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RESEARCH ARTICLE

AFLP analysis of genetic diversity and relationship among some Chinese domestic ducks and wild ducks

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Abstract The amplified fragment length polymorphic (AFLP) technique was used to analyze the genome DNA polymorphism among 8 breeds of domestic ducks and 2 species of wild ducks. Nine of the 17 selected primers pairs gave reproducible polymorphic DNA amplification bands. The amplified bands ranged from 44 to 83 per primer pair. Of the 513 AFLP markers obtained, 498 were polymorphic. The proportion of polymorphic loci was 97.1%. The genetic distance (*D*) and similarity coefficients (*GS*) were calculated based on the polymorphic data. Between domestic ducks *D* ranged from 0.331 to 0.589, while between domestic ducks and the wild ducks, it ranged from 0.298 to 0.520 (vs. *Anas Platyrhynchos*) and from 0.316 to 0.522 (vs. *A. Poecilorhyncha*), respectively. The variance analysis showed no significant difference between the two groups of data, which indicated that both mallard and spot-billed ducks made contributions to domestic duck evolution. A dendrogram was constructed according to the *D* value.

Keywords amplified fragment length polymorphic (AFLP), domestic duck, genetic diversity, wild duck

1 Introduction

China was the first country to tame wild duck and has the most abundant domestic duck germ plasm resources in the world. The rich local duck breeds are the base of the sustainable development of duck cultivation and bring great economic value. Consequently, it is important to protect local domestic duck resources. Genetic diversity research on the main breeds of China domestic duck resources has been done at various levels, such as morphological, physiological and biochemical, chromosomal, mitochondrial (Chen et al., 1999), and nuclear genomic level (Chen et al., 2001). With the help of mmolecular marker technology, such as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), simple sequence repeat (SSR), AFLP, and single nucleotide polymorphism (SNP) technique, new methods have been provided to investigate genetic diversity. Among these molecular marker techniques, AFLP, first used by Zabeau et al. (1992) based on PCR and RFLP (Zabeau and Vos, 1993), has both the advantage of stability (a feature of RFLP) and high efficiency (a feature of RAPD), and it can also provide more information than RFLP and RAPD. It is therefore considered a much more preferable molecular technique.

The aims of this study are to analyze the genomic diversity and relationship among some common native domestic ducks and wild ducks using AFLP markers, and to provide theoretical bases to solve the issue on preserving and making use of our local existing duck resources reasonably and resolving the question of the origin and evolvement of domestic ducks.

2 Materials and methods

2.1 Materials

Blood or embryo samples were collected from layer ducks including Jinding duck (strain I, II, III), Shaoxing duck, Longyan shanma duck, and Putian black duck, as well as broiler ducks including Beijing duck, Cherry Valley duck, and Muscovy duck, or used both from Lianchen white duck and two wild ducks, mallard (*A. Platyrhynchos*) and spot-billed duck (*A. Poecilorhyncha*). All the sample numbers, tissue types and collecting places are displayed in Table 1.

Blood samples collected from metatarsus vena of Jinding duck (strains I, II, III), mallard, spot-billed duck, Beijing duck, Muscovy duck, and Cherry Valley duck were stored

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Table 1 The materials for analysis

Breeds (strain)	Abbreviation	Sample number	Tissue type	Place of collection
Lianchen white duck	LC	21	Embryo	Lianchen County, Fujian Province, China
Longyan shanma duck	SM	30	Embryo	Fujian Academy of Agriculture Science Institute, China
Putian black duck	PT	10	Blood	As above
Anas Platyrhynchos	LT	14	Blood	Amoy Zoo, Fujian Province, China
A.Poecilorhyncha	BZ	5	Blood	As above
Jinding duck strain I	JD I	20	Blood	Domestic Duck Experimental Farm of Xiamen University, China
Jinding duck strain II	JD II	20	Blood	As above
Jinding duck strain III	JD III	13	Blood	As above
Shaoxing duck	SX	33	Bmbryo	Shaoxing Duck Breeding Farm, Zhejiang Province, China
Muscovy duck (black feather)	FY	20	Blood	Domestic Duck Experimental Farm of Xiamen University, China
Cherry Valley duck	YT	20	Blood	As above
Beijing duck	BJ	20	Blood	Gold Star Duck Co. Ltd., Beijing, China

at –20ºC; 14-day-old embryos collected from Putian black duck, Shaoxing duck, Longyan shanma duck, and Lianchen white duck were also stored at –20ºC.

AFLP^{TM} Reagent Kit was bought from Invitrogen life technologies; *Taq* DNA polymerase and proteinase K from TakaRa biotechnology (Dalian) Co. Ltd.; Acrylamide and N, N′-Methylencbisac, Urea, TEMED (N, N, N′, N′-Tetramethylethylenediamine), and Ammonium Persulfate from Sino-American biotechnology company (Shanghai). The other reagents were of China biotech grade.

2.2 Methods

2.2.1 Genomic DNA extraction and DNA pool construction

The preparation of genomic DNA refers to the protocols of Chen et al. (1999). The sample was processed by proteinase K digestion, phenol and chloroform/isoamyl alcohol extraction, and absolute alcohol precipitation. The genomic DNA obtained from all the individuals of every breed was mixed equivalently, and a DNA pool of each breed (or strain) was constructed. Each DNA pool was diluted with TE buffer to a final concentration of 50 ng/μL for later AFLP analysis.

2.2.2 AFLP analysis

The analysis procedure was based on the AFLP^{TM} instruction manual with some modification.

(1) Double restriction endonuclease digestion of genomic DNA and ligation of adapters: Genomic DNA (125 ng) of each accession was digested simultaneously with *Eco*R I and *Mse* I. EcoR I and Mse I adapters were subsequently ligated to the digested DNA fragments by T4 ligase. The sequence of *Eco*R I and *Mse* I adapters was as follows:

*Eco*R I adapter: 5′-CTCGTAGACTGCGTACC
CTGACGCATGGTTAA-3′

Mse I adapter: 5′-GACGATGAGTCCTGAG
TACTCAGGACTCAT-3′

(2) Restriction fragments amplification reaction: The adapter- ligated DNA (2.5 μL) was pre-amplified using the primer pairs with a selective nucleotide at the 3′ end. The cycling parameters were 20 cycles each of 30 s at 94ºC, 60 s at 56ºC, and 60 s at 72ºC. After diluting the pre-amplified DNA at a ratio of 1:50, it was used as a template for the selective amplification, which involved the use of +3 primers (*Eco*R I and *Mse* I). We selected 17 primer pairs for amplification reaction. The cycling parameters were 23 cycles each of 30 s at 94ºC, 30 s at 56ºC, and 60 s at 72ºC. The reaction mixture was stored at 4ºC.

(3) Polyacrylamide gel electrophoresis: The selective amplification product was loaded on a 4–6% denatured polyacrylamide gel in a 1×TBE electrophoresis buffer. Gels were run at 200 V, 2 W for 4–5 h and stained with silver (Sanguinetti et al., 1994). Before being loaded with samples, the gel was pre-electrophoresed for 0.5 h.

2.2.3 Data analysis

Every AFLP band was considered a molecular marker. At the same site, it was visually scored as either present (1) or absent (0) across the 12 breeds (or strains) for each primer combination. Only the clear bands were considered. The electrophoresis pattern was then changed into a binary matrix, which was used to measure pairwise genetic similarity using simple matching coefficient within SPSS version 11.

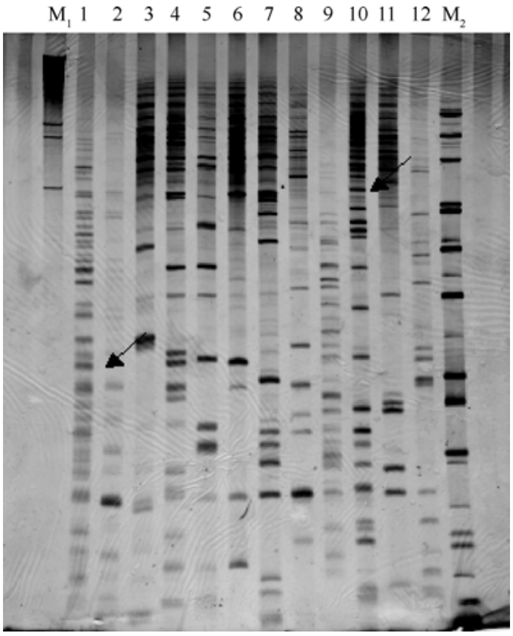
The genetic similarity calculation formula was $GS_{(ij)}=(A+D)/(A+B+C+D)$, where $GS_{(ij)}$ is the measure of genetic similarity between individuals i and j; A represents the number of polymorphic bands present that are shared by i and j; D represents the number of bands absent in both i and j; B represents the number of bands present in i but absent in j; and C represents the number of bands present in j but absent in i. The D value $[GD_{(ij)}=1-GS_{(ij)}]$ shows the genetic difference between two individuals (populations or species)

(Kim et al., 2002). According to $GD_{(ij)}$ value, a dendrogram was constructed employing the between-group linkage method within SPSS version 11. The proportion of polymorphic loci is equal to the number of polymorphic loci/number of checked loci.

3 Results and analysis

3.1 AFLP marker profile

No amplified product or only a few bands belonging to some specific breeds were detected in 8 of the 17 primer combinations. These were E-AAG/M-CAA, E-AAC/M-CAG, E-AAC/M-CTA, E-ACA/M-CTT, E-ACC/M-CTA, E-ACG/M-CAT, E-AGC/M-CAA, and E-AGG/M-CTT. The other 9 primer combinations that produced abundant polymorphic bands across accessions were E-ACT/M-CAT, E-ACA/M-CAC, E-ACC/M-CAT, E-ACA/M-CTG, E-ACA/M-CAG, E-ACG/M-CAA, E-ACA/M-CTC, E-ACT/M-CAG, and E-AGC/M-CTT. A total of 513 reproducible and clear bands were produced from these 9 primer pairs. The number of markers for each primer pair ranged from 44 to 83 with an average of 55.33 markers per pair. The amplification results showed the genetic diversity between different breeds (or strains). Of the 513 bands, 498 were polymorphic, which accounted for 97.1% of the total bands examined in this study. Some bands that were present only in specific breeds (or strains) (Table 2) were also identified. Figure 1 represents the AFLP fingerprint pattern using primer combination E-ACA/M-CTC. The arrowheads indicate the accession-specific bands.



M_1：λ DNAEcoR I/Hind Ⅲ Marker；1：LC；2：SM；3：PT；4：LT；5：BZ；6：JD Ⅰ；7：JD Ⅱ；8：JDⅢ；9：SX；10：FY；11：YT；12：BJ；M_2：pUC Mixed Marker. The arrows show accession-specific bands

Fig. 1 AFLP fingerprinting amplified with primer E-ACA/M-CTC

3.2 Genetic distance and similarity

Table 3 shows the *D* value and similarity among different samples. The smallest distance is 0.253 (between Jinding

Table 2 Genetic variation detected within accessions

Primer combination	Number of amplified fragments	Number of polymorphic loci	Proportion of polymorphic loci /%	Number of accession-specific bands*
E-ACT/M-CAT	44	39	88.6	None
E-ACA/M-CAC	46	42	91.3	None
E-ACC/ M-CAT	70	68	97.1	JD I(1), JD II(2), JD III(1),BJ(2), LT(1), FY(2)
E-ACA/M-CTG	83	83	100	SM(1), JD II (1), FY(2), LC(1)
E-ACA/M-CAG	55	55	100	LC(1), JD I(2),
E-ACG/M-CAA	51	51	100	SM(7), JD I(1), JD II(1)
E-ACA/M-CTC	68	67	98.5	LC(1), FY(1)
E-ACT/M-CAG	49	48	97.9	None
E-AGC/M-CTT	47	45	95.7	JD II(1), JD III(1), LT(1)

*Numerical values within parentheses indicate the number of specific bands.

Table 3 The genetic distance (D) and the similarity index of the samples*

	LC	SM	PT	LT	BZ	JDI	JDII	JDIII	SX	FY	YT	BJ
LC	–	0.481	0.468	0.520	0.515	0.522	0.589	0.522	0.495	0.435	0.464	0.534
SM	0.519	–	0.372	0.425	0.458	0.450	0.435	0.423	0.427	0.515	0.501	0.435
PT	0.532	0.628	–	0.329	0.351	0.374	0.382	0.394	0.386	0.462	0.398	0.366
LT	0.480	0.575	0.671	–	0.318	0.298	0.329	0.361	0.427	0.421	0.384	0.368
BZ	0.485	0.542	0.649	0.682	–	0.316	0.327	0.339	0.421	0.439	0.366	0.398
JDI	0.478	0.550	0.626	0.702	0.684	–	0.304	0.253	0.374	0.435	0.347	0.370
JDII	0.411	0.565	0.618	0.671	0.673	0.696	–	0.285	0.398	0.450	0.386	0.331
JDIII	0.478	0.577	0.606	0.639	0.661	0.747	0.715	–	0.378	0.431	0.355	0.351
SX	0.505	0.573	0.614	0.573	0.579	0.626	0.602	0.622	–	0.481	0.413	0.370
FY	0.565	0.485	0.538	0.579	0.561	0.565	0.550	0.569	0.519	–	0.388	0.450
YT	0.536	0.499	0.602	0.616	0.634	0.653	0.614	0.645	0.587	0.612	–	0.374
BJ	0.466	0.565	0.634	0.632	0.602	0.630	0.669	0.649	0.630	0.550	0.626	–

*Numbers above the diagonal represent genetic distance (D) and numbers below the diagonal indicate the similarity index.

strain Ⅲ and strain Ⅰ), followed by 0.285 (between Jinding strain Ⅱ and strain Ⅲ). The largest one is 0.589, which indicates the low genetic similarity between Jinding strain Ⅱ and Lianchen white duck. The genetic similarity index ranged from 0.411 to 0.747 among 12 accessions.

3.3 Dendrogram of genetic relationship among 12 different breeds (or strains)

A dendrogram using the between-groups linkage method was constructed according to the genetic similarity index (Fig. 2).

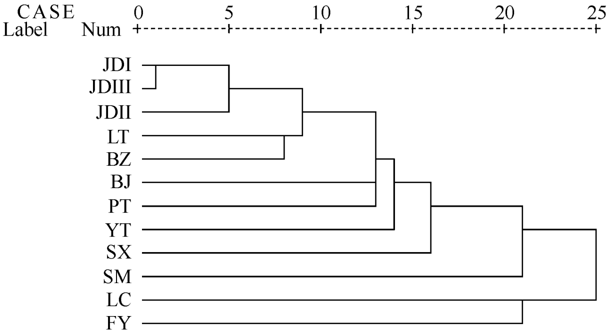


Fig. 2 Dendrogram of the ducks based on between-groups linkage cluster analysis

4 Discussion

The amplification results of each primer combination listed in Table 2 show the more or less difference between each primer pair, thus successfully reflecting the genetic variations between the accessions. The proportion of polymorphic loci varied from as high as 100% (E-ACA/M-CTG, E-ACA/M-CAG, E-ACG/M-CAA) to as low as 88.6% (E-ACT/ M-CAT), with an average of 96.6% per primer combination. The AFLP polymorphic fingerprinting revealed the genomic variations among different samples. As observed in Table 3, the *D* value ranged from 0.331 (between Beijing duck and Jinding duck strain Ⅱ accessions) to 0.589 (between Jinding duck strain Ⅱ and Lianchen white duck accessions), whereas it was around 0.281 between the three Jinding duck strains. We can therefore deduce that the domestic duck resources in China are abundant at the genomic level. A similar observation was made by Ren et al. (2002), who studied the genetic diversity and relationships among 24 Chinese and exotic pig breeds using AFLP, and detected 17.3 polymorphic markers per primer combination. They also observed some latent accession-specific bands. The dendrogram revealed that the introduced breeds and cultivated ones were clustered in one group, whereas the other Chinese local breeds were clustered in another group.

In taxonomy, the introduced breed, Muscovy duck, was different from other breeds used in the study. They are categorized in the same family but different genus. The former belongs to *Cairina*, and the latter to *Anas*. The *D* value between Muscovy duck and other breeds ranged from 0.388 (Cherry Valley duck) to 0.515 (Longyan shanma duck). Although Lianchen white duck belongs to *Anas*, which is different from Muscovy duck, Muscovy duck was the breed closest to it. The *D* value between them is 0.435. As can be seen in the dendrogram, Lianchen white duck and Muscovy duck were grouped into the same cluster, while other breeds (or strains) were segregated into another cluster. Lianchen white duck, Shaoxing duck, Putian black duck, Longyan shanma duck, and Jinding duck were the variations of tadorna. The long-term selection and development in favor of production that have led to the variance in production performance, configuration, and genomic DNA might account for the large genomic variance between Lianchen white duck and other Anas ducks, and might also be the major cause for the large differences within the variations of tadorna, as revealed by the dendrogram.

Three Jinding duck strains were selected and developed from the original breeds according to their different production and configuration characteristics. The term selected was not long, so the genetic differentiation was not remarkable, and the *D* value was smaller than 0.304. Consequently, they were grouped into one cluster.

Jinding duck strain Ⅰ is the closest domestic duck to *A. Platyrhynchos* and *A. Poecilorhyncha*, and the *D* value between them is 0.298 and 0.316, respectively. The most distant breed is Lianchen white duck, and the *D* value between them is 0.520 and 0.522, respectively, although the distance between Lianchen white duck and Jinding duck strain Ⅰ is 0.318. It is reflected in the dendrogram that *A. Platyrhynchos* and *A. Poecilorhyncha* are first clustered together, and then grouped with three Jinding duck strains. Lianchen white duck is the last one to link with the right sibling duck. Another correlative research was made by Kulikova et al. (2003). Using the RAPD technique, they discovered that low genetic variation existed between *A. Platyrhynchos* and *A. Poecilorhyncha* (D=0.401), which suggested that they were closely related in terms of evolution. When analyzed by one-way ANOVA, the *D* value between *A. Platyrhynchos*, *A. Poecilorhyncha* and all domestic duck breeds (or strains) did not vary significantly. So we supposed that both *A. Platyrhynchos* and *A. Poecilorhyncha* have contributed to the Chinese domestic duck breed formation, which is in accordance with the former study by Chen et al. (1999) using RAPD.

At present, the AFLP marker technique has been applied to the research on propagation genetic diversity, species identification, specific marker detection, and genetic linkage map construction. Fumiere et al. (2003) used 121 selective primer combinations (EcoR I/Mse I and EcoR I/Taq I) to amplify the genomic restriction fragments of slow- and fast-growing chicken strains, and found strain-specific bands. Herbergs et al. (1999) described the mapping of AFLP markers in chicken (*Gallus domesticus*), and in 19 sets consisting of three *Eco*RⅠ/*Taq*Ⅰ primer pair combinations each a total of 475 polymorphic markers were detected. From these polymorphisms, 344 markers could be mapped on the Wageningen linkage map. In our study, six of nine primer pair combinations detected breed (or strain)-specific bands that could provide data to construct a quantitative trait loci (QTL) linkage map of domestic duck in the future.

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