



## Population Structure and Biodiversity of Chinese Indigenous Duck Breeds Revealed by 15 Microsatellite Markers

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**ABSTRACT :** Duck (*Anas platyrhynchos*) is one of the most important domestic avian species in the world. In the present research, fifteen polymorphic microsatellite markers were used to evaluate the diversity and population structure of 26 Chinese indigenous duck breeds across the country. The Chinese breeds showed high variation with the observed heterozygosity ( $H_o$ ) ranging from 0.401 (Jinding) to 0.615 (Enshi), and the expected heterozygosity ( $H_e$ ) ranging from 0.498 (Jinding) to 0.707 (Jingjiang). In all of the breeds, the values of  $H_o$  were significantly lower than those of  $H_e$ , suggesting high selection pressure on these local breeds. AMOVA and Bayesian clustering analysis showed that some breeds had mixed together. The  $F_{ST}$  value for all breeds was 0.155, indicating medium differentiation of the Chinese indigenous breeds. The  $F_{ST}$  value also indicated the short domestication history of most of Chinese indigenous ducks and the admixture of these breeds after domestication. Understanding the genetic relationship and structure of these breeds will provide valuable information for further conservation and utilization of the genetic resources in ducks. (**Key Words :** Duck, Population Structure, Biodiversity, Microsatellites)

### INTRODUCTION

China has the largest duck (*Anas platyrhynchos*) population and 31% of the domestic duck breeds in the world, representing a rich genetic resource (Scherf, 2000). Because of the unique geography, complicated landform and diversified culture, many indigenous breeds have developed unique characteristics. Specific genetic and behavioral adaptations have developed to accommodate to both climatic differences and food preferences (Harley et al., 2005). The world famous meat duck "Peking" originated in northern China, while "Jinding" duck, which can produce more than 260 eggs per year, was developed in the southern coastal area. These breeds vary in body size, plumage color, and other characteristics.

Many of them, however, are often maintained in small populations, owing to their comparatively poor performance in egg production and growth rate. Facing the challenge from much more efficient commercial duck strains, almost

all of the Chinese indigenous duck breeds are decreasing in population size, and even of more concern, some of the indigenous duck breeds are on the verge of extinction. The reduction of effective population size would reduce genetic variation and the ability of a population to mount a variable response to newly introduced pathogens and parasites (O'Brien et al., 1998). The admixture among duck populations is accelerated by modern transportation and human activities, which is changing the genetic characteristics and causing the loss of the genetic diversity of these birds. There has been much concern in recent years on the loss of biodiversity in poultry (Fulton and Delany, 2003). In China, the traditional defined breeds were 26 according to local culture, plumage color and locations (Xu et al., 2003). However, some defined breeds are closely related with similar characteristics, body structure, plumage color etc. Only one paper revealed the genetic diversity of domestic ducks based on the use of molecular markers (Li et al., 2006), but the admixture and population structure of these ducks was not analyzed. The effective population size variations in recent times and the gene flow patterns, which show the admixture of these breeds being not clear for these domestic duck breeds. Hence, a comprehensive population genetic analysis is required to document the genetic relationships among the breeds and the gene flow and

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Received February 9, 2007; Accepted August 26, 2007

**Table 1.** Basic breed information, sample size (N), observed and expected heterozygosity ( $H_o$ ,  $H_E$ ) and mean number of alleles per locus ( $N_a$ )

Breeds	Location	Code	N	$H_o$	$H_E$	$N_a$
Chaohu	Village	1	31	0.429±0.025	0.638±0.057	7.333±4.938
Dayu	Farm	2	31	0.547±0.024	0.662±0.046	7.733±5.574
Enshi	Village	3	31	0.615±0.023	0.651±0.063	7.800±6.050
Gaoyou	Village	4	31	0.487±0.024	0.589±0.044	4.933±2.120
Guangxima	Farm	5	31	0.560±0.024	0.625±0.068	7.400±5.262
Hanzhong	Village	6	31	0.545±0.024	0.631±0.059	6.600±4.239
Huainan	Village	7	31	0.499±0.024	0.651±0.055	7.600±5.804
Ji'an	Village	8	31	0.527±0.024	0.597±0.055	5.933±2.434
Jianchang	Farm	9	31	0.552±0.023	0.574±0.065	6.200±4.127
Jinding	Farm	10	31	0.401±0.023	0.498±0.066	4.733±3.283
Jingjiang	Village	11	31	0.503±0.024	0.707±0.043	8.600±5.207
Jingxi	Farm	12	31	0.521±0.024	0.661±0.048	7.000±5.169
Liancheng	Farm	13	31	0.491±0.023	0.542±0.043	4.667±1.676
Linwu	Farm	14	31	0.514±0.024	0.619±0.060	6.533±4.240
Mianyang	Village	15	31	0.536±0.024	0.668±0.049	7.133±4.912
Peking	Farm	16	31	0.433±0.024	0.508±0.065	5.733±3.127
Putian	Farm	17	31	0.534±0.024	0.631±0.060	7.067±4.978
Sansui	Village	18	31	0.547±0.024	0.624±0.057	7.000±5.237
Shanma	Farm	19	31	0.495±0.024	0.564±0.053	5.333±3.244
Shaoxing	Farm	20	31	0.469±0.024	0.560±0.069	5.933±4.964
Sichuanma	Village	21	31	0.506±0.024	0.591±0.064	7.133±6.468
Weishan	Farm	22	31	0.538±0.024	0.617±0.065	7.267±5.189
Wendeng	Village	23	31	0.500±0.024	0.577±0.042	6.800±4.296
Xingyi	Village	24	31	0.465±0.023	0.598±0.052	6.533±3.852
Youxian	Farm	25	31	0.551±0.024	0.630±0.057	7.467±4.984
Yunnanma	Farm	26	31	0.477±0.024	0.594±0.049	6.067±2.631

**Figure 1.** Sample locations of the 26 breeds of Chinese indigenous duck from China.

population size variations in the duck populations. Many microsatellite markers were developed for ducks (Buchholz et al., 1998; Maak et al., 2000; Stai and Hughes, 2003), but the number and polymorphism of these markers were limited. We have previously identified over 100 duck microsatellite markers, which can be used to analyze the

duck genetic relationship, population structure and gene flow.

In current research, we used 15 polymorphic microsatellite markers to analyze the genetic diversity and admixture of 26 Chinese indigenous duck breeds from across the country. The work will provide the necessary data to understand the genetic situation of these birds and to further to conserve these genetic resources.

## MATERIALS AND METHODS

### Sampling

Blood samples of 806 individual from twenty-six indigenous duck breeds (31 individuals for each breed) were collected in this study. Samples of each breed were selected and collected from the location where the breed was originated. The names, sample sizes, and sampling locations of the duck breeds are shown in Table 1. The sampling locations for the duck populations are shown in Figure 1.

### Microsatellite markers, PCR and genotyping

Blood samples, 3 ml to 5 ml per bird, were collected from the wing vein using ACD as the anti-coagulation agent. Genomic DNA was extracted from 30  $\mu$ l fresh blood by

**Table 2.** Allele size, number of observed alleles ( $N_a$ ), observed and expected heterozygosity ( $H_o$ ,  $H_E$ ) and  $F_{ST}$  for 15 loci

Locus	Accession no.	Allele size	$N_a$	$H_o$	$H_E$	$F_{ST}$
CAUD004	AY493249	190-224	19	0.605	0.693	0.109±0.030
CAUD011	AY493256	122-154	15	0.674	0.714	0.072±0.013
CAUD017	AY493262	216-262	23	0.259	0.593	0.204±0.034
CAUD035	AY493280	216-244	14	0.793	0.740	0.109±0.020
CAUD038	AY493283	187-384	64	0.727	0.823	0.117±0.026
CAUD041	AY493286	106-136	12	0.132	0.282	0.241±0.153
CAUD044	AY493289	115-166	17	0.601	0.641	0.129±0.045
CAUD050	AY493295	246-398	50	0.782	0.883	0.066±0.013
CAUD066	AY493311	178-206	9	0.449	0.613	0.185±0.053
CAUD067	AY493312	117-151	14	0.556	0.622	0.152±0.055
CAUD068	AY493313	138-170	13	0.590	0.642	0.114±0.030
CAUD076	AY493321	100-189	27	0.291	0.464	0.417±0.051
CAUD078	AY493322	210-242	15	0.068	0.362	0.387±0.071
CAUD083	AY493328	108-156	12	0.634	0.649	0.094±0.027
CAUD089	AY493334	166-180	7	0.285	0.372	0.060±0.018
Total/average			311	0.496	0.606	0.155±0.027

following steps: haemolysis, proteinase K incubation, extraction with phenol, phenol/chloroform (1 v/1 v) and chloroform, ethanol precipitation and finally re-suspension in 300 µl TE.

A total of 15 microsatellite markers (Table 2), which are not in the same linkage group (Huang et al., 2006) with the exception of two pairs (CAUD011, CAUD089; CAUD035, CAUD078), were selected to screen all of the samples. The forward primers were 5'-end-labeled with an ABI compatible phosphoramidite dye (6-FAM or HEX). PCRs were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) as follows: an initial denaturation step at 95°C for 5 min followed by 35 cycles of 30 sec at 95°C, 1 min at the appropriate annealing temperature and 1 min at 72°C, and a final extension step at 72°C for 7 min. PCR products were diluted by 10-30 times. Then 1 µl diluted PCR product was mixed with 0.8 µl deionized formamide, and 0.2 µl Genescan-350 ROX™ or Genescan-500 ROX™ (Applied Biosystems, Foster City, CA, USA) internal standard. The mixture was denatured at 95°C for 3 minutes and run in a 4.5% denaturing polyacrylamide gel using an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The fragment sizes of the PCR products were analyzed using the Genescan 3.7 and Genemapper 1.1 software (Applied Biosystems, Foster City, CA, USA).

#### Data analysis

The number of alleles, observed and expected heterozygosity were calculated using MS Tools program (Park, 2001).

The pairwise  $F_{ST}$  values were assessed and the significance value matrices were calculated using FSTAT program with 325,000 permutations (<http://www2.unil.ch/popgen/softwares/fstat.htm>). The indicative nominal level

to determine  $F_{ST}$  significance (5%) was adjusted to  $p = 0.000154$  for multiple comparisons after Bonferroni correction. The population structure was analyzed by the STRUCTURE software (Pritchard et al., 2000) which was applied to analyze the total sample set (806 individuals, 26 sampled breeds), with  $K = 1-30$ , the admixture model for ancestral population without using any prior population information. A set of simulations was run with  $10^5$  iterations, following a burn-in period of  $10^5$  iterations. Populations or individuals were assigned to one cluster if their proportion of membership to that cluster was equal to or larger than a probability threshold of 0.80.

AMOVA (analysis of molecular variance) was conducted by using Arlequin3.01 (Excoffier et al., 2005). Isolation by distance (IBD) was analyzed for the 26 duck breeds in IBD version 2.0, where the correlation between the log-transformed geographical and Nei's standard genetic distance was estimated using the Mantel test. This test uses a one-tailed Spearman rank correlation and 1,000 permutations (Rousset, 1997; Bohonak, 2002).

## RESULTS

#### The biodiversity of the duck breeds

The size of each allele, the number of loci, observed and expected heterozygosity for each locus as well as for all of the loci of the 26 duck populations are presented in Table 2. The 15 microsatellite loci used in this study were all polymorphic, and the number of alleles per locus varied from 7 (CAUD089) to 64 (CAUD038). The average observed and expected heterozygosity across 26 duck breeds were 0.496 and 0.606 respectively.

The values of  $H_o$  and  $H_e$  for all breeds were higher than 0.4 (Table 1), with the maximum of the  $H_o$  and  $H_e$  being 0.615 (Enshi) and 0.707 (Jingjiang), and the lowest being 0.401 (Jinding) and 0.498 (Jinding) respectively. The

**Table 3.** Analysis of molecular variance (AMOVA) for duck microsatellite data

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
Among populations	25	318.054	0.18192	12.43	0
Among individuals within populations	780	1,125.871	0.16216	11.08	0
Within individuals	806	902.000	1.11911	76.48	0

**Table 4.** Bayesian clustering analysis results for 26 duck breeds. For the code of the given population, the number shows the duck breed; V or F indicates the breed was keeping in village or farm

Given pop	Population inferred clusters																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1V	0.025	0.013	0.149	0.017	0.017	0.016	0.011	0.012	0.010	0.006	0.018	0.087	0.052	0.066	0.017	0.054	0.032	0.037	0.324	0.005	0.007	0.013	0.010
2F	0.018	0.136	0.061	0.072	0.029	0.04	0.021	0.064	0.017	0.015	0.021	0.051	0.026	0.080	0.068	0.053	0.014	0.049	0.072	0.018	0.043	0.013	0.017
3V	0.116	0.006	0.013	0.009	0.012	0.018	0.031	0.017	0.211	0.276	0.007	0.019	0.009	0.014	0.026	0.020	0.024	0.016	0.015	0.019	0.013	0.079	0.030
4V	0.013	0.015	0.014	0.023	0.012	0.021	0.017	0.010	0.008	0.004	0.043	0.074	0.583	0.024	0.012	0.012	0.025	0.036	0.023	0.008	0.006	0.006	0.011
5F	0.025	0.007	0.011	0.006	0.010	0.010	0.006	0.021	0.197	0.430	0.010	0.010	0.006	0.011	0.046	0.017	0.042	0.011	0.009	0.007	0.046	0.046	0.013
6V	0.016	0.048	0.027	0.029	0.111	0.054	0.111	0.057	0.017	0.008	0.025	0.041	0.072	0.044	0.010	0.030	0.014	0.135	0.018	0.051	0.054	0.013	0.016
7V	0.027	0.020	0.111	0.049	0.020	0.023	0.019	0.009	0.007	0.022	0.014	0.078	0.070	0.058	0.029	0.055	0.012	0.029	0.306	0.010	0.009	0.013	0.010
8V	0.008	0.012	0.031	0.619	0.012	0.010	0.009	0.017	0.006	0.004	0.027	0.013	0.072	0.029	0.007	0.029	0.015	0.030	0.023	0.006	0.008	0.007	0.007
9F	0.010	0.711	0.011	0.012	0.010	0.014	0.010	0.015	0.007	0.006	0.015	0.008	0.025	0.012	0.034	0.012	0.010	0.013	0.010	0.017	0.023	0.007	0.009
10F	0.008	0.006	0.008	0.005	0.022	0.010	0.787	0.009	0.006	0.006	0.004	0.012	0.005	0.007	0.004	0.009	0.006	0.040	0.008	0.009	0.007	0.014	0.006
11V	0.012	0.014	0.097	0.084	0.058	0.160	0.058	0.033	0.013	0.053	0.015	0.099	0.017	0.085	0.007	0.057	0.018	0.025	0.018	0.032	0.014	0.013	0.019
12F	0.026	0.051	0.012	0.022	0.019	0.018	0.011	0.022	0.349	0.030	0.039	0.009	0.013	0.013	0.115	0.014	0.024	0.024	0.010	0.014	0.023	0.062	0.081
13F	0.006	0.005	0.007	0.006	0.010	0.010	0.013	0.019	0.007	0.005	0.005	0.007	0.013	0.009	0.003	0.006	0.006	0.008	0.018	0.811	0.013	0.008	0.006
14F	0.033	0.009	0.009	0.013	0.009	0.015	0.013	0.009	0.057	0.018	0.042	0.011	0.018	0.008	0.006	0.042	0.620	0.011	0.009	0.008	0.009	0.023	0.007
15V	0.013	0.034	0.070	0.033	0.040	0.061	0.056	0.055	0.013	0.007	0.052	0.076	0.057	0.110	0.012	0.057	0.022	0.110	0.028	0.044	0.013	0.020	0.017
16F	0.014	0.011	0.009	0.006	0.004	0.007	0.005	0.004	0.015	0.010	0.009	0.014	0.007	0.006	0.822	0.010	0.007	0.005	0.009	0.005	0.006	0.006	0.008
17F	0.010	0.026	0.035	0.036	0.058	0.162	0.063	0.017	0.019	0.011	0.058	0.013	0.022	0.043	0.007	0.037	0.012	0.247	0.013	0.065	0.012	0.021	0.011
18V	0.019	0.023	0.020	0.065	0.319	0.068	0.048	0.027	0.017	0.008	0.021	0.031	0.030	0.052	0.009	0.054	0.040	0.018	0.029	0.029	0.019	0.039	0.015
19F	0.012	0.006	0.005	0.005	0.013	0.007	0.010	0.007	0.055	0.007	0.012	0.006	0.006	0.006	0.007	0.006	0.009	0.012	0.007	0.008	0.006	0.773	0.014
20F	0.067	0.018	0.008	0.010	0.023	0.012	0.017	0.015	0.034	0.008	0.012	0.011	0.021	0.013	0.008	0.009	0.008	0.010	0.011	0.007	0.011	0.014	0.653
21V	0.008	0.367	0.045	0.057	0.031	0.032	0.008	0.060	0.016	0.009	0.021	0.020	0.030	0.024	0.044	0.076	0.023	0.019	0.029	0.015	0.048	0.009	0.010
22F	0.588	0.013	0.017	0.008	0.007	0.011	0.010	0.006	0.055	0.029	0.010	0.026	0.013	0.010	0.038	0.024	0.021	0.006	0.025	0.006	0.008	0.032	0.035
23V	0.017	0.028	0.014	0.018	0.009	0.012	0.011	0.012	0.013	0.008	0.675	0.009	0.020	0.014	0.034	0.018	0.014	0.013	0.017	0.007	0.008	0.009	0.017
24V	0.007	0.029	0.009	0.037	0.021	0.017	0.017	0.099	0.014	0.025	0.006	0.012	0.007	0.028	0.008	0.013	0.007	0.007	0.009	0.019	0.595	0.006	0.008
25F	0.016	0.011	0.040	0.022	0.046	0.155	0.040	0.043	0.022	0.016	0.013	0.067	0.027	0.036	0.010	0.062	0.013	0.160	0.028	0.068	0.046	0.039	0.017
26F	0.005	0.015	0.011	0.009	0.018	0.019	0.015	0.453	0.008	0.013	0.010	0.033	0.011	0.028	0.008	0.019	0.007	0.023	0.008	0.014	0.253	0.008	0.013

average number of alleles across all of the loci for each breed varied from 4.667 (Liancheng) to 8.600 (Jingjiang). Some breeds also present population-specific (private) alleles but with low frequency (Table 1).

#### Population structure and the admixture among breeds

AMOVA revealed that 11.08% of the total genetic variation was attributed to differences among individuals within populations ( $p = 0$ ), while 12.43% was attributed to differences among populations ( $p = 0$ ) (Table 3). The pairwise  $F_{ST}$  values were quite low and 10 of the 305 pairwise  $F_{ST}$  values were not significant (adjusted p values was larger than 0.000154) (data not shown). In order to confirm whether the 26 duck breeds were genetically different, we also performed Bayesian clustering analysis by using STRUCTURE. The Bayesian clustering analysis showed the division of the genetic variation into 23 clusters. The proportion for each breed which was assigned into each cluster showed that some breeds had mixed together (Table 4). Ducks on the farms could be closely clustered together while the breeds preserved in the villages were often distributed in different clusters. The Bayesian clustering analysis results were consistent with the  $F_{ST}$  analysis which showed that the differentiation of some of the breeds was

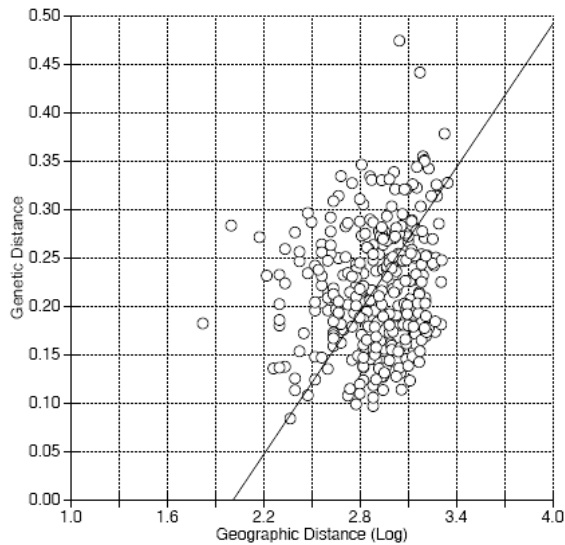
very low.

A Mantel test was performed in order to evaluate the correlation between genetic distances ( $D_A$ ) and log transformed geographical distance between breeds (Figure 2). A moderate correlation between genetic distance and geographical distance was observed ( $Z = 206.8948$ ,  $r = 0.1917$ , one-sided  $p \leq 0.0210$  from 1,000 randomizations).

#### DISCUSSION

The study reported here represents the first comprehensive genetic analysis of domestic ducks using microsatellite markers and also elucidates the genetic structures and admixtures of different breeds of Chinese local ducks.

The fifteen markers used in this study were first reported by Huang et al. (2006) and their polymorphisms were estimated only in a few resource populations by these authors. We found that these microsatellite markers had high polymorphisms with the highest number of alleles being 64 and the lowest being 7 across all of the breeds. Moreover, these markers are distributed on different linkage group with only two pairs of markers as exceptions. So these markers could be considered to have been used



**Figure 2.** Pairwise genetic distances (DA) by log-transformed geographic distance (km) showed significant isolation by distance.

successfully to analyze the biodiversity and population structure of ducks and could form the basis for further studies. Meanwhile, we detected the other eighteen polymorphic microsatellite markers which were identified by Huang et al. (2006) in some of the indigenous breeds. We did not use these markers for further analyses because the number of alleles of these markers was less than 3 and some of them could not get PCR products in most of the birds. Due to the high polymorphism of the markers used in this study, the population structure and the biodiversity can be accurately estimated by these fifteen markers.

The observed genetic diversities of the Chinese indigenous ducks ranged from 0.401 to 0.615 for individual breed, which were a little bit lower than chickens (Qu et al., 2006; Kong et al., 2006; Osman et al., 2006) and geese (Tu et al., 2006). This could be attributed to the larger effective population size of chicken and also the higher selection pressure on the duck populations. In the present study, we also found that all of the observed heterozygosity values were lower than the expected values in the 26 duck breeds, indicating the high artificial and natural selection pressure on these breeds.

We compared current results to the other work on Chinese indigenous duck breeds, in which the same 24 breeds except for Ji'an and Wendeng duck breeds were analyzed using 28 microsatellites (Li et al., 2006). Although they also found high polymorphism in all of the duck breeds, the correlation of the observed heterozygosities of the 24 breeds to our results was not high ( $r = 0.36$ ,  $p = 0.09$ ). The different methods used in the two studies might explain the low correlation. In the current study all birds were screened for the microsatellite markers using ABI PRISM 377 DNA sequencer, while silver staining of 8% polypropylene gel were used in Li et al. (2006) for

genotyping. More wrongly genotyped alleles would have been generated because artificially genotyping were required in silver staining methods. The low correlation could also be attributed to the different materials including individuals and markers they used from those in current study. Furthermore, the genetic relationship and admixture of the Chinese ducks were analyzed and more reliable population specific alleles were provided in the present study.

Population differentiations for most breeds were observed but some traditionally defined breeds were not clustered closely together (Xu et al., 2003). AMOVA analysis showed that population variations can only explain 12.43% of the variations. The pairwise  $F_{ST}$  between some breeds was very low and insignificant. Two likely causes could have contributed to this. 1) Short division time among these breeds. Compared to other domestic species (chicken, pig and cattle), duck has a much shorter domestic history, which could result in the closer genetic distance that our data showed. 2) Admixture of some of the indigenous breeds. The low pairwise  $F_{ST}$  values of the indigenous breeds indicate the admixture of them. Bayesian cluster analysis showed that some breeds especially preserved in villages mixed together with other breeds and only those ducks in protected farms would cluster closely together (Table 4). Chaohu and Huainan ducks were clustered closely to each other and the lowest pairwise  $F_{ST}$  value was observed between them (data not shown) indicating the admixture or short domestication history of these two breeds. In conclusion, the use of 15 microsatellite markers allowed the characterization of the genetic diversity of 26 Chinese indigenous duck breeds in the present study. Some previously defined breeds should be reconsidered because of their low differentiation. We also found that the admixture of these breeds were very common. Urgent actions are called for to avoid gene losses and admixture of breeds. More efficient management and genetic monitoring should be introduced to increase the effective population size of some breeds so as to enhance the genetic diversity of the indigenous duck breeds for sustainable development.

## ACKNOWLEDGMENTS

The authors would like to appreciate Mrs. Guifang Xu, Mr. Hongjie Yang and Prof. Kuanwei Chen for their helps in survey and sample collection, and Dr. Paul Siegel for critical reading of the manuscript. This work was funded by High Technology Research and Development Program of China (No. 2006AA10A121) and National Basic Research Program of China (No.2006CB102102).

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