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Evaluation of genetic diversity and relationships within and between two breeds of duck based on microsatellite markers

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Abstract

The genetic diversity of two natural populations (M, N) of Beijing duck (*Anas platyrhynchos*) and 11 artificially selected lines of Beijing duck (A, B, E–L, O) from China Gold Star Duck Production Ltd., along with two Cherry Valley duck lines (C and D) from the British Cherry Valley Livestock Division, was evaluated using 18 microsatellite markers covering 16 linkage groups. A phylogenetic tree of the 15 populations of duck, formed of four main branches, was constructed from Nei's D_A genetic distance. The mean genetic differentiation index (F_{ST}) in all loci, Nei's standard genetic distance (Ds), and the genetic distance D_A between the Beijing duck and the Cherry Valley duck were 0.075, 0.143 and 0.142, respectively. These results demonstrated a high degree of genetic similarity between the two breeds and supported the hypothesis that the Cherry Valley duck was derived from the Beijing duck. The F_{ST} matrix of seven clusters of Beijing duck suggested that the efficiency of selection was not significant to some extent and should be supplemented by marker-assisted selection.

Keywords: Duck; Microsatellite marker; Genetic diversity; Genetic distance

1. Introduction

The diversity of the genetic resource is very important to the development and sustainability of livestock and poultry production, and the evaluation of genetic diversity provides useful information to maintain and exploit genetic resources. Genetic relationships between populations can be estimated by measuring the differences in the allele number and frequency at polymorphic loci, which are very important for our understanding of the history of species and even of evolutionary processes [1]. The ability to evaluate genetic diversity and the relationships within and between populations using microsatellite markers has resulted from the rapid development of molecular genetics in recent years, and microsatellite markers have been extensively applied in the estimation of genetic diversity in animals [2–5].

The duck is of great biological interest to genetic research on molecular evolution [6] and immunology [7–9], and it is also an important food source. Compared with that in species such as humans, mice, and chickens, molecular genetic research in the duck is limited. Since Fields and Scribner [10] first isolated and characterized novel microsatellite loci from waterfowl (*Somateria fischeri*), more and more microsatellite markers for ducks have been developed and reported [11–15]. The isolated microsatellite loci, together with a recently developed genetic map of the duck, have enabled studies on the biodiversity and genetic relationships of duck breeds [6,16–18,29].

There are two main commercial duck breeds currently used for the production of meat for the Chinese market.

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One is the Beijing (BJ) duck (*Anas platyrhynchos*), which is world-famous for its roasting taste and which was exported to the United States and Britain from China for use in the development of new meat breeds in the early part of the last century. The other is the Cherry Valley (CV) duck, which is imported into China from the British Cherry Valley Livestock Division. The CV duck produces high quality meat, develops strong immunity, and is popular in the international market. It is supposed to be derived from a cross between the Chinese BJ duck and the Aylesbury duck.

In this study, the genetic diversity of the BJ duck and the CV duck was evaluated in two natural populations of BJ duck, 11 BJ duck lines, and two CV duck lines using 18 microsatellite markers covering 16 linkage groups [15]. This evaluation could be informative in the assessment of the effect of artificial selection and the understanding of the genetic mechanism behind the breeding selection program.

2. Materials and methods

2.1. Experimental populations

Forty individual ducks were collected randomly from each of 13 populations of BJ duck, including two natural populations (M and N) used for breed preservation purposes and 11 lines (A, B, E–L and O) from China Gold Star Duck Production Ltd. (Beijing, China), and from two lines of CV ducks (C and D) which were introduced from the British Cherry Valley Livestock Division and are now maintained at Gold Star Duck Production Ltd. All 11 BJ duck lines had been selected for the traits of body weight and feed conversion ratio from 9 (line I) to 22 (lines E and H) generations. BJ duck lines E and F were mainly selected for egg production, and lines I and J were mainly selected for body weight in order to meet the standard for a good meat-type duck. CV duck lines and the other BJ duck lines were all selected for their high rate of body weight gain and feed conversion with different selection pressures.

2.2. DNA isolation

Blood samples were taken from the ulnar vein of each duck and stored in 1.5 ml tubes containing EDTA as an anticoagulant at −20 °C before extraction. A routine phenol/chloroform extraction method was used to extract and purify the duck genomic DNA. The DNA was quantified using agarose gel electrophoresis, and the concentration of DNA was estimated by comparison with standard molecular markers.

2.3. PCR amplification

Eighteen polymorphic microsatellite markers distributed in 16 linkage groups (Table 1) were chosen according to the genetic map of the duck [15]. Among the microsatellite markers, seven were the flanking markers of quantitative trait loci affecting body weight, conformation, or carcass traits in BJ ducks [19,20]. The primer sequences of these microsatellite markers have been previously published [14,15]. One primer from each pair was labeled with either 6-FAM or HEX fluorescent dye at the 5′ end (AuGCT Biotechnology, Beijing, China).

First, the annealing temperature of the microsatellite primers was determined using an authorized Thermal

Table 1
Basic information of 18 microsatellite loci used in the study.

Locus[a]	Linkage group[b]	Chromosome	Affecting traits[c]	Number of alleles	Range of allele size (bp)
CAUD111	CAU5	5	–	10	64–82
Bcau3	CAU17	3	–	6	160–170
CAUD001	CAU11	Micro	–	9	315–333
CAUD039	CAU1	1	–	8	196–212
CAUD050	CAU4	4	BW	14	288–324
CAUD026	–	–	–	9	137–154
CAUD044	CAU10	10	NL	9	135–152
CAUD113	CAU13	–	ST	6	211–246
CAUD040	CAU12	–	BW, SG	14	263–13
CAUD041	CAU14	–	–	3	113–124
CAUD091	CAU3	3	–	6	170–186
CAUD016	–	–	–	11	175–219
CAUD056	CAU6	–	HTW, BW	8	119–293
CAUD060	CAU2	2	CPW	20	224–325
CAUD004	CAU16	Micro	–	9	194–223
CAUD038	CAU9	9	–	13	211–304
AMU060	CAU19	–	–	7	179–191
CAUD115	CAU7	–	ST	31	299–495
All loci				10.7	192–241

[a] See Ref. [15] for details.
[b] Microsatellite loci evenly distributed in 16 linkage groups in the duck genetic map [15].
[c] Some microsatellite markers were flanking markers of quantitative trait loci affecting 7-week production traits in BJ ducks. BW = body weight, NL = neck length, ST = fat thickness in tails, SG = girth of shank, HTW = heart weight, and CPW = crop weight [19,20].

Cycler (Eppendorf, Hamburg, Germany). The PCR amplification was conducted in a total volume of 15 μl, containing approximately 40 ng duck DNA, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.3), 1 mM tetramethylammonium chloride, 0.1% Triton X-100, 0.01% gelatin, 200 mM dNTPs, 2 pmol of each primer, and 2.5 units Taq polymerase. The PCR procedure was performed using the GeneAmp PCR System 9600 and 9700 (ABI, USA). The PCR reaction conditions comprised denaturation at 94 °C for 5 min, then 40 cycles of denaturation at 94 °C for 30 s, annealing at the primer-specific temperature (50–66 °C) for 30 s, and extension at 72 °C for 35 s followed by a final 10 min extension step at 72 °C.

2.4. Microsatellite genotyping

The PCR products of two to four markers from each individual sample were diluted 5–20 times and run in the same lane (multi-run) of the gel if their fluorescent dyes or sizes were sufficiently different (>60 bp). A mixture of 1 μl of diluted multiplex PCR product, 10 μl of deionized formamide (Amresco), and 0.2 μl of Genescan-350 ROX or Genescan-500 ROX (ABI) internal standard was run on a 3100 pop-4 (ABI) gel using a 3100 genetic analyzer (ABI). The fragment sizes of the PCR products were analyzed using the GENESCAN 3.7 and GENEMAPPER 1.1 software (ABI). Although the genotypes were scored automatically by the GENEMAPPER 1.1 software after the panel and bin had been defined correctly, all individual genotypes were checked manually twice to eliminate errors.

2.5. Statistical analysis

The genetic diversity of each duck population was evaluated by calculating the number of alleles per microsatellite locus, the observed heterozygosity (H_O), the expected heterozygosity (H_E), and the polymorphic information content (PIC) using the CERVUS program version 2.0 [21]. *F*-statistics, including the fixation coefficient of an individual within a subpopulation (F_{IS}), the fixation coefficient of an individual within all populations (F_{IT}), and the fixation coefficient of a subpopulation within all populations (F_{ST}) per locus between the BJ duck (population MN) and the CV duck (line CD) were calculated using GENEPOP version 3.4 [22]; values of F_{ST} between each pair of the seven closely related clusters of BJ ducks were also calculated. Nei's standard genetic distance (*Ds*) and the D_A genetic distance between the two breeds and among the 11 lines of BJ duck were calculated using the DISPAN computer program [23] according to Nei's methods [24,25].

$$Ds = -\ln I = -\ln \frac{JXY}{\sqrt{JXJY}}$$

$$D_A = 1 - \frac{1}{r}\sum_{j}^{r}\sum_{i}^{mj}\sqrt{XijYij}$$

Based on the matrix of the D_A genetic distances of all 15 duck populations, a phylogenetic tree was constructed by the unweighted pair group method with arithmetic mean (UPGMA) using the NEIGHBOR program in the PHYLIP software package [26], followed by the bootstrapping option with 1000 resamplings; the tree was edited using the TREEVIEW computer program [27].

3. Results and discussion

3.1. Genetic diversity

In total, 192 alleles were detected across all the duck populations for the 18 examined microsatellite loci. The allele number of the examined markers ranged from 3 (CAUD041) to 31 (CAUD115), with an average of 10.7 in the investigated samples. The allele size differences ranged from 10 bp (160–170 bp) at locus Bcau3 to 196 bp (299–495 bp) at CAUD115, with an average of 49 bp (192–241 bp) per locus, summarized in Table 1. Not all of the loci were under Hardy–Weinburg (H–W) equilibrium tested by CERVUS 2.0 in each duck population, probably owing to the limited number of samples selected or perhaps the consequences of artificial selection.

Table 2 shows the comparison of the parameters of genetic diversity, including allele number, H_O, H_E, and PIC, and the *F*-statistics (F_{IS}, F_{ST} and F_{IT}) for each locus between lines MN and CD, which represent the BJ duck and the CV duck, respectively. A total of 150 alleles, with a range from 2 (CAUD041) to 24 (CAUD115), the average being 8.33, were detected in the BJ duck breed, while 139 alleles, with a range from 2 (CAUD041) to 28 (CAUD115), the average being 7.72, were detected in the CV duck breed. All the alleles found in the CV duck also existed in all 13 populations of BJ duck. Therefore, no alleles specific to CV ducks were found in this study. According to the data shown in Table 2, differences in the genetic parameters at each locus between the two breeds were not significant, except for locus Bcau3 ($P < 0.05$). This significant difference in Bcau3 might be attributed to the selection or recombination of the chromosomal fragment; however, the relationship between this region and production traits such as body weight is unclear. The mean H_O, H_E, and PIC values per locus were 0.514 (similar to the result reported in Ref. [18], approximately 0.530), 0.604 and 0.573 in the BJ duck, and 0.527, 0.627 and 0.590 in the CV duck, respectively. This result suggests that most of the markers are highly polymorphic, and can, therefore, provide reliable results for genetic estimation. The mean H_O and PIC were greater than 0.5 [14], and the average of the H_O and PIC in the BJ duck and the CV duck were similar ($P > 0.05$).

The mean F_{IS} and F_{IT} of the two duck breeds were 0.158 and 0.221, respectively. The values of the genetic differentiation index F_{ST} ranged from −0.003 at CAUD041 to 0.479 at CAUD001, with an average of 0.075 per locus. The mean F_{ST} value of 0.075 between

Table 2
Comparison of genetic diversity parameters[a] and *F*-statistics per locus between BJ duck (MN) and CV duck (CD).

Loci	BJ duck				CV duck				*F*-Statistics[b]		
	Number of alleles	H_O	H_E	PIC	Number of alleles	H_O	H_E	PIC	F_{IS}	F_{ST}	F_{IT}
CAUD111	7	0.775	0.761	0.713	8	0.592	0.839	0.813	0.195	0.088	0.266
Bcau3	3	0.021	0.101	0.096	4	0.230	0.439	0.403	0.521	0.090	0.564
CAUD001	5	0.270	0.444	0.422	6	0.243	0.440	0.414	0.421	0.479	0.698
CAUD039	5	0.533	0.503	0.419	4	0.449	0.572	0.494	0.090	0.002	0.091
CAUD050	10	0.563	0.715	0.677	8	0.743	0.797	0.762	0.135	0.111	0.231
CAUD026	7	0.618	0.705	0.652	5	0.671	0.736	0.684	0.106	0.030	0.133
CAUD044	7	0.456	0.538	0.502	4	0.333	0.340	0.312	0.098	0.024	0.120
CAUD113	6	0.458	0.454	0.422	4	0.403	0.641	0.570	0.215	0.072	0.272
CAUD040	10	0.897	0.856	0.833	14	0.973	0.925	0.913	−0.051	0.022	−0.027
CAUD041	2	0.051	0.050	0.048	2	0.078	0.075	0.072	−0.028	−0.003	−0.032
CAUD091	5	0.813	0.780	0.739	5	0.776	0.799	0.762	−0.007	0.020	0.013
CAUD016	9	0.690	0.824	0.794	6	0.694	0.653	0.578	0.060	0.050	0.106
CAUD056	8	0.164	0.287	0.280	4	0.192	0.256	0.243	0.338	0.011	0.346
CAUD060	20	0.825	0.942	0.931	15	0.767	0.910	0.896	0.143	0.033	0.170
CAUD004	6	0.421	0.562	0.529	6	0.506	0.729	0.680	0.283	0.085	0.344
CAUD038	10	0.786	0.783	0.750	10	0.573	0.577	0.541	0.001	0.050	0.051
AMU060	6	0.449	0.629	0.577	6	0.620	0.616	0.539	0.149	0.007	0.155
CAUD115	24	0.459	0.943	0.933	28	0.641	0.948	0.939	0.417	0.018	0.427
All loci	8.33	0.514	0.604	0.573	7.72	0.527	0.627	0.590	0.158	0.075	0.221

[a] H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content. Calculated by CERVUS version 2.0 program [21].

[b] F_{IS} and F_{IT}: deviation indexes from Hardy–Weinberg equilibrium proportions within subpopulation and between populations, respectively; F_{ST}: genetic differentiation index between populations. All were calculated by the GENEPOP version 3.4 program [22].

the two breeds indicated that only 7.5% of the genetic variation was between the populations, while 92.5% was within the populations. The great similarity in the genetic variability of the two groups suggests that the CV duck is indeed a hybrid of the BJ duck and the Aylesbury duck. The seven microsatellite markers that were linked with QTL for body weight, conformation or carcass traits in duck (Table 1) showed no significant difference in allele frequency between the BJ and the CV breeds. The wide 95% confidence interval between these markers and the QTLs might have led to this result. However, the genetic variation between the BJ and the CV duck will be clarified in the future following the use of position-based cloning of economically important genes, elucidation of the whole genome sequence, and the exploitation of existing comparative genomic information.

3.2. Phylogenetic analysis

The matrix of Nei's D_A genetic distances between each pair of the 15 duck populations is shown in Table 3. The D_A value ranged from 0.046 (between BJ duck lines A and B) to 0.258 (between BJ duck lines B and I, and BJ duck lines F and M). These were clearly lower than those measured by Liu et al. [6]: in that study, the D_A ranged from 0.089 to 0.440 among 26 lines of Chinese domestic ducks, which included a BJ duck line. They used 11 microsatellite markers, four of which were also used in this study. Nei's standard genetic distance (D_S) and the D_A between the BJ and CV duck breeds (population MN vs. line CD) were calculated, respectively, to be 0.143 and 0.142, suggesting that the relationship between the two breeds is even closer than that between the BJ duck and many other Chinese domestic breeds. Fig. 1 shows a UPGMA tree constructed from the D_A matrix of the 13 populations of BJ duck and the two CV duck lines; the tree is formed of four main branches. The CV duck lines C and D are clustered together and are genetically close to the BJ duck clusters AB, GH and O; this group formed the biggest branch. The BJ duck lines E and F and the natural populations M and N were clustered apart from the other lines, forming another two branches. The BJ duck lines, I and J, and K and L, were clustered in pairs and then grouped to form the last branch.

The phylogenetic tree fitted well with the true breeding history of the duck lines. The CV duck lines C and D and the natural populations of BJ duck (M and N) were appropriately clustered together. As mentioned earlier, of the commercial lines of BJ duck, E and F were selected for high egg production, and lines I and J were mainly selected for high body weight by Gold Star Duck Production Ltd. All were clustered in the phylogenetic tree.

The calculated D_A of 0.142 between the two breeds is similar to the result reported in Ref. [17]. Table 3 shows that the D_S value reported in the current study (0.143) is significantly lower than the D_S of 0.598 between the BJ duck and the French Muscovy duck (*Cairina moschata*) [18] and is even lower than those between the BJ duck and most other Chinese domestic breeds of duck [6]. Both this result and the phylogenetic tree suggest a close genetic relationship between the BJ duck and the CV duck (Fig. 1).

Table 3
Nei's D_A genetic distances matrix between each pair of 15 duck populations.

Populations	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
A	–														
B	0.046	–													
C	0.118	0.129	–												
D	0.116	0.128	0.048	–											
E	0.225	0.234	0.210	0.230	–										
F	0.234	0.243	0.218	0.231	0.057	–									
G	0.148	0.135	0.137	0.148	0.179	0.180	–								
H	0.125	0.123	0.116	0.130	0.175	0.178	0.063	–							
I	0.255	0.258	0.241	0.249	0.213	0.233	0.197	0.203	–						
J	0.236	0.236	0.241	0.246	0.205	0.219	0.197	0.194	0.057	–					
K	0.218	0.218	0.182	0.208	0.193	0.184	0.147	0.159	0.122	0.122	–				
L	0.215	0.216	0.188	0.207	0.167	0.173	0.156	0.159	0.113	0.116	0.082	–			
M	0.193	0.187	0.178	0.199	0.252	0.258	0.166	0.155	0.233	0.225	0.199	0.211	–		
N	0.164	0.162	0.146	0.162	0.236	0.248	0.140	0.139	0.197	0.191	0.186	0.187	0.081	–	
O	0.157	0.160	0.117	0.133	0.191	0.197	0.128	0.121	0.238	0.246	0.174	0.184	0.205	0.182	–

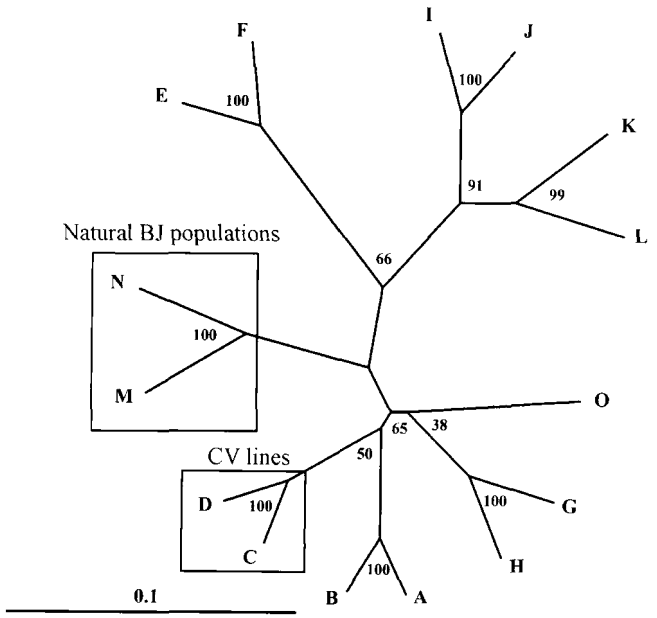

Fig. 1. An unweighted pair group method with arithmetic mean (UPGMA) tree for 13 BJ duck populations and 2 CV duck lines based on Nei's D_A distance. Numbers at the nodes represent the percentage of bootstrap values from 1000 replications of resampled loci.

3.3. Genetic relationships among BJ duck lines

The phylogenetic tree in Fig. 1 suggests that BJ duck populations are clearly divided into seven closely related clusters. The F_{ST} statistics between each pair of the seven clusters were calculated and are shown in Table 4. Values of the F_{ST} ranged from 0.0312 (between clusters IJ and KL) to 0.1109 (between clusters IJ and O), with an average of 0.0624. Values of F_{ST} between the natural populations of BJ duck, MN, and the commercial lines ranged from 0.0391 (with GH) to 0.0824 (with EF), with a mean and standard deviation of 0.0608 ± 0.0175.

Current breeding strategies for commercial poultry concentrate on specialized production lines that are derived by intense selection from a few breeds, and on very large populations with great genetic uniformity in the traits under selection [28]. The data in Table 4 indicate that long periods of artificial selection do have some impact on breeding, but not a significant effect to some extent, because after 9 to 22 generations of traditional selection, the total genetic differentiation among natural populations and selected lines is only 3.91–8.24%. This may be a good reason for the introduction of marker-assisted selection (MAS) to breeding programs, with the aim of accelerating genetic improvement at the molecular level. More markers that are linked to economic traits are needed to supplement our selection of waterfowl breeds and evaluate the effect of selection. Hopefully the information obtained in this study will cast some light on ways to develop better breeding strategies for various duck lines, in order to protect and preserve local breed resources, on the basis of the rapid development of functional genomics in waterfowl and the sequencing of the

Table 4
Genetic differentiation index (F_{ST}) statistic matrix between each pair of 7 BJ duck clusters.

Clusters	AB	EF	GH	IJ	KL	MN	O	Mean ± SD[a]
AB	–	0.0831	0.0407	0.1061	0.0859	0.0613	0.0553	0.0721 ± 0.0239
EF	0.0831	–	0.0401	0.0763	0.0419	0.0824	0.0547	0.0631 ± 0.0200
GH	0.0407	0.0401	–	0.0665	0.0403	0.0391	0.0366	0.0439 ± 0.0111
IJ	0.1061	0.0763	0.0665	–	0.0312	0.0556	0.1109	0.0744 ± 0.0304
KL	0.0859	0.0419	0.0403	0.0312	–	0.0465	0.0760	0.0536 ± 0.0220
MN	0.0613	0.0824	0.0391	0.0556	0.0465	–	0.0799	0.0608 ± 0.0175
O	0.0553	0.0547	0.0366	0.1109	0.0760	0.0799	–	0.0689 ± 0.0259

[a] SD = standard deviation.

whole genome of the duck, which is expected in the near future.

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