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Study on Genetic Diversity of Six Duck Populations with Microsatellite DNA

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ABSTRACT : In this study, we investigated the genetic diversity and phylogenetic relationship of six duck populations by employing the genetic polymorphisms of 20 microsatellites. The parameters used in this study included number of alleles, average effective numbers of alleles (E) and average rates of heterozygosity of each population. The results showed that all the microsatellite loci were highly polymorphic except that the locus AJ515896 in Muscovy duck was 0. The average PIC (0.762), average h (0.7843) and average E (5.261) of the six duck populations were all high, indicating that the gene polymorphisms and genetic diversity were high. The test of Hardy-Weinberg equilibrium showed that the six populations in this study were all in Hardy-Weinberg disequilibrium. The F-statistic analysis results showed the range of F_{ST} was from 0.0205 (AJ515895) to 0.2558 (AJ515896). The mean F_{ST} was 0.0936. Phylogenetic study revealed that Peking duck (Z1 and Z4), Shaoxing duck, Cherry Valley duck and Aobaixing duck were clustered in one group, while the Muscovy duck was clustered in one group alone. The phylogenetic relationships among different populations were in accordance with their breeding history and distribution. Our data suggested that the 20 microsatellite loci were effective markers for analysis of genetic relationships among duck populations. (**Key Words :** Domestic Duck (*Anas platyrhynchos*), Microsatellite, Genetic Diversity, Population, China)

INTRODUCTION

China was one of the earliest nations in the world to domesticate ducks (*Anas platyrhynchos*) and currently raises the largest populations of these birds. However, in past decades, China has imported foreign ducks and used them to improve native duck performance. This resulted in the decrease of native duck populations and, even worse, caused the disappearance of some native populations. Since the native duck populations are invaluable genetic resources, it remains urgent to systematically investigate their genetic diversity and genetic characteristics. There have been many reports using microsatellites to study the genetic diversity of chickens (Romanov and Weigend, 2001; Chen et al., 2003; Wu et al., 2004; Chen et al., 2004; Osman et al, 2005; Kong et al., 2006; Osman et al., 2006; Liu et al, 2006; Chang et al, 2007; Ahlawat et al, 2008). In ducks, Chen et al. (2001), Zuo et al. (2004) and Yan et al. (2005) analyzed the genetic diversity of some populations using random amplification polymorphic DNA (RAPD) and amplification fragment length polymorphism (AFLP). Stai et al. (2003), Huang et al. (2005), Li et al. (2006) and Tang et al. (2007) separately analyzed wild and domestic Muscovy ducks and some native ducks in China by microsatellites. Microsatellites are characterized by a core sequence that consists of a number of tandemly repeated units with a length of 1-6 base pairs. They have high diversity and are easily examined. With the availability of high-throughout DNA sequencing, sizing of microsatellite alleles has become efficient. Therefore, microsatellites have been widely used in many applications. Microsatellites are deemed to be one of the most valuable genetic markers (Aranguren-Mendez et al., 2002; Wang et al., 2004). In this paper, we investigated the genetic diversity of six duck populations (Peking duck (Z1 and Z4), Muscovy duck, Shaoxing duck, Cherry Valley duck and Aobaixing duck) with 20 microsatellite loci.

MATERIAL AND METHODS

Population samples and microsatellite primers

Three hundred ducks were randomly chosen from six duck populations in China (50 ducks of each population): Peking duck Z1, Z4, Cherry Valley duck and Aobaixing duck from Beijing, Shaoxing duck and Muscovy duck from

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Table 1. Microsatellite primer sequences and annealing temperature (°C)

Locus	Primer sequence	Annealing temperature (°C)	Allele size (bp)
AJ272577	CACTTGCTCTTCACTTTCTTT	55	192-212
	GTATGACAGCAGACACGGTAA		
AJ272578	AACCAAGACAGAATAATCCTTA	52	198-218
	GAACACAACTGCTTTGCTA		
AJ272579	ACATCTTTGGCATTTTGAA	57	204-278
	CATCCACTAGAACACAGACATT		
AJ272580	GGATGTTGCCCCACATATTT	57	82-138
	TTGCCTTGTTTATGAGCCATTA		
AJ272581	ATTAGAGCAGGAGTTAGGAGAC	53	120-168
	GCAAGAAGTGGCTTTTTTC		
AJ272582	GGACCTCAGGAAAATCAGTGTA	56	192-230
	GCAGGCAGAGCAGGAAATA		
AJ272583	GAATAAAGTAACGGGCTTCTCT	52	154-182
	CTGCTTGGTTTTGGAAAGT		
AJ515883	CACACGCGCAGCAGAGGA	55	86-130
	GTCGTCAGCCAGGGGTTTGAG		
AJ515884	CCTCGGTATTGTTTTCCAT	63	152-230
	GCTCTGAAGGGCATTATTTAG		
AJ515887	AAAGCCCTGTGAAGCGAGCTA	54	78-124
	TGTGTGTGCATCTGGGTGTGT		
AJ515889	CAACGAGTGACAATGATAAA	53	174-212
	CAATGATCTCACTCCCAATAG		
AJ515890	TGAATATGCGTGGCTGAA	62	178-198
	CAGTGAGGAATGTGTTTGAGTT		
AJ515891	CCTTCTGAACCTTCGTAG	53	132-180
	AAATATAGACTTTTGTCCTGAA		
AJ515893	TTCTGGCCTGATAGGTATGAG	55	216-272
	GAATTGGGTGGTTCATACTGT		
AJ515895	ACCAGCCTAGCAAGCACTGT	56	124-156
	GAGGCTTTAGGAGAGATTGAAAAA		
AJ515896	CTTAAAGCAAAGCGCACGTC	58	118-158
	AGATGCCCAAAGTCTGTGCT		
AJ515897	GTTATCTCCCACTGCACACG	59	114-198
	CGACAGGAGCAAGCTGGAG		
AJ515898	TCCTCTGCTCTAGTTGTGATGG	62	160-212
	CCTCAGCAGTCTTCCTCAGTG		
AJ515899	TCAACCAGTGGTCAGAGAAAAA	57	118-184
	AGGTCAGCCCCCATTTTAGT		
AJ515900	CCGTCAGACTGTAGGGAAGG	58	146-202
	AAAGCTCCACAGAGGCAAAG		

Zhejiang. Blood (5 ml) was collected from each duck into a tube containing 1 ml anticoagulant and was then preserved in a -20°C freezer.

Twenty microsatellite primers were designed based on the report of Maak et al. (2000, 2003). The sequences of primers are listed in Table 1.

Genomic DNA extraction and PCR condition

The hydroxybenzene-chloroform method was used to extract duck genomic DNA as described in the Molecular Cloning-A Laboratory Manual (2nd edition) (Sambrook et al., 1999). The PCR amplification was carried out in 20 μl of a mixture containing 1 μl DNA template (40 ng/μl), 10×PCR Buffer 2 μl, 0.4 μl dNTP(10 mmol/L), 2.0 μl $MgCL_2$ (20 mmol/L), 0.8 μl of each primer (10 pmol/μl) and 0.4 μl Taq DNA polymerase (2 U/μl) from Dingguo Biotech (Beijing). Double- distilled water was added to a final volume of 20 μl. After a denaturing step of 5 min at 94°C, samples were processed through 30 cycles of 45 s at 94°C, 35 s at an optimal annealing temperature and 40 s at 72°C. The last elongation step was lengthened to 5 min at 72°C.

Electrophoresis

Both agarose gel (1.5-2%) and polyacrylamide gel (8%) were used to analyze PCR products. Finally, the Alphalmager software was used to analyze the segmental length of the genes.

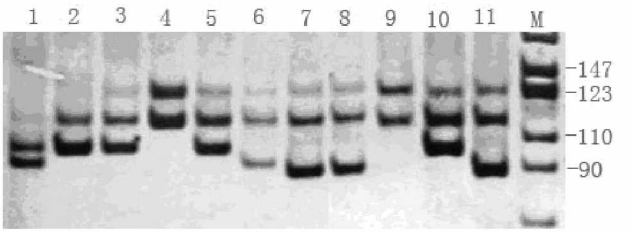

Figure 1. The un-denatured PAGE result of microsatellite AJ272580. 1 (92/106), 2 (106/116), 3 (106/116), 4 (116/128), 5 (106/116), 6 (92/116), 7 (90/116), 8 (92/116), 9 (116/128), 10 (106/116), 11 (92/116), M (marker pBR322/MSP I).

Data analysis

Allele frequencies were computed by Pi = (2 (ii)+(ij1)+ (ij2)+ …+(ijn))/2N, where Pi was the frequency of the ith allele for which i represented the allele, while j1, j2, … jn were the co-dominant alleles to i, and n was the number of the allele.

Polymorphic information content (PIC) was calculated by the following formula (Botstein et al., 1980):

$$PIC = 1 - \sum_{i=1}^{m} p_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2 p_i^2 p_j^2$$

The formulas of Heterozygosity (h) and effective number of alleles (E) (Xiao, 2002) are listed below:

$$h = 1 - \sum_{i=1}^{m} p_i^2 \; ; \quad E = 1 / \sum_{i=1}^{m} P_i^2$$

Where m was the number of the allele, and Pi and Pj were the frequencies of the ith and the jth allele, respectively.

The Hardy-Weinberg equilibrium was tested by X^2. The formula is listed below:

$$X^2 = \sum_{i=1}^{k} \frac{(O_i - E_i)^2}{E_i}$$

Where O_i showed observed times of genotypes; E_i showed expected times of genotypes; k showed the number of genotypes.

The F-statistic was calculated by the followed formula:

$$1-F_{ST} = (1-F_{IS}) \times (1-F_{IT})$$

Where F_{IT} was the fixation indices of individuals relative to its subpopulations; F_{IS} was the fixation indices of individuals relative to the total population; F_{ST} was the fixation indices of subpopulation relative to the total population.

The genetic distance (Nei et al., 1983) and the dendrogram between different populations were estimated by the neighbor-joining method (NJ) (Saitou and Nei, 1987) in the DISPAN software (Ota, 1993).

RESULTS

Gene diversity and allele frequencies

Figure 1 shows one of the polyacrylamide gels (PAGE) of PCR products. The total number of alleles in 20 microsatellite loci of six populations was 281. There were

Table 2. The PIC of 20 microsatellite sites in different duck populations

Locus	Shaoxing	Muscovy	Peking Z4	Cherry Valley	Aobaixing	Peking Z1	Mean
AJ272577	0.6294	0.7742	0.8337	0.6866	0.6649	0.6881	0.7128
AJ272578	0.7627	0.8024	0.8112	0.8512	0.8412	0.8759	0.8241
AJ272579	0.6839	0.6735	0.8278	0.5691	0.6989	0.822	0.7125
AJ272580	0.8362	0.8498	0.7378	0.7854	0.6761	0.86	0.7909
AJ272581	0.6651	0.7175	0.4559	0.7471	0.5275	0.6712	0.6307
AJ272582	0.7448	0.9111	0.8228	0.6863	0.7462	0.8259	0.7895
AJ272583	0.6204	0.7614	0.7432	0.6699	0.8014	0.6038	0.7
AJ515883	0.911	0.8859	0.7154	0.8178	0.8674	0.9515	0.8582
AJ515884	0.828	0.8499	0.7411	0.6684	0.8229	0.8336	0.7907
AJ515887	0.7309	0.7752	0.776	0.7661	0.6929	0.726	0.7445
AJ515889	0.8326	0.8046	0.8453	0.8229	0.746	0.8386	0.815
AJ515890	0.7872	0.7818	0.5711	0.8297	0.5726	0.675	0.7029
AJ515891	0.7949	0.8707	0.7128	0.6933	0.7485	0.6257	0.741
AJ515893	0.8296	0.8057	0.8633	0.8027	0.8459	0.8175	0.8275
AJ515895	0.8597	0.847	0.872	0.8703	0.886	0.8988	0.8723
AJ515896	0.6584	0	0.6831	0.7666	0.6794	0.793	0.5968
AJ515897	0.7741	0.8168	0.8963	0.8428	0.873	0.8556	0.8431
AJ515898	0.8582	0.744	0.8399	0.6826	0.849	0.8576	0.8052
AJ515899	0.8446	0.7408	0.7527	0.8009	0.721	0.8502	0.785
AJ515900	0.8147	0.7534	0.6864	0.6444	0.6963	0.5909	0.6977
Mean	0.7733	0.7583	0.7594	0.7502	0.7479	0.783	0.762

151, 176, 175, 171, 163 and 156 alleles in the Peking duck Z4, Peking duck Z1, Shaoxing duck, Muscovy duck, Cherry Valley duck and Aobaixing duck, respectively.

Polymorphism information content (PIC)

As shown in Table 2, the PIC of the microsatellite AJ515883 in the Peking duck Z1 was the highest (PIC = 0.9515), but PIC of the microsatellite AJ515896 in Muscovy duck was 0. Among microsatellite loci, the locus AJ515895 had the highest PIC (PIC = 0.8723) while the locus AJ515896 had the lowest PIC (0.5968). The average PIC of all sites and populations was 0.762. PIC of most microsatellite sites was more than 0.5. This indicated that the selected microsatellite loci had high diversity and can reflect the genetic relationship of different populations on a molecular level.

Heterozygosity (h)

The heterozygosity is summarized in Table 3; heterozygosity was the highest (0.8079) in Muscovy duck

Table 3. The heterozygosity of 20 microsatellite sites in different duck populations

Locus	Shaoxing	Muscovy	Peking Z4	Cherry Valley	Aobaixing	Peking Z1	Mean
AJ272577	0.6799	0.7893	0.8392	0.7231	0.689	0.7072	0.738
AJ272578	0.781	0.815	0.8218	0.8556	0.8483	0.879	0.8335
AJ272579	0.7188	0.6757	0.8297	0.5924	0.7066	0.8294	0.7254
AJ272580	0.8416	0.8509	0.7388	0.7856	0.68	0.8625	0.7932
AJ272581	0.7025	0.7373	0.5279	0.7588	0.5886	0.6923	0.6679
AJ272582	0.7681	0.9118	0.8316	0.7198	0.7651	0.8355	0.8053
AJ272583	0.6701	0.7823	0.7625	0.7062	0.8119	0.6597	0.7321
AJ515883	0.9123	0.8894	0.7426	0.8252	0.8712	0.881	0.8536
AJ515884	0.8364	0.8552	0.763	0.7079	0.8316	0.8405	0.8058
AJ515887	0.7435	0.7836	0.7932	0.7734	0.7274	0.752	0.7622
AJ515889	0.8407	0.8181	0.8505	0.8327	0.766	0.8472	0.8259
AJ515890	0.8022	0.7986	0.6239	0.8383	0.6153	0.7118	0.7317
AJ515891	0.8031	0.8731	0.7424	0.7245	0.7702	0.6687	0.7637
AJ515893	0.8364	0.8148	0.8678	0.8146	0.85	0.8276	0.8352
AJ515895	0.8647	0.8523	0.8755	0.8747	0.8881	0.9004	0.876
AJ515896	0.6997	0	0.7197	0.7868	0.716	0.8047	0.6212
AJ515897	0.7769	0.8268	0.8978	0.8474	0.8751	0.861	0.8475
AJ515898	0.8597	0.7622	0.846	0.701	0.8521	0.8616	0.8138
AJ515899	0.8474	0.7512	0.7626	0.8114	0.7257	0.8565	0.7925
AJ515900	0.8247	0.7618	0.7229	0.6862	0.7291	0.6289	0.7256
Mean	0.7905	0.8079	0.778	0.7683	0.7654	0.7954	0.7843

Table 4. The effective number of alleles of 20 microsatellite loci in different duck populations

Locus	Shaoxing	Muscovy	Peking Z4	Cherry Valley	Aobaixing	Peking Z1	Mean
AJ272577	3.1243	4.7451	6.2189	3.6109	3.2158	3.4154	4.0551
AJ272578	4.566	5.4051	5.6117	6.9264	6.594	8.2645	6.228
AJ272579	3.5556	3.0833	5.8716	2.4534	3.4079	5.8613	4.0389
AJ272580	6.3116	6.7086	3.828	4.6651	3.125	7.2711	5.3182
AJ272581	3.361	3.8073	2.1182	4.1456	2.4308	3.2496	3.1854
AJ272582	4.3131	11.3441	5.9387	3.569	4.2563	6.0805	5.917
AJ272583	3.0316	4.5937	4.21	3.4034	5.3166	2.9389	3.9157
AJ515883	11.407	9.0427	3.8853	5.7203	7.7645	8.4034	7.7039
AJ515884	6.1114	6.9041	4.2194	3.4239	5.9381	6.2694	5.4777
AJ515887	3.8985	4.6201	4.8356	4.4136	3.6684	4.0323	4.2448
AJ515889	6.2791	5.498	6.6872	5.9774	4.2727	6.5455	5.8767
AJ515890	5.0549	4.9641	2.6589	6.1839	2.5995	3.4699	4.1552
AJ515891	5.0782	7.8808	3.8822	3.6296	4.3513	3.018	4.64
AJ515893	6.1107	5.401	7.5622	5.3944	6.6646	5.8005	6.1556
AJ515895	7.3893	6.7712	8.0301	7.9819	8.9404	10.0409	8.1923
AJ515896	3.3295	0	3.5676	4.6915	3.5217	5.1194	3.3716
AJ515897	4.4831	5.7752	9.78	6.5549	8.0043	7.1954	6.9655
AJ515898	7.1258	4.2056	6.4935	3.3444	6.7604	7.2254	5.8592
AJ515899	6.5548	4.019	4.2123	5.3022	3.6454	6.9695	5.1172
AJ515900	5.7035	4.1984	3.6085	3.1865	3.6909	2.6947	3.8471
Mean	5.3394	5.7351	5.161	4.7289	4.9084	5.6933	5.261

Table 5. The test of HDW with χ^2 based on genotype frequency of 20 microsatellites

Locus	Shaoxing		Muscovy		Z4 Peking		Cherry Valley		Aobaixing		Z1 Peking	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
AJ272577	184.5862	0.0000	257.6276	0.0000	632.6807	0.0000	349.9426	0.0000	265.9475	0.0000	349.9426	0.0000
AJ272578	216.0555	0.0000	106.5886	0.0000	147.4315	0.0000	254.8510	0.0000	139.4438	0.0000	254.8510	0.0000
AJ272579	211.6453	0.0000	236.4039	0.0000	230.7519	0.0000	288.6522	0.0000	145.6790	0.0000	288.6522	0.0000
AJ272580	120.6654	0.0204	153.7299	0.0000	116.0988	0.0000	197.5029	0.0399	74.8153	0.0000	197.5029	0.0000
AJ272581	31.1652	0.0000	120.3796	0.0000	95.2750	0.0000	67.1336	0.0000	40.7011	0.0061	67.1336	0.0000
AJ272582	421.9551	0.0000	207.6931	0.0000	381.3644	0.0000	472.5614	0.0000	296.4712	0.0000	472.5614	0.0000
AJ272583	319.2170	0.0000	467.0140	0.0000	291.0276	0.0000	243.6853	0.0000	467.8052	0.0000	243.6853	0.0000
AJ515883	242.8270	0.0000	247.4830	0.0000	141.3094	0.0000	345.1009	0.0000	331.3248	0.0000	345.1009	0.0000
AJ515884	243.6624	0.0000	388.6876	0.0000	146.5361	0.0000	80.5982	0.0000	322.0963	0.0000	80.5982	0.0000
AJ515887	207.2695	0.0000	86.5307	0.0000	170.6410	0.0000	158.0745	0.0000	187.4259	0.0000	158.0745	0.0000
AJ515889	500.8848	0.0000	357.6970	0.0000	274.2641	0.0000	336.3496	0.0000	348.1417	0.0000	336.3496	0.0000
AJ515890	582.7834	0.0000	302.1075	0.0000	296.4126	0.0000	376.4749	0.0000	237.7415	0.0000	376.4749	0.0000
AJ515891	257.3773	0.0000	268.4272	0.0000	119.9236	0.0000	88.3250	0.0000	198.6415	0.0000	88.3250	0.0000
AJ515893	280.8617	0.0000	414.3798	0.0000	421.3526	0.0000	255.3971	0.0000	307.8604	0.0000	255.3971	0.0000
AJ515895	496.1704	0.0000	510.1399	0.0000	540.1436	0.0000	630.4136	0.0000	512.5489	0.0000	630.4136	0.0000
AJ515896	60.8402	0.0000	0.0000	1.0000	47.2842	0.0479	41.5354	0.0003	45.3371	0.0016	41.5354	0.0479
AJ515897	346.9879	0.0000	513.2889	0.0000	259.9107	0.0000	162.0440	0.0000	164.1017	0.0000	162.0440	0.0000
AJ515898	213.0434	0.0000	412.1290	0.0000	170.6788	0.0000	314.3946	0.0000	164.5541	0.0000	314.3946	0.0000
AJ515899	140.3411	0.0000	177.5165	0.0000	115.5519	0.0000	342.0581	0.0000	218.5469	0.0000	342.0581	0.0000
AJ515900	218.0968	0.0000	58.8103	0.0000	165.0098	0.0000	226.5652	0.0000	111.1092	0.0000	226.5652	0.0000

Table 6. F-statistic analysis

Locus	F_{IS}	F_{IT}	$F_{ST}(G_{ST})$
AJ272577	0.9025	0.9109	0.0868
AJ272578	0.5853	0.6039	0.0449
AJ272579	0.7307	0.7778	0.1748
AJ272580	-0.0456	0.0505	0.0919
AJ272581	0.3360	0.481	0.2185
AJ272582	0.8673	0.8767	0.0707
AJ272583	0.9644	0.9676	0.0882
AJ515883	0.5229	0.5520	0.0610
AJ515884	0.6171	0.6570	0.1043
AJ515887	0.6171	0.6532	0.0929
AJ515889	0.7950	0.8040	0.0443
AJ515890	1.0000	1.0000	0.0735
AJ515891	0.7764	0.7906	0.0635
AJ515893	0.8279	0.8337	0.0337
AJ515895	0.9192	0.9209	0.0205
AJ515896	0.3187	0.4930	0.2558
AJ515897	0.5448	0.5742	0.0646
AJ515898	0.1319	0.2179	0.0991
AJ515899	0.7043	0.7339	0.1003
AJ515900	0.7708	0.7948	0.1047
Mean	0.6477	0.6807	0.0936

and varied from 0 to 0.9118; followed by the Peking duck Z1 (0.7954) which varied from 0.6289 to 0.9004; whereas the Aobaixing duck was lowest (0.7654) and varied from 0.5886 to 0.8881. The average h of all populations was 0.7843.

Effective number of alleles (E)

The effective number of alleles is also an index used to reveal the genetic diversity of the populations. Table 4 shows the results for the effective number of alleles. The E value of 20 microsatellite loci was the highest (5.7351) in Muscovy duck and, varied from 0 to 11.3443; while it was the lowest in Charry Valley duck (4.7289) and varied from 2.4534 to 7.9819. The average E of all populations was 5.261.

The test of Hardy-Weinberg equilibrium

The Hardy-Weinberg equilibrium was used for testing whether the genotypes were maintained in balance or deviated from balance. The test results of Hardy-Weinberg equilibrium are listed in Table 5. The six duck populations were all in Hardy-Weinberg disequilibrium.

F-statistic analysis

The F-statistic was used in testing the genetic differentiation among subpopulations. The F-statistics were calculated for 20 microsatellite loci. The results are shown in Table 6. In this study, the range of F_{ST} was from 0.0205 (AJ515895) to 0.2558 (AJ515896). The mean of F_{ST} was 0.0936.

Genetic distances and clustering of ducks

The Nei's genetic distance (D_A) was calculated by the allele's frequencies and the results are summarized in Table 7. Genetic distance between Shaoxing duck and Cherry Valley duck was the shortest (0.1839), followed by Shaoxing duck and Peking duck Z1 (0.1947); the genetic distance between Muscovy duck and Peking duck Z4 was the longest (0.4558). The dendrogram was constructed using the NJ method of DISPAN software (Figure 2). The six populations were clustered into two groups. Peking duck Z4, Aobaixing duck, Peking duck Z1, Shaoxing duck and Cherry Valley duck were clustered together, while Muscovy duck was not included in the group.

Table 7. D_A genetic distances among populations

	Shaoxing	Muscovy	Peking Z4	Cherry Valley	Aobaixing	Peking Z1
Shaoxing duck	0					
Moscovy duck	0.381	0				
Peking Z4	0.3046	0.4558	0			
Cherry Valley duck	0.1839	0.4039	0.2572	0		
Aobaixing duck	0.2824	0.4477	0.2102	0.2534	0	
Peking Z1	0.1947	0.4338	0.2558	0.197	0.2463	0

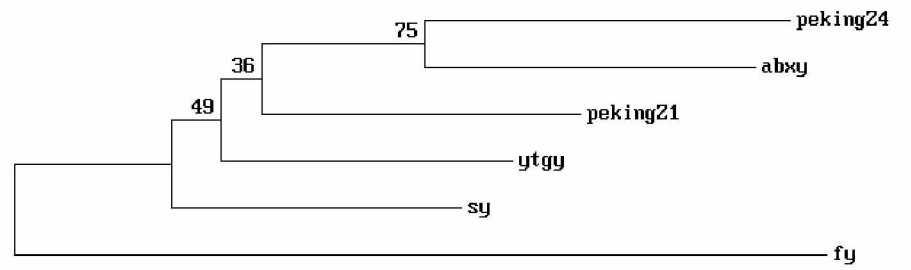

Figure 2. Dendrogram of relationships among 6 duck breeds using DA and the NJ method of clustering (abxy = Aobaixing duck; ytgy = Cherry Valley duck; sy = Shaoxing duck; fy = Muscovyduck).

DISCUSSION

Genetic diversity among loci and populations

In this study, we investigated the genetic diversity and phylogenetic relationships of six duck populations in China. The polymorphisms revealed differences among 20 microsatellite loci in the six populations. Average effective number of alleles of all microsatellite loci was 5.261, but it was different in each population and locus and varied from 0 to 11.407.

The PIC was a good index for genetic diversity evaluation. Botstein et al. (1980) first pointed out that PIC index can be used to evaluate the level of gene variation: when PIC>0.5, the locus has high diversity; when PIC< 0.25, the locus has low diversity; and the locus has intermediate diversity when PIC between 0.25 and 0.5. In this study, the PIC of 20 microsatellites in each population all showed high diversity except the AJ515896 locus in Muscovy duck. This suggested that the 20 microsatellite loci are all high diversity loci in the duck.

Heterozygosity (*h*) is one of the indices used to assay the genetic variation of each population. The values of *h* indicate the diversity level of the molecular marker. When the value is high, the molecular marker's diversity is high too. Among all loci studied, the *h* of the locus AJ515895 had the highest value (0.876), while the locus AJ515896 had the lowest *h* value (0.6212). In all populations, the *h* of Muscovy duck was the highest (0.8079), followed by Peking duck Z1 (0.7954), whereas Aobaixing duck was the lowest (0.7654). Our data indicated that genetic diversity of each population was high and there were enough gene resources in duck populations. This may be related to the breeding history and environment of each population.

Effective number of alleles (E) is used to assay the effect of alleles in each population. The result of this study revealed that the E of Muscovy duck was the highest (5.7351) and Cherry Valley duck was the lowest (4.7289) among all duck populations. Average E of all populations was 5.261. It was also shown that the E of 20 microsatellite loci in all duck populations was high. This result suggested that the populations have better abilities to keep the effective alleles when selection, mutation or genetic drift has occurred.

Taken together, each duck population used in this study had high average effective number of alleles (E), heterozygosity (h) and polymorphism information content (PIC), suggesting that genetic diversity of the 20 microsatellite loci in the six populations was high.

The structure of six duck populations

The Hardy-Weinberg equilibrium was used in testing whether the genotypes were maintained in balance or deviated from balance. In this study, the six duck populations were all in Hardy-Weinberg disequilibrium. These results showed that the structures of six duck populations were all being destroyed. Selection, non-random mating and inbreeding were the main reasons which induced the disequilibrium. In addition, in this study there were other reasons causing the disequilibrium, such as the excursion caused by mutation, genetic drift, selection, etc.

The F-statistic was used in testing the genetic differentiation among subpopulations. The F_{IS} and F_{IT} may be positive values or negative values, however, the F_{ST} values were always positive. When there is no differentiation, the value of F_{ST} is 0; when alleles among populations are quite different, the value of F_{ST} equals 1. In

this study, the range of F_{ST} was from 0.0205 (AJ515895) to 0.2558 (AJ515896). The mean of F_{ST} was 0.0936.

Phylogenesis of duck populations

There are many methods to measure the genetic distance. Nei showed that average codon margin of each locus can be estimated by the gene frequency of abundant loci. In this study, genetic distance and the dendrogram (Figure 2) between different populations were estimated by the neighbor-joining method (NJ) in the *DIASPAN* software.

The dendrogram of populations in Figure 2 reflected the genetic relationships of populations. The six populations clustered into two groups: one group included Peking duck Z4, Aobaixing duck, peking duck Z1, Cherry Valley duck and Shaoxing duck, while Muscovy duck was clustered into another group. In group one, Peking duck Z4 had the shortest genetic distance with Aobaixing duck, followed by Peking duck Z1, Cherry Valley duck and Shaoxing duck. The result of this cluster was consistent with breeding history and environment of the five populations. According to evolutional history, Peking duck appeared two hundred years ago. Peking duck Z1 and Z4 were new lines, which were cultivated in the 1980s in China. Female line Peking duck Z1 was mainly used to lay eggs, while Peking duck Z4 was the male line and used to breed with the Aobaixing duck. Cherry Valley duck was imported into China from England and Aobaixing duck from France (Zhou, 2002). They were crossed with Peking duck. Shaoxing duck was an egg-duck breed whose feather color, production performance, body size were different from Peking duck. Muscovy duck was another genera (*Cairina moschata*), and the form and biological characters were different from other duck populations. Therefore, it was clustered alone. The result was consistent with that of Yan et al. (2005).

In conclusion, our data suggested that 20 microsatellite loci could be used to investigate the genetic diversity and phylogenetic relationships among different duck populations.

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