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Measurement of Within and Between Genetic Variability in Duck Breeds by RAPD Markers

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Abstract: A total of 200 genomic DNAs were isolated from the four breeds of duck: Muscovy, Pekin, 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. Genetic similarity and genetic distance were calculated. The RAPD analysis data from 7 primers were utilized in estimating genetic similarity between four breeds, which ranged from 0.46 to 0.77 between the breeds. The maximum genetic distance was observed between Muscovy and Native duck breeds (0.76).

Key words: Duck, breeds, RAPD, genetic similarity

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 populations (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 similarity and distance among four breeds of duck by the RAPD technique.

MATERIALS AND METHODS

Sample collection and DNA extraction: A total of 200 individuals (50 individuals/breed) from four breeds of duck: Muscovy, khaki Campbell, Pekin and Native, collected in Mazandaran province-IRAN, were utilized. DNA was extracted from blood using salting out protocol modified by Miller *et al*. (1988). 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 reactions were carried out in an eppendorf thermocycler using an amplification program with 4 min at 94°C followed by 40 cycles in the following stage: a) 1 min at 94°C, b) 1 min at 35°C and c) 1 min at 72°C. At the end of 40 cycles, an additional stage of 10 min at 72°C 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 mM $MgCl_2$; 200 μM of each dNTPs; 0.4 μM of the arbitrary primers; 1 U taq and 1 μM 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 transillumination using gel document

Statistical 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

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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 samples (Nei, 1978). The method proposed by Nei (1972) is one of the most used to obtain genetic distances between populations (Lynch and Milligan, 1994) and its use is recommended with RAPD data (Apostolidis *et al*., 2001). Cluster analysis for this work were conducted using UPGMA and the resulting cluster were expressed as dendrogram. The POPGENE program also generated gene diversity indices for each breed based on Nei (1973). According to Weir (1996) this is the most adequate method for study unique populations.

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 amplified was detected in control reactions. All amplification products were found to be reproducible when reaction were repeated using the same reaction conditions. Seven of twenty seven primers (26%) were successfully amplified polymorphic bands among the four breeds studied (Table 1 and Fig. 1-4). Number of amplified bands presented in Table 2.

Genetic structure of breeds: The gene diversity index was calculated for all breeds, considering them as unique populations (Table 3). This analysis was based on the mean allelic frequency of the RAPD markers, which ranged from 230 to 2368 bp. The Muscovy breed presented lowest gene diversity (24%) when compared to the other duck breeds, while the maximum genetic diversity was obtained in Pekin duck breeds (29%).

Table 1: The sequence of the primers used and their annealing temperature

Primer	Sequence 5′-3′	AT°C
Rap 1	TCA CGA AGC C	37
Rap 6	TGG ACC GGT G	35
Rap 10	GAC CGC TTG T	36
Rap 11	AAC GCG TCG G	37
Rap 13	GAA CGG ACT C	35
Rap 14	GTG AGG CGT C	37
Rap 16	AAA GCT GCG G	36

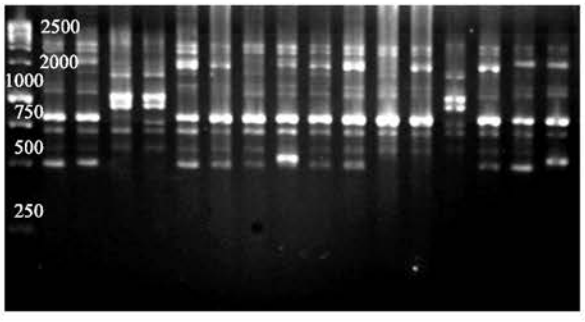

Fig. 1: RAPD amplification products generated by primer 14 in Muscovy ducks

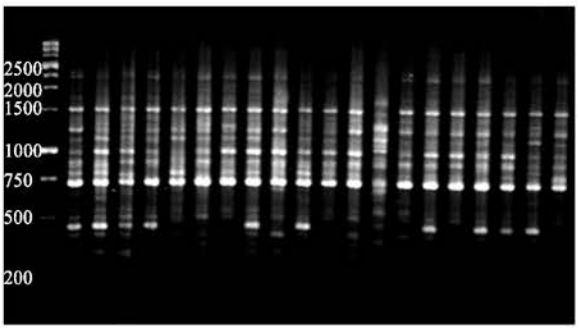

Fig. 2: RAPD amplification products generated by primer 14 in Pekin ducks

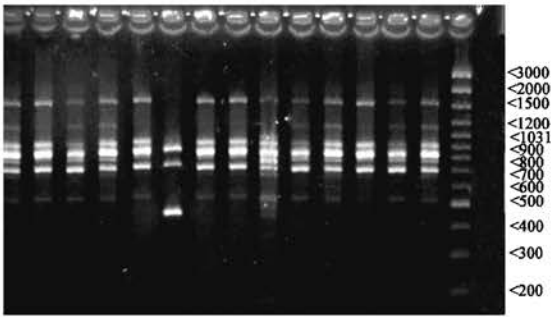

Fig. 3: RAPD amplification products generated by primer 14 in Native ducks

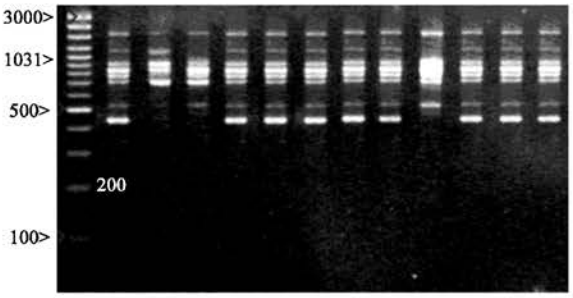

Fig. 4: RAPD amplification products generated by primer 14 in Khaki Campbell ducks

Estimation of genetic distance: The estimates of genetic distances between the breeds were calculated to help in the study of genetic relationship and genetic divergence between pairs of breeds. The lowest genetic distance (26%) was found between khaki Campbell and native duck breeds, while the maximum genetic distance (76%) was obtained between the Muscovy and Native duck breeds (Table 4).

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

	Breeds											
	Muscovy			Pekin			Khaki campbell			Native		
Primer	a	b	c	a	b	c	a	b	c	a	b	c
RAP 1	17	6	11	12	6	6	8	7	1	10	8	2
RAP 14	9	4	5	13	7	6	9	6	3	9	6	3
RAP 6	13	3	10	15	8	7	12	0	12	7	0	7
RAP 10	8	2	6	10	4	6	6	3	3	6	4	2
8 RAP 11	9	5	4	14	5	9	4	0	4	13	0	13
RAP 13	13	4	9	14	5	9	7	5	2	6	4	2
RAP 16	12	4	8	9	3	6	12	8	4	10	6	4
Total	81	28	53	87	38	49	58	29	29	61	28	33

a) Number of amplified bands, b) Number of polymorphic bands, c) Number of monomorphic bands

Table 3: Nei's (1973) gene diversity index of breeds using the popgene program (Yeh *et al*., 1999), considering them as unique population

Breed	Genetic diversity
Muscovy	24.00
Pekin	29.00
Native	27.81
Khaki campbell	27.22

Table 4: Genetic variability between the four breeds of duck based on RAPD markers

Population	Muscovy	Pekin	Native	Khaki campbell
Muscovy		0.73	0.46	0.56
Pekin	0.31		0.62	0.61
Native	0.76	0.46		0.77
Khaki campbell	0.57	0.48	0.26	

Genetic similarity (above diagonal) and Genetic distance (below diagonal)

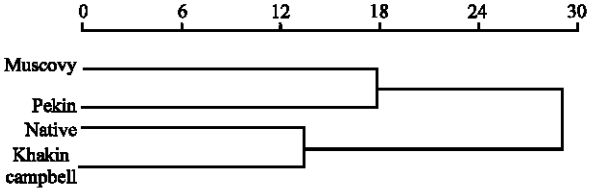

Fig. 5: Dendrogram generated by the UPGMA method for Nei (1978) genetic distance using POPGENE program

A dendrogram based on the genetic distances among four breeds is shown in Fig. 5. Two clusters were observed. The first cluster included Muscovy and Pekin while the second one included Native and Khaki Campbell duck breeds. 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).

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 populations of east Fujian and about 40.95% among populations of west Fujian.

The results 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 patterns in the entire breed studied. However, primers varied in the extent of information they generated with some producing highly polymorphic pattern whereas others produced less polymorphic products. Some DNA fragment were apparently similar in size among the four breeds, whereas others where unique to a particular breed.

CONCLUSION

In conclusion, this research has revealed that genetic diversity exist among the four duck breeds studied. With further experimentation, 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 evolution (Allard *et al*., 1992; Dinesh *et al*., 1993).

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