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Microsatellite-Based Genetic Diversity Among Three Duck Populations in Sumatera Island

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ABSTRACT

This study aimed to determine the genetic diversity among three duck populations (Bayang, Pegagan, and Pitalah) reared in Sumatera island, Indonesia, using microsatellite markers. Genetic diversity among populations (n = 90) was determined using 22 microsatellite markers, based on several indices: number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC), and Wright's *F*-statistics (F_{IS}, F_{IT}, F_{ST}). The total number of alleles detected across loci was 121. The Na per locus ranged from 2 (APH24, CAUD128, and CAUD009) to 18 (CAUD048 and CAUD040). The mean Ho (0.429) dan He (0.509) indicated that the level of genetic diversity among populations was moderate, while the mean PIC (0.46) suggested that the tested loci were informative for assessing genetic diversity. The mean *F*-statistics (F_{IS}, F_{IT}, F_{ST}) were 0.148, 0.198, and 0.060, respectively. The F_{ST} value indicated that the level of genetic differentiation among populations was moderate. The results confirms a moderate genetic diversity among populations, which could be beneficial for designing conservation and utilization of the local ducks in Sumatera island.

Keywords: Genetic diversity, Indonesian local ducks, Microsatellite marker

Introduction

Duck is a poultry species that plays a significant role for rural smallholders in Indonesia as it provides vital nutrients for humans as well as livelihoods. Up to now, there are at least three local duck breeds identified in Sumatera island, Indonesia, namely Bayang, Pegagan, and Pitalah ducks. A previous study has phenotypically characterized these local ducks and found considerable variations in qualitative and quantitative traits exist among the breeds (Maharani *et al.*, 2019), which are important for selection program for various traits of economic interest. In addition, evaluation of both genetic and phenotypic diversity is the first step for the conservation and utilization of domestic animal biodiversity.

Microsatellites are almost ideal marker for genetic diversity and phylogenetic studies as they are abundant, codominant, highly polymorphic, and spread out across the entire euchromatic part of the genome (Bennett, 2000; Schlotterer, 2000). Microsatellites are the repetitive sequences of 1 to 6 nucleotides, which are found in the eukaryotic and prokaryotic genomes (Ahmadi *et al.*, 2007). Regarding the local duck breeds, studies have been conducted to characterize their phenotypic characteristics (Sari *et al.*, 2011; Brahmantiyo *et al.*, 2002; Maharani *et al.*, 2019), but little findings are available on the genetic characterization of the breeds, especially using microsatellites (Rusfidra *et al.*, 2013; Hariyono *et al.*, 2019). Hariyono *et al.* (2019) found a considerable genetic diversity in eight duck populations in Indonesia. Rusfidra *et al.* (2013) and Ismoyowati and Purwantini (2011) only used few microsatellites to reveal the genetic diversity of Bayang and Alabio ducks, respectively. The present study was conducted to determine the genetic diversity among three duck populations in Sumatera island using 22 microsatellite markers.

Materials and Methods

Blood samples and DNA extraction. A total of 90 individual animals from three local duck populations, namely Bayang, Pegagan, and Pitalah ducks (30 animals each), were used for blood sampling. The blood samples were obtained from the ulnar vein using vacutainer tubes with K2-ethylenediaminetetraacetic acid anticoagulant. Bayang, Pegagan, and Pitalah duck samples were

obtained from Pesisir Selatan of West Sumatera, Tanah Datar of West Sumatera, and Ogan Ilir of South Sumatera. Genomic DNA was extracted using the gSYNC DNA Extraction Kit (Geneaid, New Taipei City, Taiwan), following the manufacturer's protocols and kept at -20°C until analyzed. The quality and concentration of the extracted DNA were checked by electrophoresis on 1% agarose gel, as well as by a spectrophotometer using the NanoDrop 2000C (Thermo Scientific, Waltham, MA, USA).

Microsatellite amplification and genotyping. Twenty-two microsatellite markers were chosen for genetic diversity analysis (Table 1). All microsatellite markers were modified for four types of fluorescence dye (FAM, VIC, NED, and PET) in forward primers. Polymerase chain reaction (PCR) was performed in a volume of 20 µl consisting of 10 ng genomic DNA, 2x Multi HS Primer Taq Premix (GenetBio, Korea), 8 pico mole of each forward and reverse primers, and distilled water. The PCR was performed using BIO-RAD T100 Thermal Cycler (Bio-Rad, USA) under following conditions: initial denaturation for 10 min at 95°C, followed by 38 cycles of 30 sec at 95°C, 30 sec at 60°C, and 30 sec at 72°C, with a final extension for 10 min at 72°C. The genotyping reaction consisting of 1 µl of diluted PCR products, 10 µl of Hi-DiTM Formamide (Applied Biosystems, USA), and 0.1 µl of GeneScanTM-500LIZ size standard marker (Applied Biosystems, USA) was performed on a 3130x1 Genetic Analyzer machine (Applied Biosystems, USA). The GeneMapper software version 3.7. (Applied Biosystems, USA) was used for microsatellite fragment analysis.

Statistical analysis. Genetic diversity indices, such as number of alleles per locus (Na), observed heterozygosity (Ho), expected heterozygosity (He), and polymorphism information content (PIC) was calculated using Cervus software ver. 3.0.7 (Marshall *et al.*, 1998). *F*-statistics, including inbreeding coefficient of an individual relative to the subpopulations (F_{IS}), inbreeding coefficient of an individual relative to the total population (F_{IT}), and genetic differentiation index between population (F_{ST}) were calculated using GenAlex ver. 6.501 (Peakall and Smouse, 2012).

Results and Discussion

Genetic diversity indices among the three duck populations are tabulated in Table 1. In total, 121 alleles were detected across loci. The Na values ranged from 2 (APH24, CAUD128, and CAUD009) to 18 (CAUD040 and CAUD048), with a mean of 5.5 alleles per locus. All the observed loci were polymorphic, but 7 loci had less than 4 alleles (AMU003, APH24, CAUD128, AMU123, CAUD009, CAUD086, and CAUD132). Wimmers *et al.* (2000) recommended at least 4 alleles per locus for microsatellites to be informative for estimating genetic diversity. The mean Na in this study is lower than that in other duck populations in Indonesia (Na = 6.8; Maharani *et al.*, 2018) and Asia (Na = 11.5; Sultana *et al.*, 2017).

Expected heterozygosity (He) is the best estimator for analysis of population genetic diversity (Kim *et al.*, 2002). The He values varied between 0.045 (APH24) and 0.917 (CAUD040), with a mean value of 0.509, indicating that the level of genetic diversity among populations was moderate. Meanwhile, observed heterozygosity (Ho) ranged from 0.000 (APH24) to 0.886 (CAUD040), with a mean value of 0.429. Interestingly, the Ho value for APH24 locus was 0.000, suggesting that there is no heterozygous

Table 1. Information on Genbank Accession number, microsatellite marker, and Indicators of genetic variability among populations

No	*GenBank*	Locus	N	Na	Ho	He	PIC	HWE	F_{IS}	F_{IT}	F_{ST}
1	AB180488	AMU003	87	3	0.575	0.633	0.558	NS	0.019	0.087	0.069
2	AJ515884	APH04	87	5	0.126	0.314	0.294	ND	0.550	0.594	0.099
3	AJ515895	APH20	87	4	0.345	0.598	0.511	*	0.396	0.420	0.039
4	AJ515899	APH24	87	2	0.000	0.045	0.044	ND	1.000	1.000	0.047
5	AY493256	CAUD011	87	5	0.529	0.596	0.515	NS	0.103	0.108	0.006
6	AY493276	CAUD031	87	6	0.506	0.554	0.515	NS	0.062	0.081	0.020
7	AY493280	CAUD035	87	6	0.437	0.543	0.496	NS	0.124	0.191	0.076
8	AY493284	CAUD039	87	6	0.552	0.612	0.573	NS	0.043	0.093	0.052
9	AY587030	CAUD111	87	5	0.368	0.541	0.509	NS	0.256	0.316	0.081
10	AY587047	CAUD128	87	2	0.506	0.498	0.372	NS	-0.086	-0.022	0.058
11	AY493285	CAUD040	88	18	0.886	0.917	0.905	ND	-0.015	0.027	0.042
12	AY493311	CAUD066	88	6	0.648	0.661	0.594	NS	-0.016	0.012	0.028
13	AB180602	AMU123	88	3	0.500	0.645	0.565	NS	0.150	0.217	0.079
14	AB180534	AMU52	88	4	0.045	0.045	0.044	ND	-0.022	-0.016	0.006
15	AB180549	AMU68	88	5	0.114	0.151	0.146	ND	0.197	0.243	0.058
16	AJ515887	APH08	88	5	0.727	0.721	0.663	NS	-0.032	-0.014	0.017
17	AY493250	CAUD005	88	5	0.375	0.502	0.459	NS	0.139	0.248	0.126
18	AY493254	CAUD009	88	2	0.239	0.261	0.226	ND	0.066	0.083	0.018
19	AY493289	CAUD044	88	5	0.455	0.516	0.437	NS	0.016	0.116	0.102
20	AY493331	CAUD086	88	3	0.239	0.289	0.267	ND	0.066	0.168	0.110
21	AY587051	CAUD132	88	3	0.420	0.667	0.590	***	0.254	0.363	0.146
22	AY493293	CAUD048	88	18	0.852	0.885	0.869	ND	-0.080	0.032	0.039
Total/average				121	0.429	0.509	0.462		0.148	0.198	0.060

Na: number of alleles per locus; N: number of observed individuals; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphism information content; HWE: test for Hardy-Weinberg equilibrium, *P<0.05, ***P<0.001; NS: not significant; ND: not determined; F_{IS}: inbreeding coefficient of an individual relative to the sub population, F_{IT}: inbreeding coefficient of an individual relative to the total population, F_{ST}: the effect of subpopulations compared with the total populations.

animal observed for this locus. In addition, the Ho values for 17 loci were lower than their He values, indicating heterozygote deficiency, which can be attributed by various factors such as non-random mating, unamplified alleles ("null" alleles) and subdivision in the studied populations (Wahlund's effects). However, further analysis showed that only 2 loci (APH20 and CAUD132) were in Hardy-Weinberg disequilibrium. Two alleles were detected at APH24 locus in this study, with Ho value being 0.000. Other genetic diversity indices, however, showed values close to zero for He (0.045) and PIC (0.044). There was no detected alleles at APH24, as reported by Ismoyowati and Purwantini (2011) in Alabio and Bali duck populations. The results suggest that genetic diversity of Indonesian local ducks for this locus was low.

The PIC values ranged from 0.044 (APH24) to 0.905 (CAUD040), with a mean value of 0.462. For animal traceability, microsatellites with PIC>0.5 are more informative and useful for application of genetics (Botstein, 1980). Accordingly, 12 markers were considered highly informative markers for genetic diversity and population discrimination analysis. These markers are therefore highly recommended for further genetic analysis in other Indonesian duck populations.

F-statistics were estimated in a fixation index as genetic differentiation (F_{ST}), global deficit among eight duck populations (F_{IT}), and the heterozygote deficit within duck populations (F_{IS}). The F_{IS} values ranged from -0.086 (CAUD128) to 1.000 (APH24), with a mean value of 0.148, indicating the occurrence of inbreeding. The F_{IT} values ranged from -0.022 (CAUD128) to 1.000 (APH24), with a mean value of 0.198. The F_{ST} values varied between 0.006 (CAUD011 and AMU52) and 0.146 (CAUD132), with a mean value of 0.060, indicating that the level of genetic differentiation among populations was moderate. About 6% of total genetic variation corresponded to differences between populations, while 94% was explained by differences between individuals.

Conclusions

A considerable genetic diversity was determined among the three duck populations. The level of genetic differentiation among populations was also moderate. The microsatellite panels used in this study are useful for population genetic studies in Indonesian ducks.

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