Studies on Genetic Variation of Different Chinese Duck Populations with Random Amplified Polymorphic DNA Analysis

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ABSTRACT : The genetic polymorphism and relationships of Muscovy, Cherry Valley Meat ducks, Partridge ducks and their crossbreds F_1 and F_2, respectively, were studied using a random amplified polymorphic DNA (RAPD) technique. The results showed that RAPD markers were effective for the analysis of genetic relationships among ducks. Amplification with 20-primers gave 760 reproducible amplified fragments. The percentage of polymorphic marker band was 74.70%, which indicates that the RAPD technique had higher efficiency of polymorphism detection and sensitivity in studying the genetic variations among ducks and showed that the genetic polymorphism was abundant between two species of duck populations. The average index of genetic distance in hybrid F_2 was 0.2341 and higher than that of its parents, which indicates that the genetic diversity was improved by crossbreeding with Muscovy. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 4 : 475-481)***

Key Words : Muscovy, Crossbred, RAPD, Genetic Diversity, Genetic Relationships

INTRODUCTION

Ducks, one of the avian species, have economic, social and ecological value in China, particularly in southern parts of the country. It is an important genetic resource in China. Both Cherry Valley Meat and Partridge ducks are important domestic ducks that belong to *Ares*, *Anseriforme, Anas domestica, Anas*.

Partridge duck has dark brown sparrow-colored feathers and reddish orange beak, shanks, webs and yellow skin. The tradition Partridge duck's growth speed and meat yield were rather slow in China (Zhang, 1996). Cherry Valley Meat Ducks had higher body weight approximately 3,680 g and higher growth rare (Ning, 1999). The duck has creamy white feathers and deep orange bill and legs. Better production performance although Cherry Valley Meat ducks were, they were very fat. The genetic selection programme was established to reduce fat and increase lean meat in Cherry Valley Meat ducks.

Muscovy (*Cairina moschata domestica*) is *Cairina,* which originated in South America and has capable of adapting to different climates. The breeds grazes like a goose and the males have no curled feathers in the tail, which was used for sex identification. The physical characteristics of Muscovy ducks is that no feathers on the face and the skin is bright red, while the drake has a knob on its head which gives the appearance of a crest. The feathers come in variations of black, white and blue. Both sex means of communication is by hissing. It has both claws and webbed feet. The hybrid 'mulard' is obtained by crossing a female domestic duck and a male Muscovy. The hybrids has better meat quality and lean rate and adapted in south of China and Southeast Asia environment.

According to farmers experience the F_1 stocks that crossed male selected Cherry Valley Meat duck with female lines Partridge selected had a good growth speed. Then the intergeneric hybrid F_2 stocks cross between domestic duck (male F_1 generation) and Muscovy duck (female lines) had a meat performance and growth speed. The breast muscle yield and lean of the crossing commercial duck could be significantly improved by the three-way cross. The average body weight (2,273 g) of three-way hybrid was higher than (1,684 g) of tow-way hybrid ducks when feeding and managed under the same condition (Wang, 2000). Also, had better grazing ability and demanded in the international markets in China.

Recently, the random amplified polymorphic DNA (RAPD) technique has emerged as another means for studying genetic variations at both the population and species levels (Welsh and McClelland, 1990; Williams et al., 1990; Williams et al., 1998). RAPD markers are based on amplification of genomic DNA by polymerase chain reaction (PCR) using short primers homologous to random target sites in the genome. Polymorphisms can simply be identified as the presence or absence of amplified products on ethidium bromide stained agarose gels. This provides relatively easy and rapid assessment of the differences in the genetic composition of related individuals and species (Callejas and Ochando, 2001; Simonsen and Holmstrup, 2004). Polymorphisms generated by the RAPD technique have been used for the assessment of variations in population genetic analysis, genetic mapping, fingerprinting, species identification and identification of breed of specific

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Table 1. List of arbitrary primers used in this study together with their nucleotide sequence

Primer's code	Sequence 5' to 3' flanking region	(G+C) content (%)	No. of scored bands	No. of polymorphic bands
OPA04	AATCGGGCTG	60	39	33
OPA06	GGTCCCTGAC	70	28	27
OPA07	GAAACGGGTG	60	42	30
OPA08	GTGACGTAGG	60	44	34
OPA09	GGGTAACGCC	70	38	34
OPA10	GTGATCGCAG	60	38	27
OPA12	TCGGCGATAG	70	44	49
OPA14	TCTGTGCTGG	60	27	15
OPA16	AGCCAGCGAA	60	34	25
OPA18	AGGTGACCGT	60	45	45
OPA20	GTTGCGATCC	60	31	21
OPF09	CCAAGCTTCC	60	30	12
OPV06	ACGCCCAGGT	70	39	18
OPV16	ACACCCCACA	60	44	24
OPV18	TGGTGGCGTT	60	41	33
OPZ08	GTGGCATCTC	60	44	30
OPZ09	ACAGCCTGCT	60	27	25
OPZ11	AAGGCTCACC	60	43	32
OPZ16	AGTCGCCCTT	60	39	18
OPZ17	GTGGAGTCAG	60	43	36
Total	-	-	760	568
Mean	-	-	7.60	28.40
p	-	-	-	74.70

species (Cargill et al., 1995; Smith et al., 1996; Zhang et al., 1998; Appannavar et al., 2003; Chenyambuga et al., 2004; Saifi et al., 2004; Yoon et al., 2004). RAPD may be used to detect DNA variability at different levels, ranging from single base changes to deletions and insertions. The advantages of RAPD are manifold, it does not require previous sequence information for analysis, process is simpler, faster, less expensive, and nanogram quantities of DNA are sufficient. Olowofeso et al. (2005b) had equally suggested that for a quick examination of genetic background of species especially avian, the RAPD technique could still be used to obtain first hand genetic information. However, only a limited number of duck populations have been examined by RAPD technique. In order to exploit the rich genetic resource of ducks in China, to develop prolific hybrids and to provide the baseline information for the development of a useful program for the species, we carried out a preliminary analysis on the genetic structure, genetic relationship and heterosis among five Chinese duck populations with random markers utilizing RAPD assay.

MATERIALS AND METHODS

Blood samples of the ducks

Twenty blood samples from each of the five species of ducks comprising Partridge, Cherry Valley Meat, Muscovy and their hybrids F_1 (C♂×P♀) and F_2 (F_1♂×M♀) were collected randomly from the Potou Liuwang duck farm, where male and female duck were reared in different pens with enough exercise yard and pond. The farmers adopted artificial insemination techniques for mating, in Zhanjiang city, Guangdong Province, P. R. China.

Samples were made un-coagulated with ACD and frozen in dry ice immediately after collection and then stored at -20°C in the laboratory for use.

DNA extraction and PCR amplifications and agarose gel electrophoresis

We consulted the original and made some necessary changes (Yi, 2002). The DNA concentration was estimated by photometry and electrophoresis was ethidium bromide (EB) stained. Amplifications were performed in a Hema 480 thermal cycler. Gels were photographed under UV-light with Tanon GIS-2008 system.

PCR primers

One hundred and twenty base long oligonucleotide primers obtained from Huamei Biotechnology Company, P. R. China, were used for PCR amplifications in the initial analysis. Twenty of these primers representing (16.67%) of the total initially tested were used to provide polymorphic markers for genetic diversity. The primers were randomly selected on the basis of GC-content for RAPD-PCR amplification. The sequences of the primers used are presented in Table 1.

Statistical analysis

PCR-products were scored across the lanes as discrete variables. Presence of band of amplified DNAs was scored

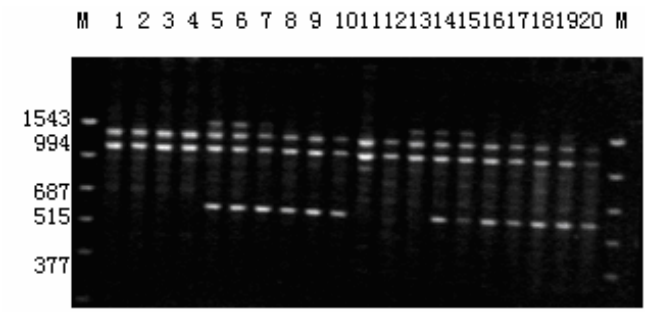

Figure 1. Primer OPZ09 with Partridge ducks.

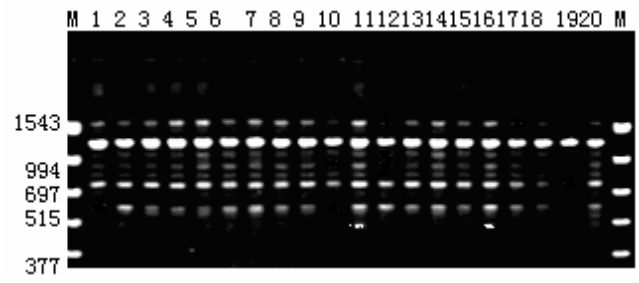

Figure 2. Primer OPZ16 with Cherry Valley Meat ducks.

as '1' and absence of band represented as '0'. Percentage of polymorphic band (p) was considered, the coefficient of band-sharing or the similarity index (F) and relative genetic distance (D) were calculated using the following formulae:

$$F = 2N_{xy}/(N_x+N_y) \quad \text{and} \quad D = 1-F$$

where N_{xy} is the number of bands shared by both individuals x and y, N_x and N_y represents the number of bands in individuals x and y (Dudley, 1994), and F denote the index of similarity (Nei and Li, 1979). Based on the relative genetic distance, the phylogenetic tree of the duck populations were reconstructed with the Un-weighted Pair-Group Method and Arithmetic Average (UPGMA) through SAS program. The genetic diversity of inter-population (H_o) was calculated using the formula:

$$H_o = -\sum X_i \ln X_i$$

where X_i denotes the phenotypic frequency of the RAPD bands in a species. For populations, the Shannon diversity index was calculated using the relation:

$$H_{pop} = 1/n\sum H_o$$

where H_{pop} is the inner diversity index of n-populations. The genetic diversity of total populations denoted as (H_{sp}) was generated with the expression:

$$H_{sp} = -\sum X \ln X$$

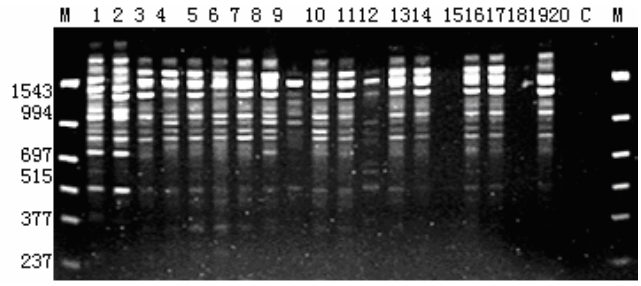

Figure 3. Primer OPZ17 with Muscovy.

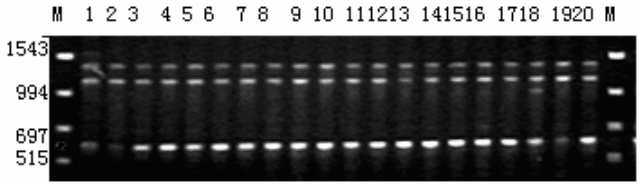

Figure 4. Primer OPZ09 with hybrids.

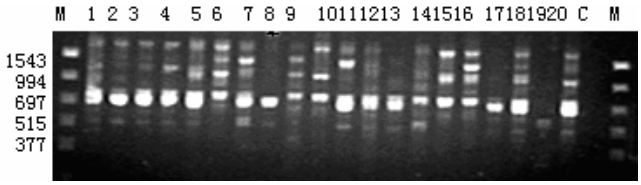

Figure 5. Primer OPZ09 with F_2.

where X is the frequency of band present in the total populations. The proportion of genetic variation (V) between the duck populations were calculated by employing the formula:

$$V = (H_{sp}-H_{pop})/H_{sp}$$

where (H_{sp}-H_{pop}) represents the diversity index between the duck populations.

RESULTS

RAPD profile

The nucleotide sequence of each primer was chosen arbitrarily with each primer made up of ten nucleotides' sequence length and possessed 60-70% G+C-content (Table 1). Series of several DNA fragments were amplified in five duck populations with each of the primer. To ensure that the amplified DNA bands originated from genomic DNA, and not primer artifacts, genomic DNA was omitted from control reactions for each primer/species combination. No amplification was detected in such control reactions. All the amplification products were found to be reproducible when reactions were repeated using the same reaction conditions.

Comparisons of genetic relationships of Muscovy duck with closely related species

For the 100 individual of ducks from the five populations, 20 primers from the tested 120 primers produced a total of 760 replicate bands, 568 representing (74.74%) were polymorphic. Although there were some variations observed between individuals of a species with a single primer, most of the bands were not variable among different individuals of a given species. Further, the number and positions of these bands were found to be dependent on the combinations of species and primer used. Depending on the primer-species combinations, the number of resolved amplified products varied from 1 to 14, with a size range varying from 200 to 2,000 bp. The individual RAPD profile of each species was found to be unique in terms of number and position (Figures 1-5). However, relatively more bands were common between Muscovy and domestic ducks, while Partridge ducks and F_1 showed higher similarity within the two species.

Genetic diversity and differentiation

The percentage of polymorphic bands (p) within population ranged from 67.90% (F_1) to 83.10% (F_2), with an average of 74.74%. The results of these are summarized in Table 1. Similarity coefficients for pairwise comparisons of these species calculated from RAPD patterns ranged from 0.4706 (Partridge ducks vs. Muscovy) to 0.6700 (Partridge ducks vs. F_1). The similarity coefficients suggested that Partridge ducks and F_1 was the closest (0.6700) and the farthest was between Partridge ducks and Muscovy (0.4706). The genetic distance values calculated ranged from 0.3300 (Partridge ducks vs. F_1) to 0.5294 (Muscovy vs. Partridge). The similarity coefficients of F_1 and F_2 with their parents are 0.6495 (father), 0.6700 (mother) and 0.5438 (father), 0.5612 (mother), respectively. All these results are summarized in Table 2.

The genetic diversity of inter-population (H_o) ranged 0.1325 to 0.1763. The inner diversity index of n-populations (H_{pop}) was 0.1593. The genetic diversity of total populations (H_{sp}) was 0.2953. Further, the ratio of H_{pop}/H_{sp}

Table 2. Genetic distances (above) and similarity coefficients (below) the diagonal among five species of Chinese ducks based on RAPD data

Population	Cherry Valley ducks	Partridge ducks	F_1 (C♂×P♀)	Muscovy	F_2 (F_1♀×M♂)
Cherry valley meat	0.0000	0.3394	0.3505	0.5256	0.4019
Partridge ducks	0.6606	0.0000	0.3300	0.5294	0.4546
F_1	0.6495	0.6700	0.0000	0.5228	0.4388
Muscovy	0.4744	0.4706	0.4772	0.0000	0.4562
F_2	0.5981	0.5455	0.5612	0.5438	0.0000

Table 3. Coefficients of genetic diversity and their partition within and between five breeds of Chinese ducks

Primer	H_0					H_{sp}	H_{pop}	H_{pop}/H_{sp}	$(H_{sp}-H_{pop})/H_{sp}$
	Cherry Valley	Partridge	F_1	Muscovy	F_2				
A04	0.1337	0.3149	0.2121	0.0973	0.1142	0.3149	0.1755	0.5573	0.4427
A06	0.1923	0.0347	0.0741	0.1990	0.1146	0.2362	0.1229	0.5203	0.4797
A07	0.1918	0.1435	0.2244	0.1520	0.1799	0.1792	0.1783	0.9950	0.0050
A08	0.3335	0.1760	0.1256	0.2638	0.1552	0.2713	0.2109	0.7774	0.2226
A09	0.1611	0.2602	0.1260	0.0726	0.2443	0.2452	0.1728	0.7047	0.2953
A10	0.2296	0.1038	0.2169	0.1257	0.0972	0.2543	0.1547	0.6084	0.3917
A12	0.0793	0.3307	0.3993	0.4013	0.1335	0.2713	0.2688	0.9908	0.0092
A14	0.0444	0.0192	0.0000	0.1098	0.2792	0.2946	0.0905	0.3072	0.6928
A16	0.3567	0.1969	0.1824	0.0000	0.0000	0.3357	0.1472	0.4385	0.5616
A18	0.3130	0.3406	0.2143	0.1275	0.2266	0.3247	0.2444	0.6753	0.3247
A20	0.0958	0.0857	0.1210	0.1325	0.1752	0.2422	0.1220	0.5037	0.4962
F09	0.0357	0.2635	0.0000	0.0000	0.0949	0.2932	0.0788	0.2688	0.7312
V06	0.1848	0.1638	0.0000	0.0000	0.1654	0.3373	0.1028	0.3048	0.6952
V16	0.1786	0.128	0.1557	0.2491	0.0680	0.2219	0.1559	0.7026	0.2974
V18	0.1759	0.2317	0.1413	0.2996	0.1700	0.3268	0.2042	0.6248	0.3752
Z08	0.1435	0.2940	0.1038	0.2379	0.1711	0.3365	0.1901	0.5649	0.4351
Z09	0.0866	0.0972	0.0098	0.2101	0.1772	0.2700	0.1162	0.4304	0.5696
Z11	0.1028	0.1897	0.1323	0.2383	0.2903	0.5228	0.1707	0.3265	0.6734
Z16	0.1578	0.0000	0.1099	0.1097	0.1009	0.2615	0.0957	0.3659	0.6340
Z17	0.1432	0.1518	0.1020	0.2526	0.2635	0.3665	0.1826	0.4982	0.5018
Mean	0.1670	0.1763	0.1325	0.1639	0.1611	0.2953	0.1593	0.5583	0.4417
p (%)	74.50	76.70	67.90	76.60	83.10	-	-	-	-

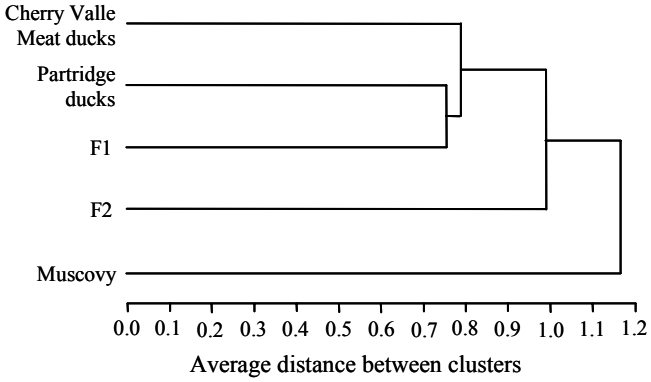

Figure 6. Phylogenetic tree based on the genetic distances among the five species studied.

and primer were found to be dependent on the primer used. Primmer numbers A07 and F09 generated the biggest and the smallest of 0.9950 and 0.2688 genetic diversity, respectively. The proportions of the genetic diversity within (H_{pop}/H_{sp}) and the proportion of genetic variation between the populations (H_{sp}-H_{pop})/H_{sp}) was 0.5583 and 0.4417, respectively (Table 3).

Cluster analysis

Using these genetic distance values, the phylogenetic tree of five Chinese duck populations was reconstructed and revealed by using UPGMA cluster analysis. The five populations were divided into three main groups: Partridge ducks, F_1 hybrids and Cherry Valley Meat ducks formed one group, F_2 hybrids population formed another and the second group, while Muscovy duck populations constitute the third and distinct group (Figure 6).

DISCUSSION

The results of this study indicate that amplified DNA-RAPD bands were polymorphic and polymorphic frequency was 72.14%. The percentage of polymorphic bands at species level was 77.44%, while at population level; it ranged between 67.90 and 83.10%, with an average of 74.74%.

The genetic diversity of total populations (H_{sp}) and the proportion of genetic variation (V) were 0.2953 and 0.4417, respectively. All these indicate that the genetic polymorphism was not only abundant, but also the genetic variation existed in inter-populations. This observation is similar to the previous results of Hao and Zhou (2000).

In a range, the higher the genetic distance between breeds of ducks, the higher the heterosis and vice-versa. The results of this trial revealed that the genetic distance between Muscovy ducks and domestic ducks (Partridge ducks, Cherry Valley meat ducks and their hybrids F_1) was larger. Muscovy and domestic ducks are of the same family, but taxonomically, they are of different genera. So, we concluded from the genetic distance values generated that the heterosis is higher between Muscovy and domestic ducks and suggests that offspring of Muscovy and Partridge will develop stronger heterosis than that of other two cross-combinations. It is therefore recommended that for stronger heterosis of these breeds in China, Muscovy and Partridge, Cherry Valley meat ducks and F_1 can be used as parents in crossbreeding activities as previously suggested by Chen (Chen et al., 2000).

The five ducks populations' genealogy was constructed by using UPGMA method based on the data of their relative genetic distance. The genealogy showed that for Partridge population and F_1 which have a closest relationship, and Cherry Valley Meat ducks which have a closer relationship and F_2 belong to one large group, and Muscovy ducks populations remain with another group. These domestic ducks crossbred with Muscovy ducks that belong to mulard to improve the genetic diversity of their offspring. The offspring (F_2) were sterile hybrid because of the difference in chromosome sizes between the two parents.

RAPD markers were also used efficiently for genetic distance and the heterosis in some other breeds such as quail (Zeng et al., 1995); pigs (Jiang et al., 2003); buffalo and cattle (Saifi et al., 2004; Sharma et al., 2004). However, Kim et al. (2002), Du et al. (2005), Li et al. (2005) used microsatellite loci to analyze the genetic variability of goat, cattle and to establish an individual identification system. Correlation of microsatellite heterozygosity with performance or heterosis was reported in a crossbreeding F1 pig population (Zhang et al., 2005).

Xing et al. (2003) using RAPD techniques could provide a new method for efficient and simple classification and identification of new strains for abalones. Of the 20 arbitrarily chosen primers, six oligonucleotides decamer primers were used on the basis of the number of the polymorphisms generated in catfish (*Silurus asotus*) from Yesan and bullhead (*Pseudobagrus fulvidraco*) from Dangjin in Korea. The average band sharing values (BS values) of all of the samples within catfish population ranged from 0.575 to 0.945, whereas 0.063-1.000 within bullhead population. The study reveals close relationships between individual identities within two species populations and individuals derived from the same ancestor, respectively. However, genetic distances between two species populations ranged from 0.124 to 0.333 (Yoon, 2004).

Gong et al. (2003) analyzed the genetic variation of 12 upgrading offspring (F_2) of Boer goat and a dairy goat by RAPD markers. The results indicated that their phenylogenetic relationship was consistent with their distribution. The genome of F_2 was rich in polymorphism. Shi et al. (2002) found that the parental genetic distance

values show positive correlation with the heterosis of feed gain ratio and dressing percentage, but negative correlation with the heterosis of loin eye area and lean percentage. Geng et al. (2003) found that the average similarity index of individuals or populations was 0.6071-0.9699 for the fowl breeds. The fowl had higher homology than other breeds and kept the breed to be pure.

In conclusion, these studies can be concluded that RAPD is a useful technique for concluding the relationship between genetic distance and the heterosis and taxonomic identification. The diversity evident among the species is presumably the result of a large amount of sequence differences among species. It is also assumed that the fragments shared by two closely related individuals of a species are allelic (Appa-Rao et al., 1996). Information on genetic relationships in line both within and between species has several important applications for its genetic improvement and in breeding programs.

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