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*Full Length Research Paper*

# Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA markers

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**A genetic analysis using RAPD markers was performed for studying variation in four breeds of sheep (Baladi, Barki, Rahmani and Saffolk). Nineteen random primers were used to amplify DNA fragments in these breeds. RAPD patterns with a level of polymorphism were detected between breeds. Results showed closer proximity of Barki to Rahmani and Baladi (95.7 and 91.3%), respectively.**

**Keywords:** Sheep, breeds, RAPD, genetic similarity.

## INTRODUCTION

The African sheep are described as thin tailed, fat tailed or fat rumped (Mason and Maule, 1960), and the thin tailed are sometimes further segregated into hairy or wooled types (Epstein, 1971). The classification based on historical, anthropological and morphological evidence is not satisfactory for the purpose of conservation and utilization. Breed characterization requires knowledge of genetic variation that can be effectively measured within and between populations (Hetzel and Drinkwater, 1992). Genetic markers may provide useful information at different levels: population structure, levels of gene of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness (Feral, 2002).

During the last few years, the great strides of molecular biology virtually gave access to the entire genome, but their complexity and high cost limited their use to precisely targeted projects in population biology. However, the polymerase chain reaction (PCR) which induced a methodological revolution (Mulis and Faloona, 1987; Sakai et al., 1988; Erlich, 1989) have been applied for genetic variations studies. The PCR technique is basically a primer extension reaction for amplifying specific nucleic acids in vitro. The use of a thermostable polymerase referred to as *taq* allows a short stretch of DNA to be amplified to about a million fold so that one can determine its size, nucleiotide sequence, etc.

The power of the existing DNA techniques to genetically define populations offers an attractive possibility toward characterization of sheep populations. The extensive genetic polymorphism revealed by DNA markers may be used as an advantage to resolve genetic difference of even closely related individuals. The main interest for population biology is that it is now possible to work with a very small initial amount of DNA (virtually, one cell is sufficient). The method is not necessarily destructive, the sample may be very tiny, preserved in ethanol or buffer, or dried. It is possible to work on

**Table 1.** List of the random primers used, their nucleotide sequence and annealing temperatures.

| Primers | Sequence 5`- 3` | Annealing Temperature °C/time (s) |
|---|---|---|
| 1 | ATG ACG TTG A | 45/30 |
| 2 | GGG CTA GGG T | |
| 3 | ACC GGG AAC G | |
| 4 | AGC AGG TGG A | |
| 5 | AGG CCC CTG T | 28/30 |
| 6 | ATG CCC CTG T | |
| 7 | AAA GCT GCG G | |
| 8 | ACC GCC GAA G | |
| 9 | GGC ACT GAG G | 45/30 |
| 10 | CGC TGT CGC C | |
| 11 | AGT CCT CGC C | |
| 12 | TGG TGG ACC A | |
| 13 | GAA TGC GAC G | |
| 14 | CTG AGG AGT G | |
| 15 | CGA GCC CTT CCA GCA CCC AC | 54/30 |
| 16 | GAA ACG GGT GGT GAT CGC AG | |
| 17 | GGT GAC GCA GGG GTA ACG CC | |
| 18 | GGA CTG GAG TGT GAT CGC AG | 58/30 |
| 19 | GGA CTG GAG TGG TGA CGC AG | |

museum samples (Higuchi et al., 1984, 1988; Ellengren, 1991) or even, under certain conditions, on fossils (Paabo, 1989; Austin et al., 1997).

Application of the random amplified polymorphic DNA technique have greatly increased the ability to understand the genetic relationships within species at the molecular level. Information on genetic relationships in livestock within and between species has several important applications for genetic improvement and in breeding programmes (Appa Rao et al., 1996). In this present paper, the genetic diversity of the four breeds of sheep was studied.

## MATERIALS AND METHODS

Blood samples of four sheep breeds were collected from the farm of the Agricultural Research Station, Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. The breeds are Black Baladi (hybrid), Barki (pure Egyptian breed), Rahmani (pure Egyptian breed) and Saffolk (foreign breed living under Egyptian conditions).

DNA extraction was carried out by method of Sharma et al. (2000) as fellows: Venous blood samples were mixed with EDTA as anticoagulant and stored at –20°C. 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, phenol-chloroform (1:1) and chloroform-isoamylalcohol (24:1). DNA was precipitated by adding 2 volumes of chilled ethanol in the presence of a high concentration of salts (10% 3 M sodium acetate). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA).

PCR amplifications were performed following the procedure of Williams et al. (1990). Nineteen random primers were used in this work (Table 1). The reaction was carried out in a 25 µL in an eppendorf tube containing 25 ng of genomic DNA. Amplifications were performed in a Perkin Elmer 9700 Cetus thermal cycler which was programmed as follows: an initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 30 s for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 s and final extension at 72 °C for 10 min were carried out. The samples were cooled at 4°C. The amplified DNA fragments were separated on 3% agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator.

PCR products were scored across the lanes as variables. The presence of a band of amplified DNA was scored as '1' and absence as '0'. The data matrix so generated was used for calculation of similarity matrix based on Jaccard's coefficients (Jaccard, 1908).

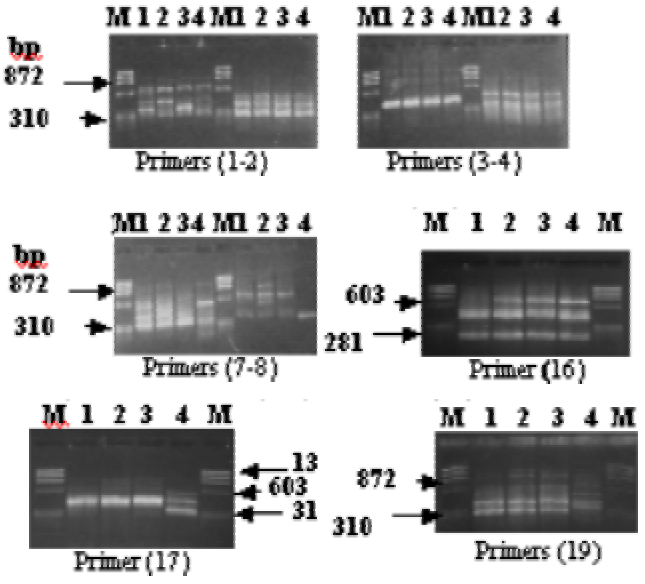


**Figure 1.** Example of RAPD patterns in four breeds of sheep obtained with different random primers. Lane M: ΦX174 DNA marker. Lane 1: Baladi, Lane 2: Barki, Lane 3: Rahmani, Lane 4: Saffolk.

## RESULTS AND DISCUSSION

To ensure that the amplified DNA bands originated from genomic DNA, and not primer artifacts, negative control

was carried out for each primer/breed combination. No amplification was detected in control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions. Five of nineteen primers (26.31%) were successfully amplified polymorphic bands among the four breeds studied (Figure 1).

RAPD analysis was used for constructing parsimony tree depicting relationships among the four breeds studied (Figure 2). Data presented in Table 2 showed closer proximity of Barki to Rahmani (95.7%) and Baladi (91.3%), while the other breed (Saffolk) was the most different. This may due to fact that Barki and Rahmani are pure Egyptian breeds, and the Saffolk is a British breed. The RAPD technique has also been used for constructing trees in other animals such as buffalo, cattle, goat and sheep (Appa Rao et al., 1996), tilapia fish (Baradakci and Skibinski, 1994) and date palm (Soliman et al., 2003).

**Table 2.** Jaccard's similarity coefficients between the four breeds of sheep based on RAPD data.

| Breeds | Baladi | Barki | Rahmani | Saffolk |
|---|---|---|---|---|
| Baladi | | 91.3 | 89.9 | 81.9 |
| Barki | 91.3 | | 95.7 | 85.1 |
| Rahmani | 89.9 | 95.7 | | 83.8 |
| Saffolk | 81.9 | 85.1 | 83.8 | |

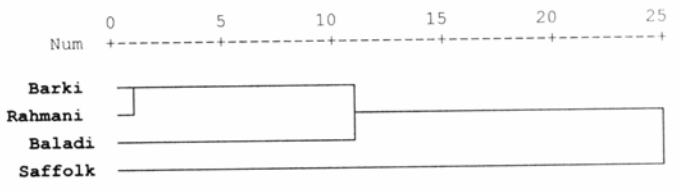


**Figure 2.** Dendrogram using Average Linkage (Between Groups) based on RAPD data analysis among the four breeds of sheep.

The results of this study demonstrate the usefulness of the RAPD approach for detecting DNA polymorphism in sheep and establishing the relationships with among different breeds. The majority of random primers used gave distinctly reproducible patterns in the entire breed studied. However, primers varied in the extent of information they generated with some producing highly polymorphic patterns whereas others produced less polymorphic products. Some DNA fragments were apparently similar in size among the four breeds, whereas others were unique to a particular breed.

In conclusion, this work has revealed that genetic diversity exist among the four Egyptian sheep populations studied. With further experimentations, the RAPD profile generated for each breed 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; Appa Rao et al., 1996).

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