# DNA microsatellite analysis of genetic diversity among Chinese indigenous laying-type ducks (*Anas platyrhynchos*)

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**ABSTRACT**: The genetic polymorphisms of 17 microsatellites were investigated in four indigenous laying-type duck breeds in China. The average number of alleles (*Na*) and average rates of homozygotes of each breed were counted. Accordingly, allele frequencies of the 17 microsatellites, polymorphism information content (*PIC*), mean heterozygosity (*H*) and genetic distances (*Ds*) were also calculated. Moreover, dendrograms using UPGMA and the neighbour-joining method were produced. The four breeds have a high average *PIC* (0.643) and *H* (0.682). *Ds* are between 0.514 and 0.662, the gene differentiation among the four breeds is 14.4%.

**Keywords**: duck; PCR; microsatellite; genetic diversity; genetic polymorphism

Microsatellites, also called simple sequence repeats (SSRs), are made up of a variable number of tandem repeats of short DNA motifs found in all prokaryotic and eukaryotic genomes (Tautz, 1989; Hancock, 1996). These motifs are present in many different chromosomes, independently of coding and non-coding regions, and their sequence information is now available from GenBank and EMBL databases. Microsatellites are abundant in copy numbers highly polymorphic in DNA sequence and have hence been employed as an important marker for genome mapping and/or paternity analysis. Examples of the use of such DNA microsatellite markers include the fields of forensics, population genetics, and evolution studies (Maak et al., 2003; Stai and Hughes, 2003; Shahbazi et al., 2007; Su et al., 2007; Li et al., 2008).

In China, particularly in its southern part, a great number of ducks have been widely bred every year. These ducks include four autochthonous laying-type duck breeds: Liancheng (LC), Jinding (JD), Jingjiang (JJ), and Shaoxing (SX). These four breeds are featured by the fact that the female is able to lay as many as 250–300 eggs per year, with an average egg weight of 62–72 g. More importantly, they have shown high levels of phenotypic variability and adaptability to a wide range of environmental conditions. In addition to their high economic and unique ecological values, these ducks also provide an invaluable genetic resource for the duck genetic management and scientific research along with the farming industry (Chen et al., 2001; Wang and Dou, 2005). Because of such importance, a protective framework has been set up in China to avoid crossbreeding so that a unique gene pool is maintained for each of these four indigenous ducks. Therefore, it is necessary to study their genetic diversity, origin, differentiation and relationships using microsatellite markers so as to provide molecular data for pure breeding, crossbreeding and preservation of important genetic resources. Recent work in this regard includes detection of polymorphism of mitochondrial DNA (Zheng et al., 1995;

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Chen et al., 1999) and RAPD and RFLP analysis of genomic DNA (Zuo et al., 2004; Xiao et al., 2004; Yan et al., 2005). In this article, the genetic diversity and the possible evolutionary relationship of the four indigenous ducks have been reported.

## MATERIAL AND METHODS

### Sampling

Blood samples were collected from 219 unrelated individuals representing four duck breed populations, including 54 LC ducks, 55 JD ducks, 60 JJ ducks, and 50 SX ducks. The blood samples collected were stored at –80°C until use.

### DNA isolation

A routine phenol/chloroform extraction method was used to extract and purify the genomic DNA. DNA concentration was estimated by comparison with molecular standard markers using agarose gel electrophoresis.

### Microsatellite loci and primers used for polymerase chain reaction (PCR)

Seventeen microsatellite loci were studied, which include *APL*577, *APL*579, *APL*580, *CMO*211, *CMO*212 (Gong et al., 2005); *AY*258, *AY*264, *AY*269, *AY*283, *AY*285, *AY*287, *AY*294, *AY*295, *AY*310, *AY*314,

Table 1. Characterisation of duck microsatellites

| | Primer sequence (5'~3') | Annealing temperature (°C) | Allele number | Allele fragments (bp) |
|---|---|---|---|---|
| *APL*577 | GAATAAA GTAACGGGCTTCTCT CTGCTTGGTT-TGGAAA GT | 55.0 | 9 | 192–272 |
| *APL*579 | ATTAGA GCAGGAGTTAGGAGAC GCAA GAA GTGGCTTTTTTC | 55.0 | 17 | 159–319 |
| *APL*580 | GGATGTTGCCCCACATATTT TTGCCTTGTTTAT-GA GCCATTA | 55.0 | 14 | 104–180 |
| *AY*258 | ATGTCTGAGTCCTCGGAGC ACA-ATAGATTCCAGATGCTGAA | 58.1 | 6 | 093–211 |
| *AY*264 | GCAGACTTTTACTTATGACTC CTTAGCCCAGT-GAAGCATG | 58.1 | 18 | 114–284 |
| *AY*269 | TCGCATTAAGCTCTGATCT ATCAACAGA-ATCCAAAATATG | 55.5 | 25 | 245–425 |
| *AY*283 | GACCACAACATCGTGCAGAG GATAATGGCT-GGCTCCTTGA | 50.9 | 17 | 211–371 |
| *AY*285 | TCCCACCCCAAACCCTGC TGTG-TAACCCGATAGACTGA | 50.3 | 14 | 231–341 |
| *AY*287 | TGCAGGTAGGTCTTCTGTTCTG GCCAGTCCTT-TGCTTCGTAA | 60.8 | 15 | 174–294 |
| *AY*294 | TGTAGTTTAGTTGCTGGATA TTAGTAAACTCTT-GCCATCT | 60.8 | 16 | 200–310 |
| *AY*295 | GGCTTCTGTGCTCCTCAGAT GCACAAGTGGCA-TGTGTCAT | 66.0 | 15 | 203–443 |
| *AY*310 | GCTTTAGTTTTTCAATTAGGTA TGGTGCGAT-GAGCTGAGAT | 58.1 | 27 | 107–477 |
| *AY*314 | CTCATTCCAATTCCTCTGTA CAGCATTAT-TATTTCAGAAGG | 50.3 | 21 | 117–317 |
| *CADU*86 | GCAGAGCGGTGTGAGAGCA AACACAGCTT-CACCCCACAG | 60.1 | 8 | 175–217 |
| *CADU*24 | CCAGCCAAGAACCTCCAGT CTTTGAATGTCCA-TGTAGCAG | 58.1 | 6 | 138–178 |
| *CMO*211 | GGATGTTGCCCCACATATTT TTGCCTTGTTTAT-GA GCCATT | 55.0 | 16 | 221–301 |
| *CMO*212 | CTCCACTA GAACACA GACATT CATCTTTGGCA-TTTTGAA G | 58.0 | 10 | 108–164 |

Table 2. The number of total alleles, common alleles and private alleles in four duck breeds

| Loci | The number of total alleles | | | | | The number of common alleles | | | | | | | The number of private alleles | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SX | LC | JD | JJ | entire sample | SX–LC | SX–JD | SX–JJ | LC–JD | LC–JJ | JD–JJ | SX–LC– | SX | LC | JD | JJ | entire sample |
| *APL*577 | 4 | 8 | 6 | 5 | 9 | 3 | 2 | 2 | 6 | 5 | 5 | 2 | 0 | 1 | 0 | 2 | 3 |
| *APL*579 | 2 | 4 | 1 | 9 | 13 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 3 |
| *APL*580 | 7 | 3 | 4 | 6 | 10 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *AY*258 | 4 | 4 | 2 | 4 | 4 | 4 | 2 | 4 | 2 | 4 | 2 | 2 | 0 | 0 | 0 | 0 | 0 |
| *AY*264 | 13 | 9 | 12 | 9 | 16 | 7 | 11 | 8 | 6 | 3 | 8 | 3 | 0 | 0 | 0 | 0 | 0 |
| *AY*269 | 11 | 18 | 10 | 13 | 21 | 10 | 9 | 9 | 10 | 11 | 10 | 9 | 0 | 3 | 0 | 0 | 3 |
| *AY*283 | 9 | 10 | 11 | 13 | 17 | 6 | 8 | 6 | 6 | 10 | 8 | 6 | 0 | 0 | 0 | 0 | 0 |
| *AY*285 | 10 | 9 | 9 | 8 | 11 | 9 | 8 | 8 | 8 | 8 | 7 | 7 | 1 | 0 | 0 | 0 | 1 |
| *AY*287 | 10 | 7 | 7 | 8 | 12 | 5 | 7 | 8 | 5 | 5 | 6 | 5 | 0 | 2 | 0 | 0 | 2 |
| *AY*294 | 10 | 5 | 6 | 8 | 13 | 4 | 5 | 6 | 5 | 4 | 5 | 4 | 2 | 0 | 0 | 1 | 3 |
| *AY*295 | 8 | 9 | 9 | 9 | 12 | 6 | 5 | 6 | 8 | 8 | 8 | 5 | 0 | 0 | 0 | 0 | 0 |
| *AY*310 | 15 | 11 | 12 | 10 | 21 | 9 | 9 | 8 | 8 | 8 | 7 | 6 | 3 | 0 | 1 | 0 | 4 |
| *AY*314 | 12 | 15 | 8 | 11 | 17 | 10 | 7 | 10 | 8 | 9 | 6 | 6 | 0 | 2 | 0 | 0 | 2 |
| *CA*086 | 6 | 6 | 5 | 5 | 7 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 1 | 1 | 0 | 0 | 2 |
| *CA*124 | 2 | 4 | 2 | 5 | 6 | 2 | 0 | 1 | 2 | 3 | 2 | 1 | 0 | 0 | 0 | 1 | 1 |
| *CMO*11 | 1 | 4 | 7 | 2 | 11 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 3 | 1 | 4 |
| *CMO*12 | 5 | 4 | 5 | 6 | 8 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 1 | 1 | 2 |
| Total | 129 | 130 | 116 | 131 | 208 | 87 | 85 | 91 | 88 | 91 | 86 | 68 | 7 | 11 | 5 | 7 | 30 |
| Range | 1–15 | 3–18 | 1–12 | 2–13 | 4–21 | 0–10 | 0–11 | 0–10 | 0–10 | 0–11 | 0–10 | 0–9 | 0–3 | 0–3 | 0–3 | 0–2 | 0–4 |
| Mean | 7.6 | 7.6 | 6.8 | 7.7 | 12.2 | 5.1 | 5 | 5.4 | 5.2 | 5.4 | 5.1 | 4 | 0.4 | 0.6 | 0.25 | 0.4 | 1.7 |
| SD | 4.15 | 4.19 | 3.43 | 3.07 | 4.9 | 3.14 | 3.46 | 2.96 | 2.74 | 3.14 | 2.90 | 2.5 | 0.87 | 0.99 | 0.77 | 0.62 | 1.5 |

*CADU*86, and *CADU*24 (from China Agriculture University). The PCR primers for each of these loci are listed in Table 1. All the primers were synthesised by the Shanghai Bioasia Bio-Tech. Co., Ltd.

## Optimisation of PCR conditions

Amplifications of microsatellite markers by PCR were performed on Biometra T gradient 1702238 Thermal Cyclers under the following conditions: total reaction volume of 20 µl containing 2 µl of 10× typical reaction buffer, 2 µl of 25 mmol $MgCl_2$, 0.8 µl of 10 mmol dNTPs, 0.2 µl of 5 U/µl *Taq* DNA polymerase, 1 µl of 10 pmol/µl each primer, and approximately 50 ng of duck genomic DNA. The reaction was carried out for 30 cycles by denaturing at 94°C for 1 min, annealing at the temperature optimised for each primer pair (Table 1) for 1 min, and polymerising at 72°C for 1 min, followed by an extension step at 72°C for 10 min.

The amplified products were separated by electrophoresis on an 8% non-denaturing polyacrylamide gel. DNA fragments were visualised by silver staining. The image data was analysed using Kodak Digital Science ID Image Analysis Software.

## Statistical analysis

The following parameters were calculated for each locus using the software Fstat (Version 2.9.3) and Genepop (Version 3.3): the distribution of allele frequencies, the presence of private alleles, the number of alleles, observed heterozygosity (*Ho*), expected population heterozygosity (*Hs*), expected total heterozygosity (*Ht*), and proportion among-population differentiation (*Gst*). We also used Genepop to test pairwise linkage equilibriums at all loci over any two groups in order to compute pairwise genetic differentiation *Fst*. Software Phylip 3.5c was used to determine Nei's standard

Table 3. Gene heterozygosity (*H*) and polymorphism information content (*PIC*)

| Locus | *H* | | | | | *PIC* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SX | LC | JD | JJ | All | SX | LC | JD | JJ | All |
| *APL*577 | 0.445 | 0.792 | 0.746 | 0.761 | 0.686 | 0.384 | 0.763 | 0.704 | 0.720 | 0.643 |
| *APL*579 | 0.058 | 0.702 | 0.000 | 0.722 | 0.370 | 0.057 | 0.645 | 0.000 | 0.689 | 0.348 |
| *APL*580 | 0.745 | 0.624 | 0.552 | 0.779 | 0.675 | 0.703 | 0.549 | 0.454 | 0.747 | 0.613 |
| *AY*258 | 0.659 | 0.637 | 0.500 | 0.615 | 0.603 | 0.594 | 0.564 | 0.375 | 0.569 | 0.526 |
| *AY*264 | 0.834 | 0.666 | 0.886 | 0.799 | 0.796 | 0.815 | 0.636 | 0.876 | 0.772 | 0.775 |
| *AY*269 | 0.854 | 0.894 | 0.825 | 0.888 | 0.865 | 0.838 | 0.885 | 0.804 | 0.877 | 0.851 |
| *AY*283 | 0.570 | 0.930 | 0.834 | 0.873 | 0.802 | 0.538 | 0.927 | 0.816 | 0.860 | 0.785 |
| *AY*285 | 0.824 | 0.707 | 0.761 | 0.816 | 0.777 | 0.803 | 0.680 | 0.735 | 0.791 | 0.752 |
| *AY*287 | 0.810 | 0.500 | 0.773 | 0.816 | 0.726 | 0.789 | 0.453 | 0.744 | 0.794 | 0.695 |
| *AY*294 | 0.824 | 0.519 | 0.891 | 0.791 | 0.756 | 0.806 | 0.487 | 0.885 | 0.760 | 0.734 |
| *AY*295 | 0.714 | 0.824 | 0.804 | 0.581 | 0.731 | 0.672 | 0.803 | 0.777 | 0.554 | 0.702 |
| *AY*310 | 0.192 | 0.815 | 0.809 | 0.843 | 0.665 | 0.019 | 0.794 | 0.787 | 0.824 | 0.606 |
| *AY*314 | 0.780 | 0.841 | 0.831 | 0.821 | 0.818 | 0.756 | 0.829 | 0.809 | 0.799 | 0.798 |
| *CA*086 | 0.752 | 0.706 | 0.721 | 0.735 | 0.729 | 0.713 | 0.650 | 0.672 | 0.693 | 0.682 |
| *CA*124 | 0.500 | 0.722 | 0.500 | 0.658 | 0.595 | 0.375 | 0.672 | 0.375 | 0.595 | 0.504 |
| *CMO*11 | 0.000 | 0.503 | 0.690 | 0.180 | 0.343 | 0.000 | 0.435 | 0.636 | 0.164 | 0.309 |
| *CMO*12 | 0.715 | 0.554 | 0.627 | 0.759 | 0.664 | 0.668 | 0.456 | 0.554 | 0.720 | 0.600 |
| Mean | 0.604 | 0.702 | 0.691 | 0.732 | 0.682 | 0.561 | 0.660 | 0.647 | 0.702 | 0.643 |
| Std. Dev. | 0.270 | 0.130 | 0.210 | 0.160 | 0.140 | 0.280 | 0.150 | 0.220 | 0.160 | 0.150 |

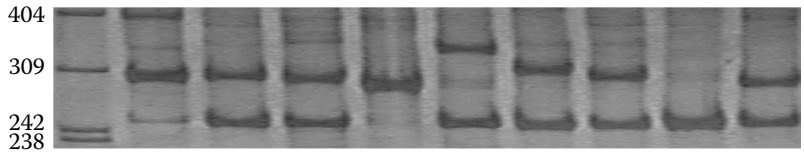


Figure 1. A portion of PCR results of *AY*295 in SX ducks

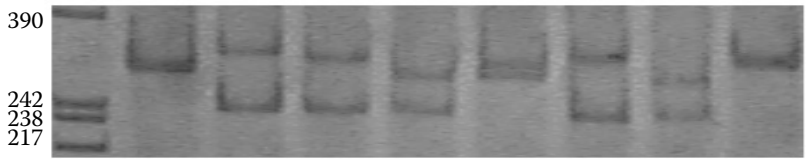


Figure 2. A portion of PCR results of *AY*294 in JD ducks

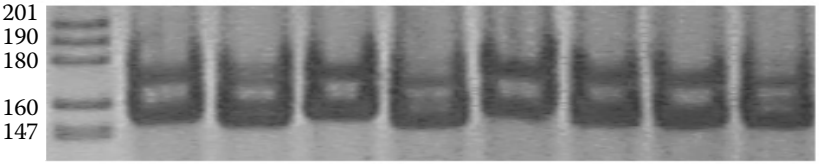


Figure 3. A portion of PCR results of *CA*124 in JJ ducks

genetic distance (*Ds*) and to draw a UPGMA tree and a neighbour-joining tree based on the allele frequency data. Polymorphism information content (*PIC*) was derived.

Table 4. Nei's estimation of heterozygosity, *Fst* and *Gst* values

| Loci Name | *Ho* | *Hs* | *Ht* | *Gst* | *Fst* | *P* ($r < 0$) |
|---|---|---|---|---|---|---|
| *AP*577 | 0.889 | 0.691 | 0.759 | 0.090 | 0.113 | 0.002 |
| *AP*579 | 0.310 | 0.375 | 0.804 | 0.534 | 0.606 | 0.002 |
| *AP*580 | 0.805 | 0.681 | 0.721 | 0.055 | 0.070 | 0.002 |
| *AY*258 | 0.927 | 0.606 | 0.697 | 0.131 | 0.166 | 0.002 |
| *AY*264 | 0.608 | 0.807 | 0.906 | 0.109 | 0.140 | 0.002 |
| *AY*269 | 0.606 | 0.877 | 0.910 | 0.036 | 0.047 | 0.002 |
| *AY*283 | 0.688 | 0.771 | 0.830 | 0.072 | 0.093 | 0.002 |
| *AY*285 | 0.512 | 0.788 | 0.841 | 0.063 | 0.083 | 0.002 |
| *AY*287 | 0.825 | 0.731 | 0.858 | 0.148 | 0.188 | 0.002 |
| *AY*294 | 0.600 | 0.725 | 0.807 | 0.101 | 0.130 | 0.002 |
| *AY*295 | 0.528 | 0.740 | 0.825 | 0.102 | 0.133 | 0.002 |
| *AY*310 | 0.741 | 0.845 | 0.904 | 0.065 | 0.086 | 0.002 |
| *AY*314 | 0.793 | 0.827 | 0.878 | 0.057 | 0.076 | 0.002 |
| *CA*086 | 0.899 | 0.734 | 0.754 | 0.026 | 0.034 | 0.002 |
| *CA*124 | 1.000 | 0.597 | 0.756 | 0.210 | 0.262 | 0.002 |
| *CMO*11 | 0.163 | 0.349 | 0.795 | 0.561 | 0.641 | 0.002 |
| *CMO*12 | 0.897 | 0.669 | 0.748 | 0.106 | 0.137 | 0.002 |
| Overall | 0.693 | 0.695 | 0.811 | 0.144 | 0.184 | 0.002 |

*Ho* = Observed heterozygosity from direct count
*Hs* = Expected population heterozygosity, from Nei (1978)
*Ht* = Expected total heterozygosity, from Nei (1978)
*Gst* = Proportion among–population differentiation, from Nei (1978)

Table 5. The genetic distances (*Ds*) and *Fst* values of four local laying-type duck populations

| | SX | LC | JD | JJ |
|---|---|---|---|---|
| SX | | 0.194 | 0.220 | 0.187 |
| LC | 0.633 | | 0.170 | 0.174 |
| JD | 0.514 | 0.526 | | 0.151 |
| JJ | 0.583 | 0.662 | 0.528 | |

*Fst* values are above the diagonal and the genetic distances are below the diagonal

## RESULTS

### Genetic diversity and genetic differentiation

Seventeen microsatellites were analyzed, all of which are polymorphic, showing 4–15 different alleles per locus (average 12.2 ± 4.9) (Table 2). Among the four duck breeds, SX ducks have totally 129 alleles, LC ducks have 130, JD ducks have 116, while JJ ducks have 131. Altogether (in all four duck breeds), there are totally 208 different alleles. Overall, the number of alleles per locus ranges from 4 (*AY*258) to 21 (*AY*269, *AY*310). Among the 208 alleles, 68 were commonly found in all the four breeds. The number of common alleles at each locus in these four duck breeds, however, varies from 0 to 9, with the average number of common alleles being 4. There were 30 private alleles, 11 in LC ducks, 7 in SX ducks, 7 in JJ ducks, and 5 in JD ducks.

Among seventeen polymorphic markers, the highest heterozygosity (*H*) is 0.865, which was observed at *AY*269, and the lowest heterozygosity(*H*) is 0.343, which was at *CMO*11. A total of 15 (88.24%) loci have heterozygosities greater than 0.50. Further calculations indicate that the average *H* ranges from 0.604 in SX ducks to 0.732 in JJ ducks. The *PIC* of 17 loci ranges from 0.309 (*CMO11*) to 0.851 (*AY269*). The average *PIC* ranges from 0.561 in SX ducks to 0.702 in JJ ducks (Table 3).

### Population genetic variations

*Gst* measures genetic divergence between different breeds and it is calculated based on allele frequencies of all the seventeen microsatellites. Our results show that *Gst* changes drastically from one microsatellite locus to another among the four duck breeds, ranging from 2.6% (*CA*086) to 56.1% (*CMO*11), with an average of 14.4% (Table 4). More than 85.6 percent of the variations were found to be due to the differences amongst the individuals within the same breeds. Population heterozygosity *(Hs*) ranges from 0.349 (*CMO*11) to 0.877 (*AY*269) in all the four breeds, and total heterozygosity (*Ht*) is from 0.697 to 0.910. *Fst* values range between 0.034 and 0.641, corresponding to moderate (0.05–0.15) to great (0.15–0.25) genetic differentiation extents. Statistically significant genetic variation ($P < 0.002$; random value < observed value) was observed at each locus analysed. When populations from different duck breeds were pair-wise associated, the highest *Fst* value of 0.220 was obtained in SX and JD ducks as compared to 0.170 in LC and JD ducks, and 0.151 in JJ and JD ducks (Table 5). Taken together, a low degree of genetic differentiation was found amongst the four breeds studied and a significantly high level of variation was observed among individuals within the same breeds. These *Fst* results suggest a relatively low gene flow between different breeds and, equivalently, a relatively high reproductive isolation within the same ones.

### The genetic distance (*Ds*) and genetic relation among the four indigenous duck populations

The genetic distance was further estimated on the basis of Nei's distance derived from Phylip software, and the calculated results are presented in Table 5. It was found that there is a short genetic distance between SX and JD ducks (0.514), and

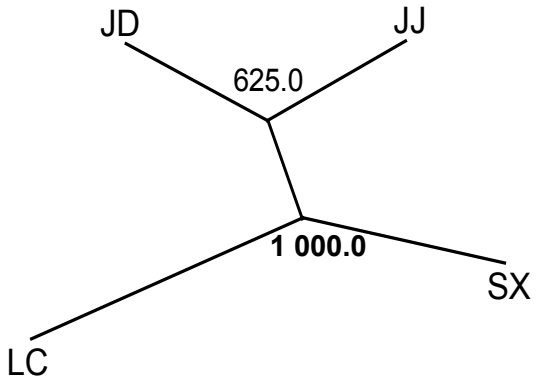


Figure 4. NJ tree for four laying-type duck breeds (the numbers at the nodes are the percent duck breeds in China occurrence in 1 000 bootstrap replicates)

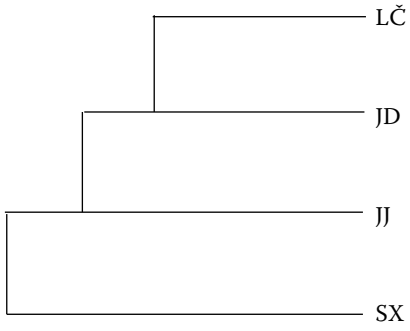


Figure 5. UPGMA tree for four laying-type duck breeds (Bootstrap values 1 000 replication)

a long genetic distance between JJ and LC ducks (0.662). These data suggest that SX ducks and JD ducks more resemble each other, with the genetic resemblance coefficient factor being 0.486.

Furthermore, UPGMA tree clustering was generated using *Ds* data (Figure 4). Bootstrap values are based on 1 000 replicates, and their support to the main clusters is as high as 62.5% and 100% (Figure 4). A similar phylogenetic tree structure was also obtained with the neighbour-joining algorithm (Figure 5). This neighbour-joining method assumes that two neighbours (the two nearest taxa on an unrooted tree) are connected by a single node. The UPGMA algorithm does not make any such assumptions as to linear descent, and it simply measures the amount of divergence among populations. The branches between SX and LC ducks are deep, and are well supported by bootstrap values, indicating an unambiguous genetic distance between them.

## DISCUSSION

The variation of genetic diversity and allele distribution was strongly dependent on the microsatellite locus that was analysed. The results obtained in this study demonstrate that the levels of genetic diversity were relatively high in these four breeds. The average heterozygosities (*H*) of microsatellite markers are similar to those in Red-Winged Blackbirds reported by Williams et al. (2004) and those in Shaoxing duck reported by Wu et al. (2006), with the average being from 0.780 to 0.800 and from 0.738 to 0.834, respectively. The *PIC* ranges from 0.513 to 0.644 in eleven duck populations (Zhao et al., 2005). *Ho* was found to be between 0.370 to 0.960 in Eider duck and their cross-species (Paulus et al., 2003). In this study, we found that *H* is 0.693. The number of alleles per locus in this study is significantly higher than that reported in the other species of ducks (Williams et al., 2004).

*Fst* are measures of the degree of resemblance between individuals within a breed. This resemblance can be interpreted as the differences between individuals in different breeds and expressed as the differences between breeds as a proportion of the total genetic variance. Genetic differentiation is a complicated issue that may result from natural selection favouring different genotypes in different subpopulations, from random processes in transmission of alleles from one generation to the next, or from stochastic differences in allele frequency among the initial founders of the subpopulations. Our study has shown that genetic differentiation has the highest value for SX and JD ducks (0.220), followed by SX and LC ducks (0.194) and SX and JJ ducks (0.185). Thus, the extent of genetic differentiation between different breeds can be extremely variable. The observed divergence most probably reflects the human selection. Williams (2005) assessed genetic variation among mottled ducks and mallards, he found there is a significant overall difference between these species within two geographic areas: *Fst* between mallards and mottled ducks in Florida is as large as 0.210.

The present study analyzes four laying-type duck breeds in China, which present high levels of genetic diversity and population genetic differentiation. Results from this study suggest that local breeds may be considered important reservoirs of genetic diversity. Phylogenetic approaches suggest that artificial selection plays an important role in genetic differentiation of duck breeds. Clearly, the knowledge of genetic relationships among breeds will be significant for the conservation of those animal genetic resources and for development of breeding programs for increased productivity.

## REFERENCES

Chen Y.X., Zhao Y., Lu L.J., Lai Y.Z. (1999): Comparative study of mitochondrial DNA restriction maps of Genus Anas ducks in Fujian Province. Journal of Xiamen University (Natural Science), 38, 108–111.

Chen Y.X., Chen X.L., Lu L.J., Zhao Y., Zuo Z.H., Lai Y.Z. (2001): Research for domestic duck resources and the St rain breeding. Journal of Xiamen University (Natural Science), 40, 642–646.

Hancock J.M. (1996): Simple sequences in a minimal genome. Nature Genetics, 14, 14–15.

Li F.E., Mei S.Q., Deng C.Y., Jiang S.W., Zuo B., Zheng R., Li J.L., Xu D.Q., Lei M.G., Xiong Y.Z. (2008): Association of a microsatellite flanking *FSHB* gene with reproductive traits and reproductive tract components in pigs. Czech Journal of Animal Science, 53, 139–144.

Maak S., Wimmers K.S., Weigend K. (2003): Isolation and characterization of 18 microsatellites in the Peking duck (*Anas platyrhynchos*) and their application in other waterfowl species. Molecular Ecology Notes, 3, 224–227.

Paulus K.B., Tiedemann R. (2003): Ten polymorphic autosomal microsatellite loci for the Eider duck Somateria mollissima and their cross-species applicability among waterfowl species (Anatidae). Molecular Ecology Notes, 3, 250–252.

Shahbazi S., Seyed Z., Mirhosseini, Romanov M.N. (2007): Genetic diversity in five Iranian native chicken populations estimated by microsatellite markers. Biochemical Genetics, 45, 63–75.

Stai S.M., Hughes C.R. (2003): Characterization of microsatellite loci in wild and domestic Muscovy ducks (*Cairina moschata*). Animal Genetics, 34, 387–389.

Su Y., Long R.J, Chen G.H, Wu X.S., Xie K.Z., Wan J.H. (2007): Genetic analysis of six endangered local duck populations in China based on microsatellite markers. Journal of Genetics and Genomics, 34, 1010–1018.

Tautz D. (1989): Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research, 17, 6463–6471.

Wang G.C., Dou X.H. (2005): The idea of development on future waterfowl in partly city of china. China Poultry, 27, 36–43.

Williams C.L., Homan H.J., Johnston J.J., Linz G.M. (2004): Microsatellite variation in red-winged blackbirds (*Agelaius phoeniceus*). Biochemical Genetics, 42, 35–41.

Williams C.L., Brust R.C., Fendley T.T., Tiller Jr. G.R., Rhodes Jr. O.E. (2005): A comparison of hybridization between mottled ducks (*Anas fulvigula*) and Mallards (*A. platyrhynchos*) in Florida and South Carolina using Microsatellite DNA Analysis. Conservation Genetics, 6, 445–453.

Wu Y., Hou S.S., Liu X.L., Huang W., Jiang F. (2006): Genetic relationships among six ducks populations using four microsatellite markers. Acta Veterinaria et Zootechnica Sinica, 37, 389–395.

Xiao T.F., Zheng X.C., Wu Q.E. (2004): Genetic diversity of Fujian local duck populations indifferent ecological type. Chinese Journal of Applied Ecology, 15, 879–882.

Yan F.H., Zuo Z.H., Chen M., Song Y.Q., Lv L.J., Chen Y.X. (2005): Analysis of genetic diversity and relationship among some Chinese domestic ducks and wild ducks. Journal of Xiamen University (Natural Science), 44, 729–733.

Zhao X.T., Duan X.J., Yang T.G., Chen Z.Y., Gong D.Q., Zhang J., Gao Y.S. (2005): Analysis of genetic variation in duck breeds using microsatellite markers. Journal of Yangzhou University (Agricultural and Life Science Edition), 26, 34–38.

Zheng W.Z., Chen Y.X., Wu L. (1995): Restriction analysis on mtDNA from Anas platyrhynchos. Journal of Xiamen University (Natural Science), 34, 614–618.

Zuo Z.H., Chen Y.X., Lv L.J. (2004): Studies on genetic diversity and molecular marker of Jinding Ducks. Journal of Xiamen University (Natural Science), 43, 256–259.

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