

# Microsatelit

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# MICROSATELLITES AND MORPHOMETRIC DIVERSITY AMONG INDONESIAN LOCAL DUCK POPULATIONS IN LOMBOK ISLAND

Maskur Maskur

## Abstract

The genetic diversity of three breed Indonesian local duck in lombok island west nusa tenggara province locus were investigated base on morphometric traits and four microsatellites marker. Experimental ducks consist of 75 Sasak ducks, 75 Khaki campbell ducks and 70 Mojosari ducks. Morphometric traits were recorded and statistically analyzed, whereas genetic parameters of microsatellite loci were calculated using PopGene32. In this study, the mean PIC of the 4 microsatellite loci in all populations was 0.472, wherein AY287 is the most polymorphic locus with a PIC value of 0.735 and APL579 is a monomorphic locus with a PIC value of 0 (zero). The mean value of observed heterozygosity ( $H_o$ ) is lower than expected ( $H_e$ ), with the value 0.393 and 0.472 (Khaki Campbell); 0.433 and 0.532 (Mojosari); and 0.330 and 0.451 (Sasak), respectively. This indicates that the population is heading towards a heterozygosity deficit and proving that the three populations are not in Hardy-Weinberg equilibrium. The inbreeding coefficient in the three populations was quite high with a positive  $F_{is}$  value ranging from 0.000 – 0.422. Meanwhile, the value of the genetic differentiation indeks ( $F_{st}$ ) in all populations is zero (0), indicating that there is no differentiation between breeds at the observed loci.

**Keyword:** local duck, morphometric, microsatellites, heterozygosity, inbreeding and differentiation

## 1. INTRODUCTION

In some countries including Indonesia, the practice of breeding and hybridization are conducted by importing livestock with higher productivity to increase the productivity of local livestock. These practiced seems to increase genetic diversity, but the consequences can threaten the existence of some native livestock. The genetic resources of livestock should be viewed as a future insurance, which has important meaning to improve the socio-economic life at present or in the future. Therefore, it is essential to maintain a balance of breeding and hybridization practices with the conservation of genetic resources of livestock (Oskam et al., 2004).

In Indonesia, There are several commercial duck breeds currently used for meat and egg production for local markets. These local ducks are named according to their location, such as on the Java island, there are Tegal duck, Mojosari duck and Magelang ducks; in Bali island, known as balinesse duck; in Lombok island, known as sasaknesse duck and others. Breeders develop local ducks through crosses with imported ducks and produce various hybrids with distinctive morphological characteristics and productivity levels (Hetzel, 1985 and Wilson et al., 1997). The enormous genetic diversity of local ducks in Indonesia is the

result of breeding practice and production systems developed over time by breeders to fulfill diverse needs in various environmental conditions (Maharani et al., 2017). The availability of wide biodiversity is an important element in the sustainable use of animal genetic resources (AnGR) as it enables livestock keepers to adapt their animals to changing conditions (FAO, 2007). Evaluation of the genetic diversity of ducks is essential for the development and sustainability of production and is very useful for maintaining and exploiting the genetic resources of local ducks.

Molecular techniques have provided a number of new genetic markers for the study of genetic variation among domestic animal. Among the genetic markers, microsatellites have been comprehensively exploited to access genetic variability as they contribute information on every region of the genome (Sharma et al., 2015) and many clear evidence of the usefulness for genetic diversity studies (Hariyono et al., 2019). In the recent study, microsatellites were recommended as the most appropriate choice marker for the analysis of the diversity and population structure, or study the evolution of related species (Sultana et al., 2017; Seo et al., 2016). microsatellites are abundant in copy numbers and widely distributed in the genome, codominant and relatively easy to produce and analyze, especially after successfully amplifying these microsatellite loci by PCR technology.

In this paper, we investigated the genetic diversity of 3 local duck population based on phenotype variability and 4 microsatellite loci as DNA markers. The previous studies in order to analyze the genetic diversity among Indonesian local duck populations have been reported by researchers such as Ismoyowati and Purwantini, (2011); Rusfidra et al., (2013); and Maharani et al., (2017). This study was conducted to evaluate the genetic diversity of the Indonesian local duck population on the island of Lombok based on morphometric and microsatellite markers. The results of this study are expected to provide scientific information for designing breeding strategies and conservation plans.

## MATERIAL AND METHODS

All procedures carried out with the use of animals had been approved by the board of ethics committee of Faculty of Medicine, Mataram University, Indonesia.

### 2.1. Phenotype Traits Measurement

Two hundred and twenty local ducks consist of 75 Sasak ducks, 75 Khaki campbell ducks and 70 Mojosari ducks were reared in a semi-open house cages at the Experimental Farm of Animal Science Faculty, University of Mataram. All experimental ducks were given

the same feed, namely starter feed (containing 21% Crude Protein and 2806 kcal/kg Metabolic Energy) for 1-3 weeks old duck and grower feed (containing 15% Crude Protein and 2806 kcal/kg Metabolic Energy) for 4-10 weeks old duck. The observed variables were body weight, Average Feed Conversion, shank length and diameters and middle finger, feather colour, shank and bill color. Morphometric was measured during one week when duck start to production to 10 weeks (Ismoyowati *et al.*, 2006). The data collected were analyzed using an analysis of variance of one way classification with mathematical models by Gomez and Gomez (1984)

## 2.2. DNA extraction, PCR and genotyping

Blood samples for DNA genotyping were collected approximately 1.0 ml from 220 unrelated individuals representing 3 duck breed populations, including 75 Sasak ducks, 75 Khaki Campbell ducks, and 70 Mojosari ducks. Blood samples was collected on Venoject tube with K2EDTA\_ and preserved at -25°C for several weeks. The isolation of Genomic DNA from blood sample was performed using Wizard Genomic kit following manufacturer instructions (Promega, Madison, WI, USA).

The PCR analysis was carried out in eppendorf mastercyclers nexus gradient (Eppendorf - Hamburg, Germany). The reaction mixture was conditioned in a total volume of 25 µl and contained approximately 100 ng of genomic DNA, 0.5 µM of each primers (forward and reverse), 1 x PCR buffer (10mM Tris-HCl pH 9.0), 50 mM KCl and 1.5 mM MgCl, 5% deionized Formamide, 200 µM dNTP, and 0.025 U of Taq polymerase (Pharmacia). The PCR programs was performed in the following cycles: initial cycle at 94°C for 5 minutes followed by 33 cycles 94°C for 30 seconds, 60°C for 60 seconds, and 72°C for 90 seconds, then ended the next step at 72°C for 7 minutes. Sequence and position of the primer is presented in Table 1.

The amplification products were separated by electrophoresis on 2.5% agarose and visualized by EtBr staining. The images data was analyzed using the Kodak Digital Science ID Image Analysis Software. The position of the DNA band on the agarose gel is determined manually. The size and amount of the allele that appears on the gel is determined on the assumption that all DNA bands with the same migration rate are homologous, whereas the alleles with the fastest migration are defined as allele A, the next is the B allele and so on (Leung *et al.*, 1993).

Table1. Sequence and annealing temperatures of the primer (Su Y. & Chen G.H., 2009)

Lokus	Primer sequence (5'→3')	Annealing Temperature (°C)	Allele fragment
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CAD U086	F: GCAGAGCGGTGTGAGAGCA R: AACACAGCTTCACCCACAG	60.1	175 – 217
CMO212	F: GGATGTTGCCCCACATATT 3 TTGCCTTGTTTATGA GCCATT	55.0	221 – 283
APL579	F: ATTAGA GCAGGAGTTAGGAGAC 7 R: GCAA GAA GTGGCTTTTTTC	55.0	159 – 289
AY287	F: TGCAGGTAGGTCTTCTGTTCTG R: GCCAGTCCTTTGCTTCGTAA	60.8	154 – 294

## 2.2. Statistical analysis

4 The allele frequencies were calculated directly from the observed genotype. The results of calculating genetic parameters (Table 2 and 3) were obtained using the Microsatellite-Toolkit software. PopGene software (Version 32) was used to determine 7 Effective numbers of alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), Shannon's Information Index ( $I$ ), fixation index ( $F_{is}$ ) and differentiation index ( $F_{st}$ ) for data as depicted in Tables 4 and 5.

## RESULTS AND DISCUSSION

### A. Phenotype and Morphometric Variability

6 The average body weight of the three ducks breed at weeks 1 to 10 is listed in table 2. The analysis of variance results showed that the body weight and average daily gain of the Sasak ducks was not significantly different from the Khaki Campbell ducks and was significantly smaller ( $P < 0.05$ ) compared to with Mojosari duck. The results of this study showed that the increase in body weight gain in the three breeds of ducks occurred until the age of 5-6 weeks, then growth slowed down the following week. Growth in ducks is generally the fastest in the starter period, then slows down in the next growth period (Rosilawati et al., 2010). In crossbreed ducks (alabio x peking), the highest body weight gain occurred at the age of 6 weeks (Susanti, 2012).

Tabel 2. Average Daily Gain and Feed Conversion of 3 breed local duck in Lombok, Indonesia

Breed	Average Body Weight (grams)		Average Feed Conversion	Average Daily Gain (grams/day)
	1	2		
Sasak	52,98 <sup>a</sup>	917,84 <sup>a</sup>	3,60 <sup>a</sup>	13,73 <sup>a</sup>
Khaki Campbell	62,50 <sup>b</sup>	907,09 <sup>a</sup>	3,90 <sup>b</sup>	13,40 <sup>a</sup>
Mojosari	66,30 <sup>b</sup>	1124,69 <sup>b</sup>	4,04 <sup>b</sup>	16,80 <sup>c</sup>

Keterangan: superscript yang berbeda pada kolom yang sama menunjukkan perbedaan yang nyata ( $P < 0.05$ )

6 The difference in body weight and average daily gain between the three breeds of ducks indicates the type of production of the ducks. Khaki Campbell ducks and Sasak ducks

are true laying ducks, where the characteristics of laying ducks include small bodies (Soegeng P. et al., 2018), while Mojosari ducks are dual-purpose ducks that produce eggs and produce meat with a larger body size. The different of body weight and average daily gain in this study was due to genetic factors because environmental effects has been made uniformly. Ismoyowati et al. (2006) reported that when the affects of the environment are uniform, phenotypes that appear will showed the genetic capability.

Quantitative and qualitative trait such as feather, shank and bill color, *shank length and diameters and middle finger length* were the basis of a selection process and breeding duck. According to Yakubu and Ugbo (2011), comparison of phenotype based on morphological characteristics could be representing of genetic differences. The results of morphometric traits measurement is listed in table 3.

Table 3. Morfometric and Colours of Bill, Shank and Feathers at 10 weeks

Variable	Sasak	Khaki Campbell	Mojosari
<b>Shank and Middle finger</b>			
shank length (cm)	5,78±0,56	5,70±0,53	5,83±0,59
shank diameters (cm)	1,10±0,09	1,09±0,08	1,14±0,07
middle finger length (cm)	6,72±0,71	6,52±0,69	6,53±0,74
<b>Postur Tubuh</b>			
	slightly upright posture, cylindrical-shaped body and slender (elevation angle ± 45 drajat)	slightly upright posture with a long and slender neck (elevation angle ± 35 <sup>o</sup> )	the body showed like bottle, relatively big and nearly vertical (elevation angle ± 70 <sup>o</sup> )
<b>Colours of</b>			
Bill	Black, Black and Chocolate combination	Yellow	Black
Shank	Black	Yellow	Light yellow
Feathers	In general, the whole body is light brown and the tips of the wings are white flanked by dark brown / black	The whole body is dominated by khaki color	The body is striated–reddish brown, while the head, neck and chest are black and the bottom is whitish

In general, the coat color pattern of Mojosari ducks is dominated by a striated - reddish-brown color. However, other colors are also found in the population such as the combination of striated color with white stripes on the neck and chest area. The appearance of color variations is thought to be caused by a recessive color segregation pattern, because the chances of emergence are relatively small (Suparyanto A., 2003). The color pattern of

the Sasak duck tends to be more varied than the Khaki Campbell and Mojosari ducks. In general, Sasak duck have a light brown color on the whole body and the tips of the wings are white flanked by dark brown/black. However, several researchers also reported that there were several variations in the color of the Sasak duck, such as black - dark brown on the whole body with different variations (Tamzil MH and Indarsih B., 2017). Phenotypic variation is thought to be due to the intensity of unstructured out-crossing, although one of the parental sources comes from one family.

The *shank length and diameters and middle finger length* were morphometric traits that reflects the duck's size and height. Data of shank length of Sasak, Khaki Campbell and Mojosari ducks gathered in this study are  $5,78 \pm 0,56$  cm ;  $5,70 \pm 0,53$ ;  $5,83 \pm 0,59$  cm, respectively (Table 3). The score is shorter than those of Tegal duck ( $6.79 \pm 0.56$  cm), Magelang ducks ( $7.10 \pm 0.51$  cm), and Cirebon ducks ( $6.55 \pm 0.50$  cm) (Setioko et al., 2005), but the same as Damiaking ducks and Turi ducks ( $5.88 \pm 0.44$  cm) (Sofiyana et al., 2003). Therefore, based on the length of the shank, the three breeds of ducks are classified as medium size

## B. Genetic parameters of microsatellite loci

In the present study 4 microsatellite markers were chosen for further analysis the genetic diversity of 3 breed Indonesian local ducks population. The genetic diversity of the 3 local duck breeds at the 4 microsatellite loci were estimated. A portion of amplification results showed in Fig.1–4. The results of calculating genetic parameters (Table 2 and 3) were obtained using the Microsatellite-Toolkit software. Effective numbers of alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), Shannon's Information Index ( $I$ ),  $F_{is}$ = fixation indeks and  $F_{st}$ = differentiation index were calculated using PopGene32.



Fig 1. A portion of PCR result of CMO211 on agarose gel. M = 100 bp marker



Fig 2. A portion of PCR result of CADU86 on agarose gel. M = 100 bp marker

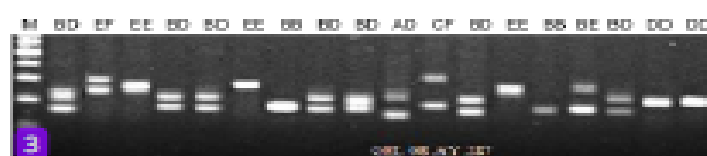


Fig 3. A portion of PCR result of AY267 on agarose gel. M = 100 bp marker

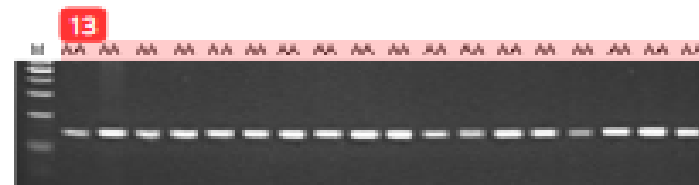


Fig4. A portion of PCR result of APL579 on agarose gel. M = 100 bp marker

The effective number of alleles is the number of equally frequent alleles that would take to achieve the same expected heterozygosity in population and often used to assay the effect of alleles in each population and to reflect the genetic variation expressed by inverse homozygosity. Estimating the value of heterozygosity has an important meaning, namely to get an idea of genetic variability (Marson et al. 2005) and to know the level of polymorphism of an allele and the prospect of the population in the future (Falconer & Macay 1996). Genetic diversity can be estimated as the average level of heterozygosity in the population, the number of alleles per locus and the percentage of polymorphic loci (Soysal 2004).

Table 4. Genic Variation Statistics For All Loci (Nei, 1987)

LOCUS	N	Na	Ne	I	Ho	He	Fis	Fst	PIC
AY287	220	5.000	4.355	1.532	0.641	0.773	0.169	0.000	0.735
CMO211	219	4.000	3.115	1.222	0.493	0.681	0.274	0.000	0.618
CAUD086	220	3.000	2.599	1.014	0.407	0.617	0.339	0.000	0.536
APL579	219	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
Mean	219.5	3.250	2.767	0.942	0.385	0.518	0.181	0.000	0.472

Sample size; Na=Observed number of alleles; Ne=Effective number of alleles [Kimura and Crow (1964)]; I=Shannon's information index [Lewontin (1972)]; Ho=Observed heterozygosity; He=Expected heterozygosity; PIC=polyomorphic information content, Fis= fixation index and Fst= differentiation index.

The number of alleles for the 4 microsatellites in the 3 duck breeds analyzed was 13, with a mean of 3.25 (Table 2). The highest number of alleles was 5 (AY287) and the lowest was 1 (APL579). The mean number of alleles of the four loci in the analyzed population ( $N_a = 3.25$ ) was lower than that of the four breeds of Chinese indigenous laying-type ducks at the same four loci ( $N_a = 5.25$ , Su et al. 2009). The mean number of alleles of the CAUD086 locus in the 3 analyzed breeds ( $N_a = 3$ ) was also found to be lower than that of the 4 Javanese duck breeds ( $N_a = 6$ , Maharani et al., 2017). According to Barker (1994), microsatellite markers used in genetic distance estimation should have more than four alleles to reduce standard errors in distance estimation. Meanwhile, according to FAO standards, a minimum of four different alleles per locus is required for the assessment of genetic variation and differences within and between populations. Thus, the 2 microsatellite loci AY287 and CMO211 used in this study showed sufficient polymorphism to evaluate genetic variation within and between local duck populations in Indonesia.

By definition, expected heterozygosity represents the probability that a randomly selected individual from a population in Hardy Weinberg equilibrium is heterozygous,



whereas observed heterozygosity indicates the effective proportion of heterozygous individuals at each locus (Carco et al., 2018). The results of measuring the heterozygosity value of each locus in all samples are presented in table 2. The  $H_e$  value is higher than  $H_o$  for 3 polymorphic loci except APL579 which is a monomorphic locus. The mean value of observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) in all loci and populations also revealed medium to high genetic diversity, with the value 0.385 and 0.518, respectively. The expected mean heterozygosity of 0.518 indicates that the genetic diversity being observed in the local duck population on the island of Lombok is the same as the previous study on local ducks on the island of Java reported by Maharani, et al., 2017. This moderate genetic diversity is a good substance in determining breeding strategies, especially in small populations.

PIC measures the quantity of information per microsatellite and depends on the number of identified alleles and allele frequency (Purwantini and Purwantini 2010). Polymorphism Information Content (PIC) is the ability of a genetic marker to detect the polymorphism among individuals of a population, and often used to measure the informativeness of a genetic marker for linkage studies. In this study, the mean PIC of the 4 microsatellite loci in all populations was 0.472, with 3 microsatellite showing high diversity (Table 2) and 1 locus producing monomorphic for all three races. AY287 is the most polymorphic locus with a PIC value of 0.735, and APL579 is a monomorphic locus with a PIC value of 0. Bolstein et al. (1980) classified the PIC value as highly informative ( $PIC > 0.50$ ), reasonably informative ( $0.50 > PIC > 0.25$ ), and slightly informative ( $PIC < 0.25$ ). Thus, three loci (AY287, CMO211 and CAUDO86) were highly informative loci and could be used for further genetic analysis in the studied population and one locus, APL579, could not be used. The results of this study did not differ from Su et al. (2009), which included AY287, CAUDO86, CMO211, and APL579 for genetic diversity analysis of Chinese indigenous laying-type ducks. In the study AY287 had the highest PIC (0.695) compared to CAUDO86, CMO211 and APL579 at 0.682 respectively; 0.309 and 0.348. The APL579 locus was also found as a monomorphic locus (1 allele) in Jinding breed ducks with PIC 0. Analysis conducted by Carco et al. (2018) in the population of Italian and Polish duck breeds, the CAUDO86 locus also showed a high PIC value of 0.735.

### C. Genetic diversity within the breeds

Genetic diversity among breeds is indicated by the number of alleles,  $H_o$ ,  $H_e$  and PIC values which are basic measures and provide important information for individual and

population discrimination (Seo et al., 2016). The results of measuring the value of genetic diversity among the 3 local duck breeds are listed in table 3. Lokus AY287, CAUDO86 and CMO211 showed a wide variation among populations. Different microsatellite loci showed high variation within each population and the same locus also showed high variation between populations. AY287 has the highest observed heterozygosity values for 3 breeds namely 0.711 (Khaki Campbell), 0.627 (Mojosari) and 0.580 (Sasak). Meanwhile, APL579 was found as a monomorphic locus in 3 populations, where there were no heterozygous individuals. Carco et al, (2018) showed the average observed heterozygosity for the Polish population, 0.692 and 0.652, respectively, while the Italian breeds had lower values: 0.450 for AGV and 0.372 for AMG. Khan Ahmadi et al. (2007) also found similar levels of genetic variation within the Peking and Muscovy populations with values of heterozygosity of 0.530 and 0.440, respectively.

Table 5. Genetic diversity measures in Indonesian duck populations across 4 microsatellite loci

DUCK	LOCUS	N	Na	Ne	I	Ho	He	HWE	PIC	Fis
Khaki Campbell	AY287	75	5,000	3,785	1,439	0,711	0,743	0,002	0,692	0,032
	CMO211	75	2,000	1,923	0,673	0,480	0,482	<b>0,943</b>	0,364	0,000
	CAUD086	75	3,000	2,919	1,085	0,380	0,664	0,000	0,583	0,422
	APL 579	73	1,000	1,000	0,000	0,000	0,000	0,000	0,000	0,000
	Mean	<b>74,5</b>	<b>2,750</b>	<b>2,407</b>	<b>0,799</b>	<b>0,393</b>	<b>0,472</b>	<b>0,236</b>	<b>0,410</b>	<b>0,114</b>
Mojosari	AY287	70	5,000	4,879	1,597	0,627	0,803	0,002	0,762	0,211
	CMO211	70	4,000	3,577	1,326	0,625	0,728	0,004	0,672	0,132
	CAUD086	70	3,000	2,438	0,980	0,480	0,596	0,000	0,516	0,186
	APL 579	70	1,000	1,000	0,000	0,000	0,000	0,000	0,000	0,000
	Mean	<b>70,0</b>	<b>3,250</b>	<b>2,974</b>	<b>0,976</b>	<b>0,433</b>	<b>0,532</b>	<b>0,002</b>	<b>0,488</b>	<b>0,132</b>
Sasak	AY287	75	4,000	2,614	1,108	0,580	0,624	<b>0,204</b>	0,550	0,060
	CMO211	74	3,000	2,630	1,033	0,380	0,626	0,001	0,550	0,386
	CAUD086	75	3,000	2,204	0,857	0,360	0,552	0,003	0,442	0,340
	APL 579	75	1,000	1,000	0,000	0,000	0,000	0,000	0,000	0,000
	Mean	<b>74,75</b>	<b>2,750</b>	<b>2,112</b>	<b>0,750</b>	<b>0,330</b>	<b>0,451</b>	<b>0,052</b>	<b>0,386</b>	<b>0,197</b>

N= Sample size; Na=Observed number of alleles; Ne = Effective number of alleles [Kimura and Crow (1964)]; **11** Shannon's Information index [Lewontin (1972)]; Ho=Observe heterozygosity; He=Expected heterozygosity; PIC=polymorphic information content; HWE= Hardy-Weinberg equilibrium; and Fis= fixation indexs.

In our study, the **3** observed heterozygosity for all locus **5** and all population around 0,000 to 0,711 , and the expected heterozygosity 0,000 to 0.803. The mean value of observed heterozygosity (Ho) and expected heterozygosity (He) in all loci and each population revealed medium to high genetic diversity, with the value **0,393 and 0,472** (Khaki Campbell); **0,433 and 0,532** (Mojosari); and **0,330 and 0,451** (Sasak), respectively. The mean value of observed heterozygosity obtained in this study is lower than expected, indicating that the population is heading towards a heterozygosity deficit and proving that the three populations

are not in Hardy-Weinberg equilibrium (Table 3). The Hardy-Weinberg equilibrium test (HWE) confirmed that all loci in the three populations deviated significantly from HWE, except for the CMO211 locus in khaki Campbell ducks and AY287 in sasak ducks. Maharani et al., 2017 also reported heterozygosity deficit and Hardy-Weinberg equilibrium deviation in a population of 4 Javanese duck breeds where the mean value of observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) in all loci and population were 0.465 and 0.580, respectively. Several factors can contribute towards a heterozygotes deficit. First, the locus is under selection pressure. Second, inbreeding may be common in the population. Third, the Wahlunds effect because the presence of a population substructure (Nei, 1987; Peter et al., 2007) and genotyping errors are likely due to low sample quality (Morin et al., 2009). In our research, the deviations from HWE suggest that these loci may be under selection pressure and inbreeding occurs at some loci in all populations.

In addition to the HWE test, we also evaluated genetic diversity among populations using the F Wright statistic.  $F_{st}$  is calculated to estimate the proportion of total genetic variation due to differentiation between populations, while  $F_{is}$  provide an explanation in terms of inbreeding coefficients. The individual inbreeding coefficient relative to the subpopulation ( $F_{is}$ ) can be positive or negative, while the estimated value of the genetic differentiation index between populations ( $F_{st}$ ) is always positive. Wu et al. (2008) reported that when there is no differentiation, the  $F_{st}$  value is 0 and when the alleles between populations are quite different, the  $F_{st}$  value is equal to 1. In our study, the inbreeding coefficient in the three populations was quite high with a positive  $F_{is}$  value ranging from 0.000 – 0.422. This indicates the presence of inbreeding and low heterozygosity at this locus. Meanwhile, the value of the genetic differentiation indeks ( $F_{st}$ ) in all populations is zero (0), indicating that there is no differentiation between breeds at the observed loci. These  $F_{st}$  results suggest that there is no gene flow between different breeds and, equivalently, relatively high reproductive isolation within the same breed. In contrast to our study, several reports showed moderate genetic differentiation in duck populations in Java with  $F_{st}$  value = 0.093 (Maharani, at al., 2017), Beijing and Cherry Valley duck,  $F_{st}$  = 0.075 (Wu et al., 2009), Chinese indigenous laying-type ducks,  $F_{ST}$  = 0.184 (Su dan Chen, 2009), and asia duck,  $F_{st}$  = 0.135 (Sultana et al., 2017).

In general, in the three populations (breeds) studied, deficits heterozygote and a low to moderate level of inbreeding have been observed. Some of the most likely reasons related to this phenomenon are: 1) uncontrolled mating with an unbalanced sex ratio (male: female), 2) breeders do not have a pedigree record/reference family structure, and 3) selection at the

breeder level based on the plumage color and morphometric traits to improve the performance of duck production. The use of limited stock of males to mating practices in the population and selection at the phenotypic level resulted in a deficit heterozygote.

## CONCLUSION

Characterization of three local duck breeds using a panels of 4 microsatellite showed an acceptable level of genetic diversity from the perspective of genetic conservation. The analyzed microsatellites had moderate to high PIC values with the number of alleles in accordance with the FAO minimum standard, except for the APL579 locus. The mean value of observed heterozygosity is lower than expected, indicating that the population is heading towards a heterozygosity deficit and inbreeding coefficient of an individual relative to the subpopulations (FIS) obtained in this study was quite high with positive value in three local duck populations. Therefore, it is necessary to rearrange the breeding system properly to avoid inbreeding depression. The genetic index of population based on microsatellite data can provide basic information in developing breeding and conservation strategies to protect the germplasm of local Indonesian ducks. However, in the utilization of genetic resources, phenotypic information is also very much needed in relation to efforts to increase productivity. Therefore, the combination of morphometric with microsatellite data will be very necessary in conducting breeding and conservation programs in a sustainable manner.

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