



Genetic diversity among Indonesian local duck populations in Java Island assessed by microsatellite markers

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ABSTRACT

To date, a number of local duck breeds has been widely raised across Java island of Indonesia, namely Mojosari, Magelang, Rambon, and Turi. In order to evaluate the genetic diversity among them, 22 microsatellite markers were used to genotype of 120 individuals (30 individuals per population). Such indicators, including *number of alleles* (Na), *observed heterozygosity* (Ho), *expected heterozygosity* (He), *polymorphism information content* (PIC), and Wright's F-statistics (F_{IT} , F_{IS} , F_{ST}) were analyzed. A total of 139 alleles were detected in all loci, ranging from 3 (AMU123, CAUD128, CAUD009) to 21 (CAUD048). The mean value of Ho, He, and PIC were 0.465, 0.580, 0.524, respectively, indicating relatively medium genetic diversity among populations. Only 8 loci were not in accordance to Hardy Weinberg equilibrium ($P < 0.01$). Among the four populations, the global heterozygosity deficit (F_{IT}) was 0.197, global inbreeding of individuals within population (F_{IS}) was 0.112, and population differentiation index (F_{ST}) was 0.093. In conclusion, relatively medium genetic diversity and differentiation among populations were successfully estimated using 22 microsatellite markers. The considerable genetic diversity among populations will allow us for future breeding improvement, as well as conservation strategies.

Keywords: Genetic diversity, Microsatellite markers, Indonesian local ducks

Introduction

Because of centuries of domestication and breeding, a wide range of livestock breeds exists today. Duck is one of the most widely distributed livestock species in Indonesia. It plays an important role in providing income and food sources. To date, more than eight local duck breeds have been raised by livestock keepers across Indonesian archipelago. Indonesian local duck breeds seem to possess enormous genetic diversity as the result from various populations and production system developed over time by breeders to fulfil diverse needs in various environmental conditions. Genetic diversity within farm animal species refers to the extent of genetic variation within and among the breeds, strains, and lines in order to preserve the highest intraspecific variability (Lenstra *et al.*, 2012). The availability of wide range of biodiversity allows us for implementing genetic improvement for the local breeds. It is also an important element in sustainable use of animal genetic resource (AnGR) as it allows livestock keepers to adapt their animals to changing conditions (FAO, 2007). Effective management of

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this AnGRs therefore, is needed to be implemented. In addition, understanding the genetic diversity among local breeds or populations, as well as the biological mechanism underlying genetic diversity can increase the successful preservation and utilization of these valuable genetic resources.

Recently, modern technique in molecular genetics has provided a number of genetic markers which can be used to assess the genetic diversity among domestic animals. The genetic markers have been comprehensively exploited to assess genetic variability as they contribute information on every region of the genome, regardless of the level of gene expression (Sharma *et al.*, 2015). Among them, microsatellite marker is the most widely used in genetic diversity studies. Microsatellites, or simple sequence repeats (SSRs), represent codominant molecular genetic markers, which are abundantly distributed across genomes (Chistiakov *et al.*, 2006). Because of the relative ease of scoring and ability to exhibit high level of polymorphisms as well as higher heterozygosities, its application as a genetic appraisal tool is quite significant (Olowofeso *et al.*, 2005). Microsatellite variations have been commonly used for assessing genetic diversity, differentiation, structure, and relationship among indigenous duck breeds, as well as inbreeding levels in many countries (Wu *et al.*, 2008; Ismoyowati and Purwantini, 2011; Mukesh *et al.*, 2011; Gaur *et al.*, 2016; Seo *et al.*, 2016).

To date, a few studies have reported the genetic diversity among Indonesian local duck populations (Ismoyowati and Purwantini, 2011; Rusfidra *et al.*, 2013). The objective of this study was to evaluate the genetic diversity among Indonesian duck populations across Java island using microsatellite markers. Study on genetic diversity of Indonesian local ducks is expected to provide scientific information for designing breeding strategies and conservation plans.

Materials and Methods

Duck samples and DNA Isolation

A total of 120 individuals from 4 Indonesian local duck populations were examined (30 individuals by population). The samples were collected from different breeding regions, Mojosaari (MJ) from BPTU-HPT Pelaihari, South Kalimantan; Magelang (MG) from Magelang, Central Java; Rambon (RM) from Cirebon, West Java; and Turi (TR) from Bantul, Special Region of Yogyakarta region (Figure 1). MJ population was reared under controlled breeding program, while the remaining populations were reared by traditional production system by local farmers. The blood samples were obtained from the ulnar vein. Genomic DNA was extracted from blood samples using the gSYNC DNA Extraction Kit (Geneaid, Taiwan) following the manufacturer's instructions and stored at -20°C before doing PCR amplification.

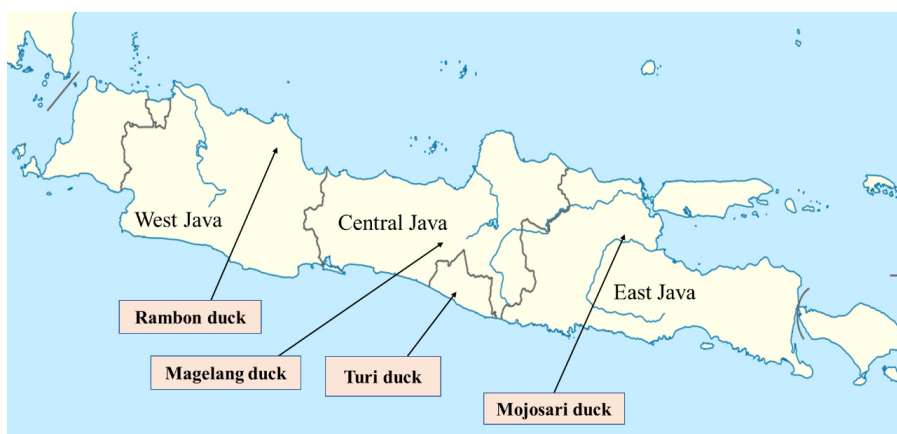


Figure 1. Distribution of four local duck breeds across Java island.

PCR and microsatellite genotyping

Twenty two microsatellite markers were used to assess the genetic diversity (Table 1). The microsatellites were amplified with multiplex PCR using extracted genomic DNA in total volume of 20 μ L. The PCR mixture contained 10 ng/ μ L of duck genomic DNA, 2X Multi HS Prime Taq Premix (Genetbio, Korea), 8 pmol of each forward primers modified by four types of fluorescent dye (FAM, VIC, NED, PET) and reverse primer (Applied Biosystems, USA), and distilled water. The PCR was performed in an initial denaturation at 95°C for 10 min, followed by 38 cycles of 30 sec of denaturation at 95°C, 30 sec of annealing at 60°C, 30 sec of extension at 72°C, and final extension at 72°C for 10 min using BIO-RAD T100™ Thermal Cycler. The genotyping reaction contained of 1 μ L of diluted PCR products, 10 μ L of Hi-Di™ Formamide (Applied Biosystems, USA), and 0.1 μ L of GeneScan™-500 LIZ™ size standard marker (Applied Biosystems, USA). Fragment analysis was performed using the Genetic Analyzer 3730 xl (Applied Biosystems, USA). The fragment size of the PCR products were identified using GeneMapper ver.3.7 (Applied Biosystems, USA).

Table 1. Information of *Genbank Accession number* and indicators used for assessing genetic diversity among populations using 22 microsatellite loci

No	GenBank	Lokus	N	Na	Ho	He	PIC	HWE	F_{IS}	F_{IT}	F_{ST}
1	AB180488	AMU003	117	4	0.590	0.673	0.598	NS	0.047	0.120	0.077
2	AJ515884	APH04	117	5	0.308	0.593	0.508	***	0.438	0.481	0.075
3	AJ515895	APH20	117	4	0.256	0.589	0.512	***	0.499	0.565	0.132
4	AJ515899	APH24	117	5	0.051	0.123	0.119	ND	0.564	0.581	0.040
5	AY493256	CAUD011	117	5	0.521	0.590	0.513	NS	0.088	0.105	0.019
6	AY493276	CAUD031	117	7	0.462	0.502	0.469	NS	0.030	0.072	0.044
7	AY493280	CAUD035	117	5	0.487	0.711	0.667	***	0.281	0.315	0.047
8	AY493284	CAUD039	117	5	0.701	0.724	0.674	NS	-0.003	0.026	0.029
9	AY587030	CAUD111	117	6	0.359	0.720	0.672	***	0.482	0.499	0.035
10	AY587047	CAUD128	117	3	0.513	0.507	0.386	NS	-0.046	-0.019	0.025
11	AY493285	CAUD040	118	17	0.831	0.902	0.890	ND	0.021	0.078	0.058
12	AY493311	CAUD066	118	5	0.610	0.608	0.542	NS	-0.055	-0.010	0.043
13	AB180602	AMU123	118	3	0.466	0.539	0.431	NS	0.044	0.133	0.093
14	AB180534	AMU52	118	6	0.339	0.631	0.557	***	-0.093	0.456	0.502
15	AB180549	AMU68	118	5	0.271	0.266	0.250	ND	-0.048	-0.024	0.023
16	AJ515887	APH08	118	6	0.322	0.659	0.613	***	0.108	0.513	0.454
17	AY493250	CAUD005	118	9	0.610	0.639	0.601	NS	-0.024	0.038	0.060
18	AY493254	CAUD009	118	3	0.288	0.451	0.404	**	0.311	0.359	0.069
19	AY493289	CAUD044	118	4	0.475	0.449	0.378	NS	-0.124	-0.061	0.056
20	AY493331	CAUD086	118	6	0.415	0.362	0.314	NS	-0.215	-0.157	0.048
21	AY587051	CAUD132	118	5	0.475	0.611	0.527	**	0.157	0.233	0.078
22	AY493293	CAUD048	120	21	0.875	0.915	0.905	ND	-0.005	0.040	0.035
Total/mean				139	0.465	0.580	0.524		0.112	0.197	0.093

Na: number of alleles per locus; N: number observed individuals; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphism information content; HWE: test for Hardy-Weinberg equilibrium, ** $p < 0.01$, *** $p < 0.001$; 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.

Genetic diversity analysis

The genotyping data were used to analyze genetic diversity among populations. Number of allele (N_a), expected heterozygosity (H_o), observed heterozygosity (H_e) and polymorphism information content (PIC) were analyzed using Cervus 3.0 program (Marshall *et al.*, 1998). The 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}), genetic differentiation index between population (F_{ST}) were calculated using GenAlex version 6.501 (Peakall and Smouse, 2012).

Results

Number of alleles per locus (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e), and polymorphism information content (PIC) for each locus across all populations were summarized in Table 1. A total of 139 alleles were detected across 22 microsatellite loci, ranging from 3 (CAUD128, AMU123, CAUD009) to 21 (CAUD048), with a mean value of 6.32 alleles per locus. The H_o and H_e values in all loci ranged from 0.051 (APH24) to 0.875 (CAUD048) with a mean value of 0.465, and 0.123 (APH24) to 0.905 (CAUD048) with a mean value of 0.580, respectively. The PIC values for each locus varied from 0.119 (APH24) to 0.905 (CAUD048), with a mean value of 0.524. For the Wright's F -statistics, the mean value of F_{IS} , F_{IT} , and F_{ST} among all loci were 0.112, 0.197, and 0.093, respectively (Table 1). All twenty two loci utilized in the present study were found to be polymorphic in all populations studied.

Discussion

To evaluate the genetic diversity among duck populations, the number of alleles, H_o , H_e and PIC values are the basic measures, providing important information for the discrimination of individuals and populations (Seo *et al.*, 2016). The level of variation depicted by the number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of breeds within a species (Buchanan *et al.*, 1994). The mean number of alleles further reflected the medium to high level of genetic diversity among studied populations. The mean number of alleles in all loci across all analyzed populations ($N_a = 6.95$) were lower than those of Beijing duck ($N_a = 10.7$, Wu *et al.*, 2009), Southeast Asian ducks ($N_a = 9.38$, Seo *et al.*, 2016), and Asian ducks ($N_a = 11.5$, Sultana *et al.*, 2017).

In our study, 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 0.465 and 0.580, respectively. Furthermore, based on the PIC value, there were 14 microsatellite loci which had high PIC values ($PIC > 0.5$), indicating that these microsatellite loci had high diversity and can be employed to reflect the genetic relationship among duck populations on molecular level. Bolstein *et al.* (1980) classified the PIC value as highly informative ($PIC > 0.50$), reasonably informative ($0.50 > PIC > 0.25$), and slightly informative ($PIC < 0.25$). Using the same microsatellite loci, the PIC values in this study (Table 1) was lower than reported by Seo *et al.* (2016) in Southeast Asian ducks ($PIC = 0.584$) and Sultana *et al.* (2017) in Asian duck populations ($PIC = 0.602$). Interestingly, the CAUD048 had the highest PIC value (0.905) in this study. The results were similar to those of some studies in which PIC values of the CAUD048 were also high with value 0.932 (Seo *et al.*, 2016) and 0.942 (Sultana *et al.*, 2017). It suggested that CAUD048 had high diversity and discriminating power to distinguish among individuals and populations. In contrast, the APH24 had the lowest PIC value (0.119), whereas the number of alleles detected in this locus was 5. In previous study, null (0) alleles and 0.000 for PIC in the APH24 of Alabio and Bali ducks had been reported (Ismoyowati and Purwantini, 2011). Moreover, the PIC value of APH24 was 0.502 in Southeast Asian ducks (Seo *et al.*, 2016), and 0.602 in Asian duck populations (Sultana *et al.*, 2017), suggesting that the APH24 had lower variation in

Indonesian duck populations compared to Asian duck populations.

However, the observed heterozygosity in all loci were lower than expected value, indicating that microsatellite loci pointed towards the deficit of heterozygotes and a departure from Hardy-Weinberg equilibrium (HWE). The test for HWE confirmed that in 8 out of 22 loci significantly deviated from HWE (Table 1). Various factors can contribute towards deficit of heterozygotes. First, the locus is under selection. Second, 'null alleles' may be present which are leading to a false observation of excess homozygotes. Third, inbreeding may be common in the population. Fourth, the presence of population substructure may lead to Wahlunds' effect (Nei, 1987; Peter *et al.*, 2007) and genotyping errors likely due to low sample quality (Morin *et al.*, 2009). The deviation from HWE indicated that these loci might were under selection pressure and the occurrence of inbreeding in some loci across all populations.

In addition, we also evaluated the genetic diversity among populations using Wright's F -statistic. The F -statistic (F_{IS} , F_{IT} , F_{ST}) give an explanation in terms of inbreeding coefficient and population differentiation. The inbreeding coefficient of an individual relative to the subpopulations (F_{IS}), and inbreeding coefficient of an individual relative to the total population (F_{IT}) may be positive values or negative values, whereas, the estimated value of genetic differentiation index between population (F_{ST}) were always positive. Wu *et al.* (2008) reported that when there is no differentiation, the value of F_{ST} is 0; when alleles among populations are quite different, the value of F_{ST} equals 1. In this study, the overall F_{IS} value (0.112) showed that the loci observed were higher than zero. Only 7 out of 22 loci had negative value for F_{IS} (Table 1). The APH24 had the highest coefficient inbreeding with positive F_{IS} value (0.659) indicating the presence of inbreeding and low heterozygotes in this locus.

Moreover, the mean F_{ST} value of 0.093 among all duck populations indicated that only 9.30% of the genetic variation was between the populations, while 90.70% was within the populations. Furthermore, based on the results suggested that there was a moderate genetic differentiation among these duck populations. The level of genetic differentiation among Indonesian duck populations was higher to those reported in Chinese (Beijing and Cherry Valley) ducks, $F_{ST} = 0.075$ (Wu *et al.*, 2009) and Chinese native chicken, $F_{ST} = 0.013$ (Ding *et al.*, 2010), but lower than Chinese indigenous laying-type ducks, $F_{ST} = 0.184$ (Su and Chen, 2009), and quite similar to Asian duck populations, $F_{ST} = 0.135$ (Sultana *et al.*, 2017).

Overall, among duck populations studied, deficit heterozygotes and low inbreeding level have been observed. The most possible reason being related to this phenomenon was that in all populations (except Mojosari) were likely in uncontrolled mating by using unequal sex ratio of breeding ducks. In farmer's level furthermore, to improve the performance of the ducks in producing egg, they also practiced a selection based on plumage colour or production performance. The farmers selected the plumage colour as they expected the ducks which have a certain plumage colour, would produce higher eggs in quantity and quality. Therefore, this non random mating might could result on deficit heterozygotes. Mating system between the ducks was also still unclear as many of them didn't have any pedigree record, as well as limited stock of males in the flocks, leading to the uncontrolled use of males and females in mating practices.

In conclusion, all microsatellite markers used in this study could reflect medium genetic diversity and differentiation among duck populations of Indonesia in Java island. Inbreeding coefficient of an individual relative to the subpopulations (F_{IS}) was positive in several duck populations. It is therefore needed to establish a well-planned and organized breeding system to avoid inbreeding depression. The gene diversity level in all populations furthermore, can provide basic information for breeding and conservation strategies to protect this prestigious gene diversity among Indonesian duck populations. However, combining the valuable genetic information from molecular analysis using these microsatellite markers and phenotypic traits analysis should be taken together as a valuable information to initiate the conservation and preservation program of genetic resources among these duck populations.

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References

1. Bolstein D, White RL, Skolnik M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 32:314-331.
2. Buchanan FC, Galloway SM, Crawford AM (1994) Ovine microsatellites at the OarFCB5, OarFCB19, OarFCB20, OarFCB48, OarFCB129 and OarFCB226 loci. Ovine microsatellites at the OarFCB5, OarFCB19, OarFCB20, OarFCB48, OarFCB129 and OarFCB226 loci. *Anim Genet* 25:60.
3. Chistiakov DA, Hellemans B, Volckaert FAM (2006) Volckaert. Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Aquaculture* 255:1-29.
4. Ding FX, Zhang GX, Wang JY, Li Y, Zhang LJ, Wei Y, Wang HH, Zhang L, Hou QR (2010) Genetic diversity of a Chinese native chicken breed, Bian chicken, basen on twenty-nine microsatellite markers. *Asian-Aust J Anim Sci* 23:154-161.
5. FAO (2007) Status of animal genetic resources. In: The state of the world's animal genetic resources for food and agriculture (Ed. B. Rischkowsky and D. Pilling). Commission on Genetic Resources for Food and Agriculture, Rome, Italy.
6. Gaur U, Tania MS, Mishra B, Kumar STB, Vijn RK, Chaudhury A (2016) Population structuring of land and coastal ducks (*Anas platyrhynchos*) using microsatellite markers. *Curr Sci* 110:1977-1983.
7. Ismoyowati, Purwantini D (2011) Genetic variability of Bali and Alabio ducks on the basis of phenotypic and microsatellites. *Asian J Poult Sci* 5:107-115.
8. Lenstra JA, Groeneveld LF, Eding H, Kantanen J, Williams JL, Taberlet P, Nicolazzi EL, So'lkner J, Simianer H, Ciani E, Garcia JF, Bruford MW, Ajmone-Marsan P, Weigend S (2012) Molecular tools and analytical approaches for the characterization of farm animal genetic diversity. *Animal Genetics* 43:483-502.
9. Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* 7:639-655.
10. Morin PA, Leduc RG, Archer FI, Martien KK, Huebinger R, Bickham JW, Taylor BL (2009) Significant deviations from Hardy-Weinberg equilibrium caused by low levels of microsatellite genotyping errors. *Mol Ecol Res* 9:498-504.
11. Mukesh, Javed R, Gaur U, Jianlin H, Sathyakumar S (2011) Cross-species applicability of chicken microsatellite markers for investigation of genetic diversity in Indian duck (*Anas platyrhynchos*) populations. *Afr J Biotechnol* 10:17623-17631.
12. Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York, USA.
13. Olowofeso O, Wang JY, Dai GJ, Yang Y, Mekki DM, Musa HH (2005) Measurement of genetic parameters within and between Haimen chicken populations using microsatellite markers. *Int J Poult Sci* 4:143-148.
14. Peakall R, Smouse PE (2012) Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and reasearch-an update. *Bioinformatics* 28:2537-2539.
15. Su Y, Chen GH (2009) DNA microsatellite analysis of genetic diversity among Chinese indigenous laying-type

ducks. *Czech J Anim Sci* 54:128-135.

16. Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G, Erhardt G, the ECONOGENE Consortium (2007) Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Anim Genet* 38:37-44.
17. Sultana H, Seo D, Choi NR, Kim YS, Manjula P, Bhuiyan MSA, Heo KN, Lee JH (2017) Genetic diversity analysis of Asian duck populations using 24 microsatellite markers. *Korean J Poult Sci* 44:75-81.
18. Rusfidra Y, Heryandi, Jamsari, Rahman EY (2013) Variasi genetik Itik Bayang berbasis marka mikrosatelit pada lokus AY287 dan lokus AY283. *Sains Peternakan* 11:91-98.
19. Seo D, Bhuiyan MSA, Sultana H, Heo JM, Lee JH (2016) Genetic divesity analysis of South and East Asian duck populations using highly polymorphic microsatellite markers. *Asian Australas J Anim Sci* 29:471-478.
20. Sharma R, Kishore A, Mukesh M, Ahlawat S, Maitra A, Pandey AK, Tania MS (2015) Genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers. *BMC Genetics* 16-73.
21. Wu Y, Liu XL, Hou SS, Huang W (2008) Study on genetic diversity of six duck populations with microsatellite DNA. *Asian-Aust J Anim Sci* 21:776-783.