# Evaluation of Genetic Diversity Within and Between Native and Khaki Campbell Duck breeds Using RAPD Markers

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**Abstract:** A total of 100 genomic DNAs were isolated from two breeds of duck: Khaki Campbell and Native , through a modified salting out procedure. The samples were used in a Polymerase Chain Reaction (PCR) with 27 RAPD Markers. Amplified PCR-products with the markers were separated on a 2% agarose gel and stained with ethidium bromide. To evaluate the bands , polymorphic and monomorphic bands were described. The RAPD analysis data from 5 primers were utilized in estimating genetic diversity and genetic distance. The genetic distance between two population was 36/06. The genetic diversity within Native and Khaki Campbell breeds was 51/08 and 66/01 respectively.

**Key words:** Duck, breeds, RAPD, genetic diversity

## INTRODUCTION

From ancient times domestic ducks have served as a source of food and income for people in many parts of the world (Wiliam and Sandhu, 2001). Breed characterization requires knowledge of genetic variation that can be effectively measured within and between population (Hetzel and Drinkwater, 1992). The classification based on historical, anthropological and morphological evidence is not satisfactory for the purpose of conservation and utilization (Ali ,2003). With the advent of molecular biology, random amplified polymorphic DNA (RAPD) generated by Polymerase Chain Reaction (PCR) with single 10 base oligonucleotide primers of arbitrary sequence are used as a preliminary approach to identify possible patterns of inter and intra population genetic variation on threatened or endangered species (Cardoso *et al*.,1998; Welsh and Celland, 1990; Williams *et al*., 1990).
The objective of this study was to evaluate genetic diversity and distance in two breeds of duck by the RAPD technique.

## MATERIALS AND METHODS

**Sample collection and DNA extraction:** A total of 100 individuals (50 individuals/breed) from two breeds of duck: Khaki Campbell, Native, collected in Mazandaran province-IRAN, were utilized. DNA was extracted from blood using salting out protocol modified by Miller *et al.* (1998). After the DNA was diluted, quantified by spectrophotometer and in agarose gel at 0.8% and stored at 20 C until use.

**RAPD-PCR analysis:** Twenty seven random primers were used in this work. The PCR reaction were carried out in and eppendorf thermocycler using an amplification program with 4 min at 94C, followed by 40 cycles in the following stage: a) 1 min at 94C, b) 1 min at 35C and c) 1 min at 72C. At the end of 40 cycles, an additional stage of 10 min at 72C was added for complete extension of amplified products.RAPD-PCR reactions were carried out in a final volume of 25 µL,with buffer PCR 1x; 4.5 µM $MgCl_2$; 200 µM of each dNTP; 0.4 µM of the arbitrary primers; 1 U taq and 1 M of template DNA. The amplified DNA products were resolved by electrophoresis on a 2% agarose gel with Tris-borate EDTA buffer and stained with ethidium bromide, for 20 min and photographed under UV transllumination using gel document.

**Statsical analysis:**To evaluate the bands, polymorphic and monomorphic bands were described. Data were recorded in a binary matrix (1 = presence of band, 0 = absence). The level of polymorphism was quantified by using Nei's estimator of similarity, base on the probability that an amplified fragment from one duck will also be found in another according to the formula $S_{xy} = 2n_{xy} /(n_x + n_y)$ where $n_{xy}$ is the number of fragment shared by individuals x and y and nx and $n_y$ are the number of fragments scored for each individual (Nei and Li, 1997; Lynch, 1991). The genetic distances between breeds were calculated using the POPGENE program (Population Genetic Analysis) version 1.31 (Yeh *et al*., 1999). This program establishes standardized genetic distance matrices (Nei, 1972) and matrices of genetic distance corrected for small sample (Nei,1978). The method proposed by Nei (1972) is one of the most used to obtain genetic, distance between population (Lynch and Milligan1994) and this use is recommended with RAPD data (Apostolidis *et al*., 2001). Cluster analyses done this work were conducted using UPGMA and the resulting cluster were expressed and dendrogram. The POPGENE program also generated gene diversity indicate for each breed base on NEI (1973). According to Weir (1996) this is the most adequate method for study unique population.

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## RESULT 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 amplified was detected in control reactions. All amplification products were repeated using the same reaction condition. Five of twenty seven primers (18/5%) were successfully amplified polymorphic bands between two breeds studied (Table 1 and Fig 1,2).Number of amplified bands presented in Table 2

**Genetic structure of breeds:** The gene diversity within Native and Khaki Campbell breed was 51/08 and 66/01 respectively (Table 3). This analysis was based on the mean allelic frequency of the RAPD markers, which ranged from 434 to 2607 bp.

Table1: The sequence of the primers used and their annealing temperature

| Primer | Sequence 5 - 3 | ATºC |
|---|---|---|
| Rap 1 | TCA CGA AGC C | 37 |
| Rap 10 | GAC CGC TTG T | 36 |
| Rap 13 | GAA CGG ACT C | 35 |
| Rap 14 | GTG AGG CGT C | 37 |
| Rap 16 | AAA GCT GCG G | 36 |

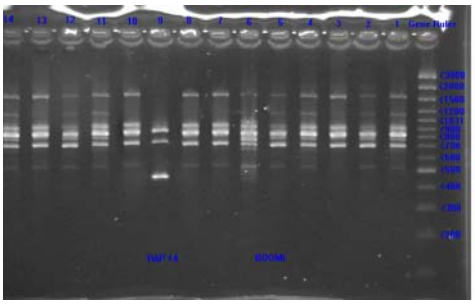

Fig 1:RAPD amplification products generated by primer 14 in Native ducks

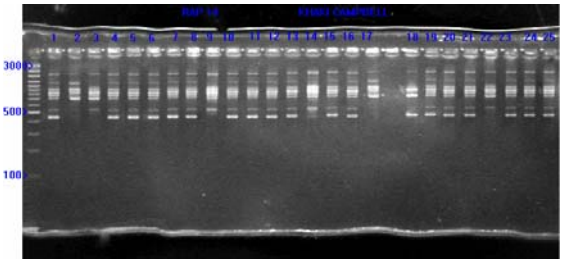

Fig 2: RAPD amplification products generated by primer 14 in Khaki Campbell ducks

Table 2: Number of RAPD bands for each primers using agarose gel in duck breeds

Breeds

| | Khaki campbell | | | Native | | |
|---|---|---|---|---|---|---|
| Primer | a | b | c | a | b | c |
| RAP1 | 8 | 7 | 1 | 10 | 8 | 2 |
| RRP14 | 9 | 6 | 3 | 9 | 6 | 3 |
| RAP10 | 6 | 3 | 3 | 6 | 4 | 2 |
| RAP13 | 7 | 5 | 2 | 6 | 4 | 2 |
| RAP16 | 12 | 8 | 4 | 10 | 6 | 4 |
| Total | 42 | 29 | 13 | 41 | 28 | 13 |

a) number of bands , b) number of polymorphic bands c) number of monomorphic bands

Table3:Nei's (1973) gene diversity index of breeds using the popgene program (yeh et al.,1999).

| Breed | Genetic diversity |
|---|---|
| Native | 27/81 |
| Khaki Campbell | 27/22 |

**Estimation of genetic distance:** The estimates of genetic distances between two breeds were calculated to help in the study of genetic relationship and genetic divergence between breeds. The genetic distance between two population was 36/06. 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 (Bardakci and Skibinski, 1994) and date palm (Soliman *et al.,* 2003). Xiao *et al.* (2004) used the RAPD technique to evaluate the genetic diversity of Fujian local duck populations in different ecological type. They showed that the genetic diversity in east Fujian (67.97%) was higher than that in west Fujian (59.05%). Genetic differentiation was estimated to be about 32.03% among population of east Fujian and about 40.95% among population of west Fujian.

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

## CONCLUSION

In conclusion, this research has revealed that genetic diversity exist between two breeds studied. With further experimentation, the RAPD profile generated for each breed can be effectively used as supporting marker for taxonomic identification. In taxonomic and molecular systematic, species-specific RAPD markers could be an invaluable tool for species evolution (Allard *et al.,* 1992; Dinesh *et al*., 1993).

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