 2007).

The UPGMA dendrogram, based on genetic distance, was constructed to show phylogenetic relationships among the buffalo population Figure (5). The lower Egypt population appeared to be most distant from the other population whereas the upper Egypt and El-Delta populations were closely related with the highest genetic similarity as shown by an UPGMA dendrogram based on Nei's standard genetic distance.

The RAPD technique has also been used for constructing phylogenetic relationships in other farm animals such as; cattle (Kemp and Teale, 1994; Gwakisa et al., 1994; Glazko et al., 1999; Horng and Huang, 2000; Rincon et al., 2000; Zubets et al., 2001 and Jarina Joshi et al., 2007) , goat (Ahmed, 2004; Chen Xiang et al., 2004 and Li et al., 2006) , horse (Baily and Lear, 1994) and sheep (Mel'nikova et al., 1995; Cushwa et al., 1996; Stephen et al., 2000; Gong et al., 2002; Ali, 2003; Paiva et al., 2005 and Mahfouz et al., 2008) .

Conclusion

In conclusion, this work has revealed that genetic diversity exists among the three Egyptian water buffalo populations studied. With further experimentations, the RAPD profile generated for each flock can be effectively used as a supporting marker for taxonomic identification. In taxonomic and molecular systematic, species-specific RAPD markers could be an invaluable tool for species variation and establishing the status of organisms and its evolution (Allard et al., 1992; Dinesh et al., 1993; Rao, 1996).

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Table (1): Primer codes, sequences and CG content used to variation in Egyptian buffalo populations

Primers	Sequence 5`- 3`	CG%
A12	TCGGCGATAG	60
B03	CAT CCC CCT G	70
B07	GGT GAC GCA G	70
B09	TGG GGG ACT C	70
B10	CTG CTG GGA C	70
B11	GTAGACCCGT	60
B12	CCT TGA CGC A	60
B14	TCC GCT CTG G	70
B18	CCA CAG CAG T	60
B20	GGA CCC TTA C	60
C02	GTG AGG CGT C	70
C05	GAT GAC CGCC	70
C15	GACGGATCAG	60
D01	ACCGCGAAGG	70
D05	TGA GCG GAC A	60

Table (2): Total number of bands, polymorphic bands, % of polymorphic loci and their size ranges from the random primers.

Primer	Total No of bands	No. of polymorphic bands	% of polymorphic loci	Size range (in bp)	
				min	max
OPA12	13	12	92.31%	64	1389
OPB03	12	10	83.33%	91	1493
OPB07	6	5	83.33%	458	1454
OPB09	4	4	100%	288	1444
OPB10	10	9	90%	152	1675
OPB11	10	7	70%	72	1349
OPB12	15	10	66.67%	76	1416
OPB14	12	9	75%	190	1646
OPB18	9	7	77.78%	203	1479
OPB20	8	8	100%	139	1407
OPC15	6	5	77.78%	310	1371
OPD01	10	10	100%	134	1358
OPD05	11	10	90.91%	118	1129

Table (3): Chi square significance of differences revealed by random primers in Egyptian buffalo population

Primer	OPA12	OPB03	OPB07	OPB09	OPB10	OPB11	OPB12	OPB14	OPB18	OPB20	OPC15	OPD01	OPD05
Fragment (bp)	888 752 734 354 303 299 271 245 138 127 112 85 64	589 447 390 304 280 271 242 206 175 149 101 91	682 458 145 143 134 133	303 288 144 133	999 780 520 339 290 286 271 167 152 142	517 400 361 307 303 231 134 104 85 72	1358 1114 641 280 249 231 211 191 167 144 134 133 101 85 76	1646 1078 1015 789 690 603 540 373 334 278 252 190	1479 1397 1387 585 555 453 233 218 203	1407 1397 939 420 327 233 216 139	1371 1106 903 810 511 310	1358 1078 771 564 528 432 354 331 214 134	1129 919 631 432 303 271 247 240 234 224 118
P-value	0.34 1.00 0.56 0.03* 0.74 0.00* * 0.56 0.01* * 0.03* 0.25 0.74 0.76 0.25	0.34 0.28 1.00 0.12 0.12 0.25 0.09 0.28 0.28 0.34 1.00 0.25	1.00 0.34 0.34 0.56 0.76 0.34	0.34 0.76 0.28 0.12	0.76 1.00 0.25 0.71 0.76 0.25 0.56 0.71 0.12 1.00	1.00 0.25 1.00 0.56 0.28 0.12 0.74 1.00 0.09 0.76	0.02* 0.09 0.09 0.09 0.02* 0.09 0.34 0.34 0.34 0.34 1.00 1.00 1.00 1.00 1.00	1.00 0.15 0.34 0.56 0.76 1.00 0.09 1.00 0.76 0.74 0.74 0.28	0.43 0.25 0.25 0.74 0.43 1.00 0.74 0.25 1.00 0.43	0.25 0.25 0.34 0.56 0.74 0.74 0.74	1.00 0.34 0.76 0.34 0.76 0.76	0.25 0.74 0.43 0.74 0.30 0.74 0.74 0.74 0.34 1.00	0.43 0.34 1.00 0.76 0.56 0.12 0.74 0.76 0.43 0.74 1.00

*Number of bands significantly different ($P \leq 0.05$) among three Egyptian population of buffalo

Table (4): Genetic diversity in investigated buffalo populations breeds based on RAPD markers.

Buffalo population	No. of polymorphic loci	% of polymorphic loci	Genetic diversity Nei ,s (1973)	Shannon's diversity (Lewontin 1972)
Upper Egypt	75	59.52	0.2590	0.3718
El-Delta	78	61.90	0.2654	0.3824
Lower Egypt	80	63.49	0.2603	0.3794
All	106	84.13	0.3086	0.4574

Table (5): Genetic identity (above diagonal) and genetic distance (below diagonal) between the investigated buffalo populations (Nei's 1972)

Pop	Upper Egypt	El-Delta	Lower Egypt
Upper Egypt	****	0.9312	0.8641
El-Delta	0.0713	****	0.9186
Lower Egypt	0.1461	0.0849	*****

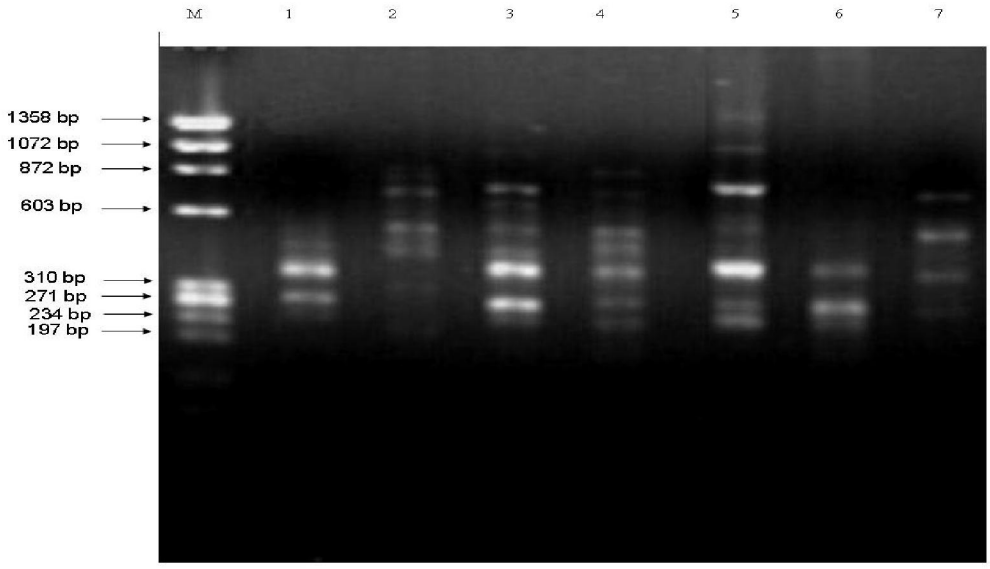

Fig. (1): Random amplified polymorphic DNA (RAPD) profile generated by primer OPB-14 in individual buffalo of different populations, Lane M= molecular marker(ϕ x 174 DNA Hae III digest. lane (1,2) represents DNA of pop1, lane (3,4) represents DNA of pop2 and lane (5,6,7) represents DNA of pop3.

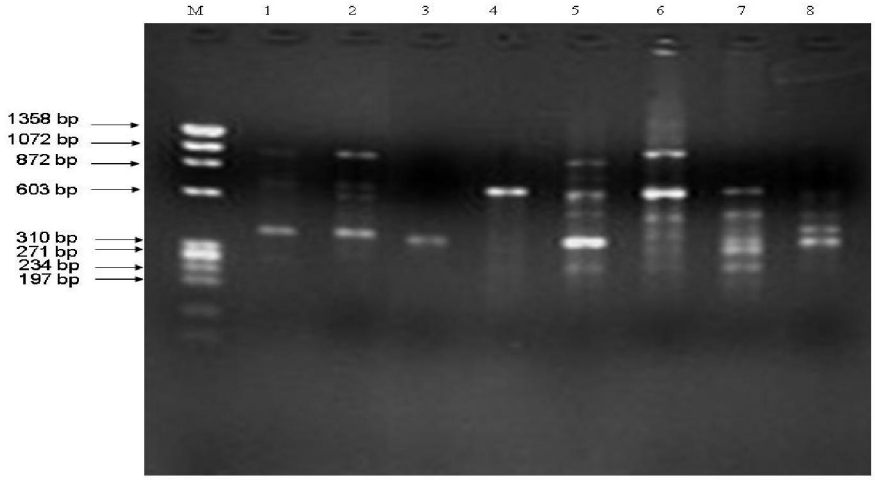

Fig. (2): Random amplified polymorphic DNA (RAPD) profile generated by primer OPC-15 in individual buffalo of different populations, Lane M= molecular marker(ϕ x 174 DNA Hae III digest. lane (1,2,3) represents DNA of pop1, lane (4,5) represents DNA of pop2 and lane (6,7,8) represents DNA of pop3.

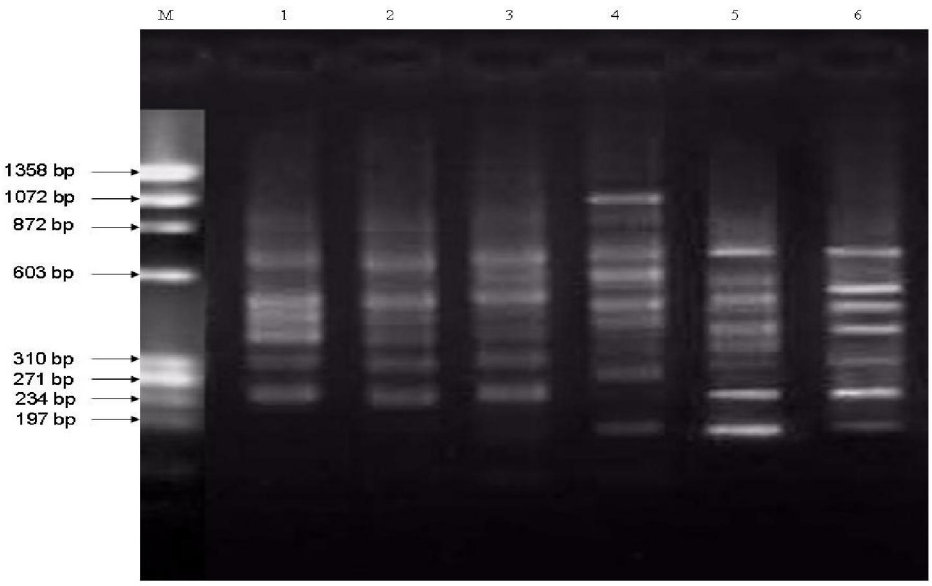

Fig. (3): Random amplified polymorphic DNA (RAPD) profile generated by primer OPD-05 in individual buffalo of different populations, Lane M= molecular marker(ϕ x 174 DNA Hae III digest. lane (1,2) represents DNA of pop1, lane (3,4) represents DNA of pop2 and lane (5,6,) represents DNA of pop3.

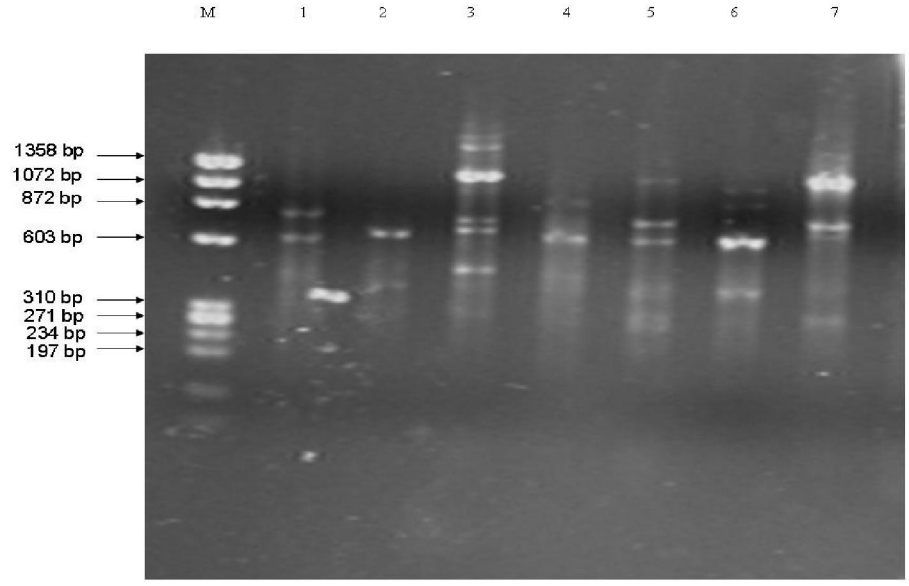

Fig.(4): Random amplified polymorphic DNA (RAPD) profile generated by primer OPD-01 in individual buffalo of different populations, Lane M= molecular marker(ϕ x 174 DNA Hae III digest. lane (1,2) represents DNA of pop1, lane (3,4) represents DNA of pop2 and lane (5,6,) represents DNA of pop3.

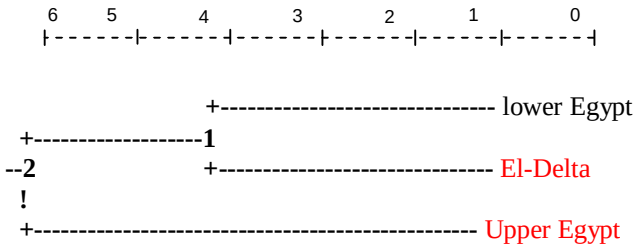

Fig. (5): Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA
--Modified from NEIGHBOR procedure of PHYLIP Version 3.5

6/18/2009