Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Poultry Science 4 (4): 198-204, 2010
ISSN 1819-3609

An Estimation of Genetic Variation in Indonesian Local Duck using Microsatellite Marker

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Abstract: This research was aimed to evaluate genetic variation as well as genetic relationship among three breeds of local duck originated from Tegal, Magelang and Mojosari, Java, Indonesia. Six microsatellite markers were used in the analysis. All DNA marker profiles derived from six microsatellite primers showed polymorphisms with allele sizes ranged from 80-760 bp. The smallest allele was APH-24 (90-290 bp). Among all loci obtained from the whole population, ADL-231 showed the highest (0.828) and APH-23 showed the lowest (0.412) Polymorphic Information Content (PIC) values. The average PIC values of duck populations from Mojosari, Magelang and Tegal were 0.620, 0.699 and 0.76, respectively. There was a great variation in estimation of heterozygosity (He) values in various duck populations, i.e., 0,509, 0,695 and 0,728 each for duck populations from Mojosari, Tegal and Magelang. The Hardy-Weinberg Equilibrium (HWE) analysis showed deviations in all loci analyzed due to selection and migration occurred in the three populations. Genetic distance analysis indicated that the closest genetic relationship was found between Tegal and Magelang duck populations (0.1722) while the farthest one occurred between Tegal and Mojosari populations (0.3251). In conclusion, estimation of genetic diversity in duck population in Java using microsatellite marker in this research showed a great variation based on PIC and heterozygosity values. In addition genetic relationship between duck populations from Tegal and Magelang was closer compared to that between Tegal and Mojosari.

Key words: Microsatellite, Indonesia duck, polymorphism, genetic distance, heterozygosity

INTRODUCTION

Microsatelite marker has been widely used to evaluate genetic structure, variation and relationship in various organisms. The advantages of the technique include its ability to detect polymorphisms in many loci and the codominan nature of generated markers (Tadano *et al*., 2007; Groenen *et al*., 2000; Crooijmans *et al*., 1993). Identification and characterisation of local duck populations is important since the data can be used as sources of Indonesian germplasm and help the breeding program.

There are various local ducks in Indonesia. These include those located in Java which consist of Tegal, Magelang and Mojosari ducks; in Bali known as Balinese duck and in South Borneo, known as alabio duck. Indonesian ducks are the offsprings of hybrids

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between local and imported ducks; therefore, the colours and names are vary (Hetzel, 1985; Yuwanta *et al.*, 2001; Wilson *et al.*, 1997). Therefore, genetic variations are high among Indonesia ducks as observed in morphology and productivity. The ducks showed variations in feather (plumage) colour, body size and egg production. For breeding purpose, It is important to analyse the genetic variation using a molecular marker technology such as microsatelite.

Christel *et al.* (2006) reported 122 microsatelite markers polymorphic between Common and Manila (muscovy) ducks and 51 markers identified from female ducks were candidates of QTLs. Microsatelite marker gives better results for evaluating genetic variation and genetic relationship in various domestic poultries due to high polymorphism and easier for identification as compared to allozyme assay and RAPD (Zhang *et al.*, 2002). Huang *et al.* (2008) stated that genetic mapping in duck using microsatelite and Single Nucleotide Plymorphism (SNP) is important to identify genes associated with characteristics of high economic values. This research was aimed to evaluate genetic variation in local duck of Java, Indonesia based on PIC value, heterozygosity and genetic distance.

MATERIALS AND METHODS

Sample Size and Collection

Materials used were blood samples of various local ducks in Java, i.e., Tegal, Magelang and Mojosari ducks obtained from local farmers. Sample collection was done from June 21st to August 17th in duck farming centres: Cirebon West Java for Tegal ducks, Muntilan, Magelang Central Java for Magelang ducks and Mojosari Mojokerto, East java for Mojosari ducks. Blood samples were obtained from thirty ducks for each location such that in total there were 90 samples analyzed.

DNA Isolation

DNA was extracted following the salting out procedure (Miller *et al.*, 1988). Because DNA concentrations of obtained were varied among blood samples, DNA concentrations were adjusted to 50 μg μL^{-1} before they were subjected PCR cycles.

PCR and Electrophoresis Procedures

Six pairs of microsatellite primers were used in this study (Table 1). The PCR reaction consisted of 15 μL master mix, 1 μL forward primer, 1 μL reserve primer, 2 μL DNA and 11 μL H_2O such that the total volume was 30 μL. PCR was performed using a thermo cycler with the following program: 94°C for 5 min, 94°C for 1 min, 50°C for 1 min, 72°C for 1 min and 72°C for

Table 1: Microsatelite primers used in this study

No.	Primer	Sequence
1	ADL-115F	5'-GGA TGA GAA GAA AGG CA-3'
	ADL-115R	5'-CAA TGG TGG TTC AGG TAA TC-3'
2	ADL-209F	5'-GGT TAG CTC CCT CCT TCC AG-3'
	ADL-209R	5'-AAG GAA ACA AAG AGA AAT CC-3'
3	ADL-231F	5'-ACT ATT AGC CTG GGG AGA GC-3'
	ADL-231R	5'-TCA CTC CAG CTT GAG ACA GG-3'
4	APH-23F	5'GGA TGA GAA GAA GAA AGG CA-3'
	APH-23R	5'AAG GAA ACA AAG AGA AAT CC-3'
5	APH-24F	5'GGA TGA GAA GAA GAA AGG CA-3'
	APH-24R	5'CAA TGG TGG TTC AGG TAA TC-3'
6	APH-09F	5'GGA TGT TGC CCC CAC ATA TTT-3'
	APH-09R	5'TTG CCT TGT TTA TGA GCC ATTA-3'

F: Forward primer; R: Reverse primer, Primer 1-5 (Slavenaite *et al.*, 2004), Primer 6 (Li *et al.*, 2006)

5 min. A total of 30 PCR cycles were conducted. Amplification products were separated by electrophoresis using 2% agarose gel in 1X TBE buffer using ethidium bromide banding. The results were visualized using UV light and photos were taken for documentation.

Data Analysis

Total genetic variation in various local ducks and the average of genetic variation was measured based on DNA polymorphism and mid value of heterozygosity (H). Gene frequency was measured according to Pirchner (1981).

$$F_{An} = \frac{\sum \text{locus}_{An}}{\sum \text{locus}_{A1} + \sum \text{locus}_{A2} + \sum \text{locus}_{An}}$$

Where:
F_{an} = Frequency of gene A at n^{th} locus

Calculation of genetic variation was determined using the heterozygosity formula of Nei (1987).

$$h = 1 - \sum_{i=1}^{m} x_i^2$$

Where:
h = Heterozygosity
m = No. of allele
x_i = Frequency of ith gene

Average of heterozygosity (H) was the average of h value against all loci, or

$$1 - \sum_{i=1}^{m} x_i^2$$

$$H = \frac{h}{Rr}$$

Where:
r = No. of loci observed

Genetic distance value was obtained from allele frequencies of all loci observed using the formula of Nei (1987):

$$D = - \log_e I$$

I represent genetic similarity value between two populations, calculated using the following formula:

$$I = \Sigma X_{ij} Y_{ij} / \sqrt{(X_{ij}^2)\,(Y_{ij}^2)}$$

Where:
X_{ij} = Frequency of Ith allele, jth locus on X population
Y_{ij} = Frequency of Ith allele, jth locus on Y population

Polymorphism Information Content (PIC) is a value represents polymorphism in a population measured using a particular marker. The PIC value depends on number and distribution of detected allele.

$$PIC = 1 - \sum_{i=1}^{m} P_{ij}$$

Where:
P_{ij} = Frequency of i marker allele

RESULTS AND DISCUSSION

The six microsatellite primers all showed polymorphisms in local duck populations (Table 2). The allele size range varied from 80 to 760 bp with the smallest allele size APH-24 (90-290 bp). Number of allele identified from each locus varied with the range of 5 to 7 allele. The total numbers of allelic pairs identified from six primers were 37 pairs in all populations. PIC values based on loci obtained from the whole population was highest at ADL-231 (0.828) and lowest at APH-23 (0.412) loci. Ahmadi *et al.* (2007) reported that from 13 microsatelite markers used to amplify DNA of Pekin duck (*Anas platyrhynchos*) and Manila duck (*Cairina moschata*), 4 microsatelites were not identified, 3 were monomorphic and 6 showed DNA polymorphism. Li *et al.* (2006) reported that, in 24 local ducks of China, the most alleles was found in APH09 primer, which were 13 alleles; whereas in this research 6 alleles were obtained from APH09 primer from Tegal, Magelang and Mojosari ducks. The results of this research supported the finding of Li *et al.* (2006) that the primer was an effective marker to analyse genetic relationship among duck breeds.

Estimation of expected heterozygosity (He) and Polymorphic Information Content (PIC) values were obtained using data on each duck population locus. The He values were considerably high with the range of 0.528 (APH-24) to 0.795 (ADL-209). The average He value from the whole population was 0.644±0.096 (Table 2). Estimation of He value on different locus and duck populations showed a wide variation, i.e., 0.509 for Mojosari duck, 0.695 for Tegal duck and 0.728 for Magelang duck (Table 3). The He value showed that genetic variation in Magelang duck population was highest. The high He value was due to low level of inbreeding and selection as well as many numbers of alleles was being detected. Heterozygosity reflects the heredity and mutation of loci in each group that effective parameter to assess the heredity and mutation of population (Li *et al.*, 2006). This finding is in agreement with Huang *et al.* (2008) that microsatellite markers can be used for genetic mapping on ducks and other water fowls due to its high precision.

The average numbers of alleles identified in the whole duck population was 6.167±0.555, while for the three sub population the numbers were 4.167±0.555 for Tegal duck; 4.500±1.167 for Magelang duck and 3.500±0.500 for Mojosari duck. The numbers of alleles identified

Table 2: Analysis result of 6 microsatelites in local duck population

Locus	Allele size (bp)	No. of allele	Expected Heterozygosity (He)	Polymorphic information conten (PIC)
ADL-115	90-500	6	0,552	0,744
ADL-209	80-400	6	0,795	0,817
ADL-231	100-760	7	0,625	0,828
APH-23	90-600	7	0,583	0,412
APH-24	90-290	5	0,528	0,742
APH-09	100-350	6	0,780	0,716
Average		6,167	0,644	0,710
Standart error		0,556	0,096	0,099

Table 3: Estimation of the average polymorphism information content and expected heterozygosity on Tegal, Magelang and Mojosari ducks

Locus	Variables	Tegal	Magelang	Mojosari
ADL-115	bp	150-500	150-300	90-250
	N	17	11	12
	PIC	0,768	0,562	0,611
	He	0,795	0,427	0,435
ADL-209	bp	80-400	80-400	80-250
	N	11	15	20
	PIC	0,796	0,818	0,7
	He	0,799	0,97	0,617
ADL-231	bp	200-700	100-700	100-500
	N	14	14	18
	PIC	0,693	0,806	0,636
	He	0,527	0,829	0,519
APH-23	bp	90-600	90-600	150-400
	N	7	13	10
	PIC	0,815	0,698	0,72
	He	0,545	0,671	0,531
APH-24	bp	150-290	150-290	90-290
	N	14	8	12
	PIC	0,709	0,594	0,625
	He	0,542	0,519	0,525
APH-09	bp	100-350	100-350	100-350
	N	22	21	15
	PIC	0,778	0,716	0,551
	He	0,963	0,953	0,425
Average	N	14,167	13,667	14,5
	PIC	0,76	0,699	0,62
	He	0,695	0,728	0,509

bp: Base pairs, N: No. of sample, PIC: Polymorphic information content, He: Heterozygosity

showed moderately high variations, especially in Magelang and Tegal populations. Li *et al.* (2006) reported genetic variation in local duck in China ranged from 0.514 to 0.617 with the average of 0.569, while Vafaei *et al.* (2009) reported that Pekin and Muscovy ducks had relatively low He values, i.e., 0.4329 and 0.4069, respectively. The low He value showed the present of inbreeding, selection repression and low level of alleles identified, i.e., for each locus in average only 2.22 and 2.44 alleles were found. Microsatellite loci used in studies of genetic distance should have more than four alleles in order to reduce the standard errors of distance estimate (Barker, 1994), thus, the microsatellite marker used in this study were effective markers for genetic diversity analysis. Tadano *et al.* (2007) stated that high heterozygosity showed the occurrence of mating between individuals with no close genetic relationship and considerably wide area of the poultry population.

The average PIC values were 0.620, 0.699 and 0.76 for Mojosari, Magelang and Tegal ducks respectively. PIC represented the value of a marker used to detect polymorphism in a population. PIC depends on the number of alleles identified and distribution of allele frequencies. Microsatelite markers used in this research were species nonspecific markers being used across chicken, duck and other water fowls. Microsatelite marker for chicken was ADL, while for duck and other water fowls were APH. Huang *et al.* (2005) stated that microsatellite for conserved DNA in poultry can be used across various species. There were 20.42% conserved microsatellite loci between duck and chicken.

The Hardy-Weinberg Equilibrium (HWE) test showed deviation in all identified loci. The deviation was due to selection and migration occurred in Tegal, Magelang and Mojosari duck populations. The selection and migration occurred as farmers selected the dods as well as male and female parents. Migration occurred by bring in ducks from other regions to improve egg production. Vanhala *et al.* (1998) stated that selection and migration result in

loss of alleles and miss genotyping. This research showed several alleles with zero (0) values, therefore several individuals with homozygote genotype were not found (0). Deviation of HWE was also due to non random mating among the ducks (Tadano *et al*., 2007).

Genetic distance calculated according to Nei (1987) showed that genetic relationship between Tegal and Magelang ducks was closest (0.1722), while that between Tegal and Mojosari ducks was farthest (0.3251) (Table 3). These results proved that genetic relationship was affected by geographical location. Ducks reared around Pantura area (Tegal ducks) was geographically closer to those reared in Magelang compared to those reared in Mojosari, East Java. Genetic distance was measured based on gene frequencies showing genetic variation in a breed. These results support the finding of Li *et al*. (2007) that phylogenetic pattern and genetic distance on the population level, suggests that large genetic divergence has occurred between or within breeds and limitation of lower mobility or geographic isolation between breeds and abundant diversity within breeds have been considered to be the main for genetic relationship. Estimation of genetic variation using microsatellite marker on duck population in Java, Indonesia showed a high level of variation based on PIC and heterozygosity values. Genetic relationship among ducks in Java showed that Tegal and Magelang ducks had a closer genetic relationship (0.1722) compared to Tegal and Mojosari ducks (0.3251).

ACKNOWLEDGMENTS

The authors thanks The Direktorat Jenderal Pendidikan Tinggi (DIKTI) for funding this research based on Surat Perjanjian Pelaksanaan Penelitian Fundamental Nomor: Kept. 191/H23/PL/2009 25 April 2009. Thanks to Alice Yuniaty for proofreading this study.

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