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Paternity assessment: application on estimation of breeding value in body-weight at first egg trait of egg-laying duck (*Anas platyrhynchos*)

Jindong Ren · Lizhi Lu · Xiaolin Liu · Zhengrong Tao · Congcong Zhang · Deqian Wang · Junda Shen · Weimeng Liu · Yong Tian · Zhigang Zhu

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Abstract Paternity index was analyzed using five microsatellite loci among Chinese egg-laying ducks (*Anas platyrhynchos*). Based on the paternity relationship that was identified by paternity index analysis, the estimated breeding value (EBV) was calculated using BLUP (best linear unbiased predictor) method. Body weight at first egg (BWF) is the only considered trait in this study. In total, 12 sires, 31 dams and 77 daughters were involved in the EBV calculation. The results demonstrated that five microsatellite loci's polymorphism information content (PIC) ranged from 0.795 in locus AY493338 to 0.957 in locus AY493264 with average 0.899; the parent–offspring relationships were built by these microsatellites' genotype, 12 families of half sibling and 2 families of full sibling were involved, and the relationship error is smaller than 10^{-7}. The EBV results suggest that the average EBV was significantly higher in females (average EBV is 10.234 and 0.1045 for mother and daughter, respectively) than males (average EBV is just −26.44). The EBV results on BWF were in good agreement with the principle of GH (growth hormone) expression in poultry. These results show that paternity analyses of Chinese egg-laying ducks were basically resolved using the five microsatellite loci selected. The paternity relationships can apply in Chinese egg-laying duck breeding to quicken the improvement of genetic progress.

Keywords Microsatellite loci · Paternity · *Anas platyrhynchos* · Estimated breeding value

Abbreviations

EBV	Estimated breeding value
BWF	Body weight at first egg
BLUP	Best linear unbiased predictor
GH	Growth hormone
PAGE	Poli-acrylamide gel electrophoresis

Introduction

Estimated breeding value (EBV) is an estimate of the ability of an individual to produce superior offspring; based on one or more measurements of performance, using phenotypic values, taken on the animal itself or, more commonly, on a number of its relatives [1]. EBV is a useful tool to quicken the progress of breeding in animals [2–4]. Although EBV has so many advantages in breeding work, it was difficult to apply it in breeding of poultry because there is little available data on parentage, especially in Chinese egg-laying ducks. Recently, studies on paternity and maternity greatly benefit from the use of microsatellite makers. These will become available on Chinese egg-laying duck. This report concerns the isolation of five highly

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J. Ren · X. Liu (✉)
College of Animal Science and Technology, Northwest A & F University, 712100 Yangling, Shaanxi, China
e-mail: liuxiaolin@nwsuaf.edu.cn

J. Ren · L. Lu · Z. Tao · C. Zhang · D. Wang · J. Shen · W. Liu · Y. Tian
Zhejiang Province Academic Agriculture Science, Animal Husbandry and Veterinary Institution, 310021 Hangzhou city, China

C. Zhang · W. Liu
Animal Science Department, Nanjing Agriculture University, 210095 Nanjing, China

Z. Zhu
Shaoxing Duck Original Farm, 312000 Shaoxing, China

polymorphic microsatellite makers, which were developed particularly for paternity analysis to calculate the EBV in Chinese egg-laying ducks.

Microsatellites or simple sequence repeats (SSR) are tandem repeated motifs of 1–6 bases found in all organism genomes and are present in both coding and non-coding regions. Because of microsatellites' existence in organism genomes, they become the efficient tool being widely employed in genetic research, such as genetic mapping, population genetics evolutionary studies, investigation of social systems [5–8]. Because of the hereditary characteristics of high polymorphism and segregation, they also are used for paternity assessment in wild mallards, spiders, Chinese Holstein, pigs, goats [9–13]. Their precisions of identification can reach more than 90%. Ellegren has reported that five microsatellite loci, each locus has more than six alleles, can make the precision of identifying reach 98% [14]. Based on this theory, this study has chosen five unlinked highly polymorphic microsatellite loci from mapped duck genetic maps [15] to assess paternity in Chinese egg-laying ducks.

Many methods of estimating breeding value are often used in breeding research such as index of individual phenotype, full- and half-sib family averages and best linear unbiased prediction (BLUP) [16]. In 1994 Reverter has reported a method *R* variance components applying to simple breeding value model to estimate breeding value [17]. Among these methods of estimate breeding value, however, the BLUP is more convincing and precise. Under the same pedigree error, BLUP has an advantage over any other methods [16].

The Chinese egg-laying duck is a famous duck breed for egg production in China. Because of its excellent egg-laying performance (lay more then 300 eggs in the first laying cycle). It recently becomes the target of growing interest in heritability of laying performance and hereditary characteristics for several reasons. First, the Chinese egg-laying duck is one of the highest egg producing breeds in the first laying cycle, but we have no idea about how this egg producing genetic trait is expression in the genetic. The second, better understanding of the genetics of egg production can optimize selection strategy and management within the selection programs. Finally, to compare the hereditary characteristics between egg-laying ducks and laying chickens by finding the key locus responsible for the domestication of egg production [18]. The parentage–offspring relationships is an exhaustire work using traditional ways (cage management and artificial insemination or only cage management), and moreover, cage management affects egg production in ducks. Considering these conditions, the microsatellite marker assessment for paternity and maternity was chosen in calculating of EVB to get a precise result.

Materials and methods

Resource population

In collaboration with a company named Original Shaoxing egg-laying Duck Production (Shaoxing city, China), an experimental population with 100 female and 20 male ducks, as parental population, and 77 daughters. A routine phenol/chloroform extraction method was used to extract and purify the duck genomic DNA. The DNA was quantified using agarose gel electrophoresis. The DNA concentration was estimated by ultraviolet (UV) spectrograph (the method's detail can be seen on http://bitesizebio.com/2007/08/22/dna-concentration-purity/ website).

Data collection

The body weight at first egg (BWF) was recorded when 5% the egg-laying ducks began laying. Descriptive statistics of the trait is summarized in Table 1.

PCR amplification

The annealing temperature of microsatellite primers was from Genbank (the detail information in Table 2). DNA amplification was performed in total volume of 20 μl containing 2.5 U Taq DNA polymerase (Takara, Dalian, China), 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of each primer and 50 ng duck DNA. The PCR reaction conditions were denatured for 5 min at 95°C, followed by 94°C for 40 s, 56.5–60°C for 30 s, and 72°C for 30 s, with a final 10 min elongation step at 72°C. The PCR products' electrophoresis was carried out for 5–7 h at the voltage of 100–200 V on 8% PAGE (poli-acrylamide gel electrophoresis). PCR products were visualized following silver staining.

Microsatellite genotyping

The length of microsatellite alleles were calculated by using the software Quantity One and Bio-Rad (Alpha

Table 1 Descriptive statistics for BWF of parental population and offspring population

	Parental population		Offspring population	Total
	Male parent	Female parent	Female	
# recorded	20	100	77	197
Mean	1,428.0	1,575	1,582.2	1,494.1
SD	132.9	146.5	116.5	156.9
CV(%)	9.3	9.3	8.3	10.5

Table 2 Characterization of five microsatellite loci from Anas platyrhynchos

Accession	Repeat type	Primer sequence (5′-3′)	Annealing temperature	No. of alleles	Size-range(bp)
AY493338	Tandem	F: ACAGCTTCAGCAGACTTAGA R: GCAGAAAGTGTATTAAGGAAG	60.0	12	194–262
AY493294	Tandem	F: TTAGTAAACTCTTGCCATCT R: TGTAGTTTAGTTGCTGGATA	58.7	23	121–490
AY493301	Tandem	F: TGGTGCGATGAGCTGAGAT R: GCTTTAGTTTTTCAATTAGGTG	56.5	37	64–490
AY493264	Tandem	F: CTGGAACAAGGAATTAGAAGT R: TATGTGGTGCTGGGCTGTT	58.8	26	121–287
AY493285	Tandem	F: TGTGTAACCCTGATAGACTGA R: TCCCACCCCAAACCCTGC	58.7	29	239–402

Technologies Ltd, Laren, Co. Antrim, Ireland) matched with the DNA ladder 100 (TaKaRa Biotechnology Dalian Co., Ltd).

Analysis of microsatellites' diversity and paternity index

The genotyping data were formatted in Excel worksheets. The allele frequency, effective number of alleles, the PIC (polymorphic information content) and heterozygosity to evaluate the diversity of these microsatellite loci were calculated using SAS software (statistics analysis system). exclusion probability (PE) also was calculated when the genotype of one parent is absent, as follow:

$$PE = 1 - 4\sum_{i=1}^{n} p_i^2 + 2\left(\sum_{i=1}^{n} p_i^2\right)^2 + 4\sum_{i=1}^{n} p_i^3 - 3\sum_{i=1}^{n} p_i^4$$

where p_i is the allele frequency.

Paternity index is the likelihood ration (L). It was calculated by followed formula:

$$\frac{p_{ij}(\text{offspring})}{p_i(\text{sire}) \times p_j(\text{dam}) + p_i(\text{dam}) \times p_j(\text{sire})} \quad (i \text{ or } j = 1 \text{ to } n)$$

where p_{ij}(offspring) is the probability of genotype ij in offspring population, when putative father or mother mated with random dams or sires; the denominator is the probability of genotype ij in offspring population under random mating. The cumulated paternity index is,

$$L = \prod_{n} L_i$$

where n is the number of genotype in offspring population. In general, it is also denoted by the logarithm, in this case, LOD score. When the LOD>3, we can say that the assumed sire or dam is the father or mother with 99.9% accuracy.

Method of analysis

The EBV was calculated by animal model BLUP method following Henderson's classical mixed-model equations (MME), the animal model as followed linear model,

$$y_{ij} = h_i + a_j + e_{ij}$$

where y_{ij} is the phenotypic record of the jth duck in the ith generation effect ($i = 0, 1$); h_i is the fixed effect of the ith generation; a_j is the EBV of the jth duck; e_{ij} is the random environmental effect associated with record y_{ij}. The model above is expressed in matrix notation as followed:

$$y = Xh + Za + e$$

where y is a vector of the observation of trait; h is a vector of fixed effects; a is a vector of random individual EBV; e is a vector with random residual effect, and X and Z are the incidence matrices relating the observations to the respective fixed and random effects. The assumed variance–covariance structure is:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1}k \end{bmatrix} \begin{bmatrix} h \\ a \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

according to Henderson's mixed-model equations (MME), the matrix A contains additive genetic relationships among all ducks σ_a^2 and σ_e^2 are additive genetic and residual variances, respectively; where $k = \frac{\sigma_e^2}{\sigma_a^2} = \frac{1-h^2}{h^2}$, and h^2 is heritability of the trait. The heritability (h^2) was estimated by the method of genetic correlation between parental and offspring using followed formula:

$$h^2 = 2r_{W(O,D)}$$

where $r_{W(O,D)}$ is the coefficient of genetic correlation between parental and offspring.

Results

Polymorphism of microsatellite loci

All the five microsatellite loci were detected in parental population and offspring population, 197 individuals in total. Each microsatellite locus was highly polymorphic, and average effective number of alleles can reach to 22.778, the maximum one is 35.094 in AY493301; heterozygosity (*H*) is 0.948; PIC is 0.899; PE is 0.6272 for average value. All of the information details were listed in Table 3. In this study, high polymorphism microsatellite loci are selected for the purpose of precise assessment paternity, and low polymorphism microsatellite loci were not taken account, such as, AY493342 which just has a few alleles and each allele is not easy to distinguish. Fig. 1 shows its part of genotype PAGE (middle of the PAGE is 100 Ladder Maker).

Component analysis and test of normality

According to generation and sex, all individuals in all parent–offspring relationships were grouped. And then, their data were tested for normal distribution and analyzed of T-TEST between each both of groups. The result of T-TEST was showed in Table 4.

The means of BWF, *T* value and *P* value were listed in Table 4. The largest mean of BWF and second largest mean of BWF were 1,629.6 and 1,582.2 g, respectively. They come from the parental female group and the progeny group, respectively. The lowest mean of BWF is 1,452.6 from the parental male. The T-TEST results indicate that the BWF score is significantly higher in the parental female group and the progeny group than in the parental male group. There is no significant difference between the parental female group and the parental male group.

Table 3 Polymorphic information of five microsatellite loci and exclusion probability

Accession no. dsadsasdsad	Ne	*H*	PIC	PE
AY493294	16.208	0.938	0.935	0.571
AY493264	24.142	0.959	0.957	0.674
AY493285	27.262	0.963	0.919	0.705
AY493301	35.094	0.972	0.89	0.780
AY493338	11.185	0.911	0.795	0.406
Average	22.778	0.948	0.899	0.6272

The normal distribution fitting degree can reach greater than 80%. This result indicates that the collection of the sample is large enough. The normal frequency distributions in different groups are shown in Figs. 2 and 3.

Assessment for paternity

According to the genotypes of individuals, the parentage–offspring relationships were first built. The paternity index was calculated between every individual and assumed father/mother. When the LOD<3, the assumed sire and dam was rejected from our study. After paternity assessment, 120 individuals were involved in parent–offspring relationship. In total, 12 sires, 31 dams and 77 daughters were involved in these parentage–offspring relationships. The other individuals and their phenotypes were removed from this experiment, because they haven't found relationships with other individuals.

Heritability and EBV

The heritability of BWF equals 0.207 and the coefficient, *k*, is 3.825 in Henderson's MME, and then a formula substitution was mach to get the EBV of individuals. The average EBV using T-TEST was determinate by comparing each pair of groups. The result was given in Table 5.

Table 5 was shows means, *T* value of EBV of different groups and *P* value. The results show that EBV of the paternal male group is significantly lower than other groups, and no evidence indicates that EBV of the paternal female group is higher than progeny group. This result supports that the genetic of BWF follows the law of maternal inheritance.

Discussion

Polymorphism of microsatellite loci

All of these microsatellite markers have more allele high polymorphism than indicated in other research. The average number is >25. Among these microsatellite loci, AY493301 has the maximum number of alleles, 37 alleles, (the reason caused maximum number of alleles is the repeat sequence which involved (TTTCCCTCTTTC)18, A5, A3, G3 and C3 in it. The large repeated sequences have not shown in other microsatellite); whereas, AY493338 has the minimum number of alleles, 12 alleles, and the average effective number of alleles is 22. The Yinhua Huang genetic map report shows that these markers PIC value range from 0.3–0.9, with an average under the value 0.5 [15]. In this study, AY493085 has the maximum PIC value of 0.963; the average PIC value is 0.899, and the

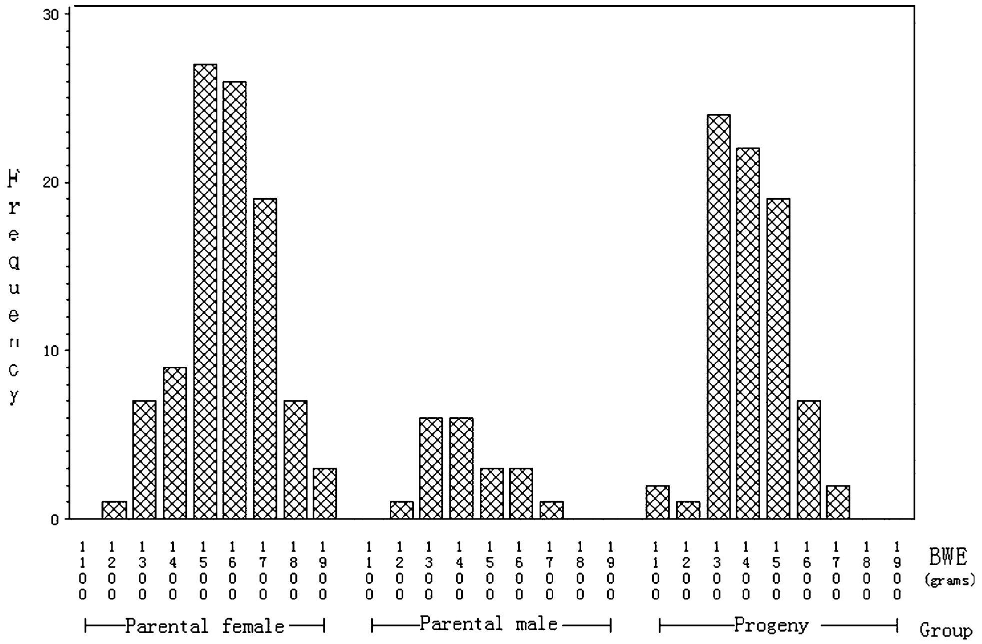

Fig. 1 The frequency of BWF in different groups

Table 4 The result of Students' test of BWF in different group

Group	Body weight at first egg		
	Mean (g)	*T* value	*P*
Parental male	1,452.6	3.37	0.0017
Parental female	1,629.6		
Parental male	1,452.6	2.807	0.0010
Progeny	1,582.2		
Parental female	1,629.6	1.606	0.0556
Progeny	1,582.2		

average heterozygosity is 0.948. Compared to the other studies [19–21], our results show higher polymorphism, which may be caused by the following reasons: (1) the phenomenon of hybridization is so common among Chinese egg-laying duck strains. This tends to make them heterozigotes; (2) in this study, highly polymorphic microsatellite loci were selected to raise the precision of assessment paternity. So low polymorphism microsatellite loci were not taken into account, such as, AY493342 (part of its PAGEs show in Fig. 3), which just has a few alleles and each allele is not easy to distinguish.

These parameters of PIC, heterozygosity and other polymorphism parameters are the statistical parameters that reflect the polymorphic level, and several studies showed that there is a strong interaction between each pair. In our study, the correlation coefficient between the effective number of alleles and heterozygosity is 0.952, while the correlation coefficient of heterozygosity and effective numbers of alleles with PIC were lower, 0.682 and 0.434, respectively. Therefore, as for PIC and heterozygosity, either of them can be used to assess the polymorphism in each locus, whereas, the exclusion probability and either of PIC and heterozygosity are necessary for the selection of microsatellite markers in paternity identification.

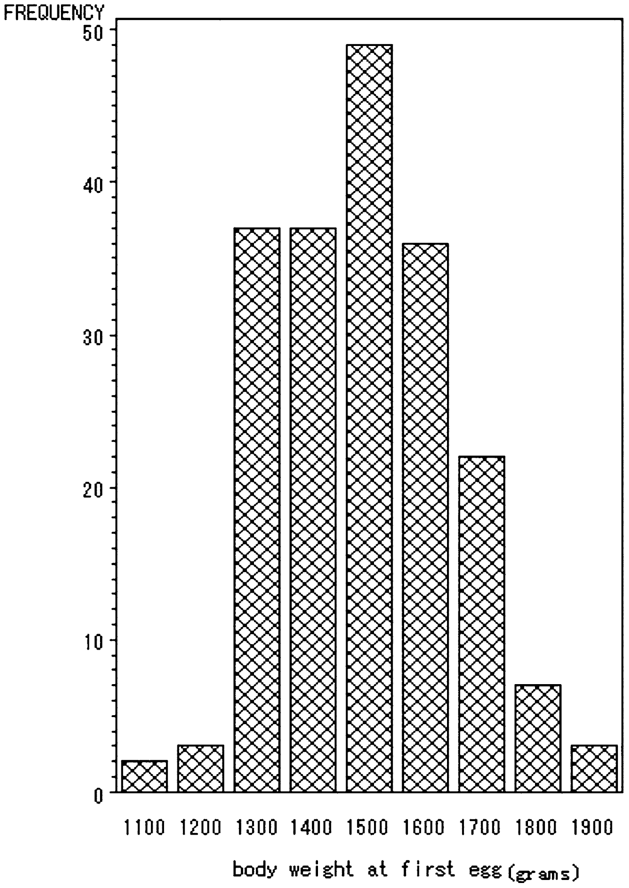

Fig. 2 The normal distribution of BWF with total observation

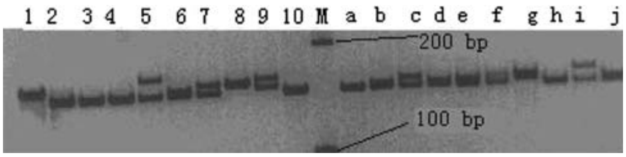

Fig. 3 The ay493342 part of genotype page

Table 5 The result of Students' test of EBV in different group

Group	EBV		
	Mean	*T* value	*P*
Parental male	−26.44	3.37	0.0017
Parental female	10.234		
Parental male	−26.44	−3.11	0.0072
Progeny	0.1045		
Parental female	10.234	1.56	0.1206
Progeny	0.1045		

Assessment for paternity

In this report, five microsatellite makers were used in paternity assessment. The parentage–offspring relationships, involving 12 half-sibling families, were built, and the average pedigree error less than 10^{-3} (LOD>3). Though, the precision was lower than that of Birus report the 10^{-5} in 2003 [22]. But the precision was only used when the conditions were considered, such as time, place, and other conditions of disappearance. In our experiment, parentage and offspring were raised at the same place and in the same season. Therefore, the precision degree is reliable in our experiment. The paternity result shows that these microsatellite makers were advantageous in Chinese egg-laying duck paternity assessment.

Analysis of differential BWF

The BWF (body weight at first egg) trait is a trait which is closely related with the age at first egg in laying poultry. There is evidence that indicates BWF increased by 75 g/d will delay the age for first egg [23], and furthermore, BWF is an index which can be used to predict the egg production [24]. So, we can know that the BWF is a very important trait in egg-laying birds, and positively correlated with age at first egg [25]. In this experiment, BWF has a significant difference between males and females. We can conclude that the inherited trait follows the maternal effect in Chinese egg-laying duck. The conclusion was supported by the results of EBV calculation.

Heritability and EBV

From the past reports about the heritability of egg production, we found that a majority of egg traits had lower heritability, for example, the heritability of age at first egg, egg weight and hen laying rate per day, were 0.34, 0.55, 0.1, respectively [26]. In this study, BWF's heritability was 0.207 lower than juvenile body weight (the body weight at 6 weeks of age), 0.64 in Koerhuis's report [26]. From the results of EBV calculation, we found that EBV has the same distribution with BWF in parental females, parental males and progeny groups.

The conclusion of BWF following the maternal effect is proven again on EBV. There are some evidences to support this conclusion. First, females were significantly higher than males on EBV in this study; but there is no different between mothers and daughters; second, GH is associated with chicken body weight [27], the ovary is an extrapituitary site of GH (growth hormone) gene expression during sexual maturation in egg layers [28], from these phenomena we can conclude that GH is a resource of the different BWF between male and female in Chinese egg-laying ducks. In brief, these results also indicate that the five microsatellite loci can be efficient applied in paternity assessment serving for Chinese egg-laying duck's BLUP breeding.

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