The Genetic Variation and Distance of Local Duck in West Sumatera

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Abstract

The basic difference between the qualitative and quantitative characteristics were involves a number of genes that contribute to phenotypic variability. For conservation purpose, identification of phenotype and genetic variations for future development is required. The aims of this research are to determine: (1) heterozygosity value and (2) genetic distance of three population local duck in West Sumatera (Pitalah, Kamang and Bayang). For measurement of heterozygosity and genetic distance, 25 ducks were selected randomly from each population group for DNA analysis by using DNA microsatellite markers which used Popgene32 and MEGA 5 for get Heterozygocity value and dendogram genetic distance. The genetic variation analyses showed that heterozygocity value of Bayang was largest (0,56) than Kamang (0,49) and Pitalah duck was smallest (0,44) .The genetic distances analyses showed that Kamang had relatively closer distance with Bayang (0,1393) when compared to Pitalah (0,1649). In the other hand, genetic distances analyses between Bayang and Pitalah was further distance (0,2590). This experiment suggest that Pitalah had more differences characteristics, but Kamang and Bayang had more similarity.

Keywords*: Qualitative Character, West Sumatera Duck; Genetic Distance; Plumage Color*

INTRODUCTION

Indonesia has long been known as one of mega biodiversity countries in the world. However, some of the plant and animal populations have been decreasing in the last decades likes West Sumatera Local duck, namely *Pitalah, Kamang and Bayang* (Suhaemi et al., 2016). Ducks are a source of Indonesian livestock biodiversity which has the opportunity to be developed as a producer of eggs and meat. They are originated and distributed in West Sumatera Indonesia. Pitalah and Kamang duck represents ones of the Indonesia fauna species which has started to decrease and undoubty will extinct, despite the glorifying efforts. This reason also allows ducks to be developed on an industrial scale(Onba & Erdem, 2011). The concervation and management practices of this species could be improved through a better understanding of the genetic diversity and structure of them. Yet, their potential has not been fully developed. Therefore, this study was conducted to improve the genetic quality of Pitalah,

kamang and Bayang ducks to be used as economic resources for the community (Suhaemi et al., 2023).

One possibility is the use of RAPD and DNA markers, because DNA marker behaves like a microsatellite can be found in a large numbers. DNA is a compound that contains the genetic information of living things from one generation to the next. The entire DNA in a cell will form the genome. The genome includes the functional and non-functional parts of the genes in an organism's cells. Genomic DNA includes both genes and intergens (Yuwono, 2006). In the other hand, it is well distributed in genom with high polymorphism. Unlike microsatellite for chicken, the microsatelite for duck is rare and depends on the types of duck. Microsatellites are deemed to be one of the most valuable genetic markers (William, 2005). In this paper, we investigated the genetic diversity of three duck population (Pitalah, Kamang and Bayang) and Java duck just for compare with 17 microsatellite loci.

MATERIAN AND METHODS

Population samples and microsatellite loci

Twenty of duck each groups were randomly chosen for phylogenetic relationship analisys used microsattelite loci. Twelve microsatellite primers were designed based on the report of (Yan; et al., 2008), but 3 of them can not used for local duck, sequences of primers are listed in Table 1. Each duck blood (1 ml) was collected into a tube containing anticoagulant and then preserved in a -20 °C freezer.

Genomic DNA extraction and PCR condition

Genomic DNA was isolated with (Su et al., 2007) method. Blood was digested in 300 µL lysis buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, 100 mmol/L NaCl, pH 8.0) with 8 µL proteinase K (10 mg/mL) for 12 h at 55^0C. The extraction was repeated three times. After precipitation by adding two volumes of ice-cold ethanol, DNA was isolated by centrifugation and then stored at -20^0C for future use. DNA pellets were re-suspended in 30 µL TE buffer, and the total genomic DNA was quantified using agarose gelelectrophoresis. The DNA concentration was calculated according to the standards.

The PCR primers are listed in Table 1 that were synthesized by the Eurofins MWG GmbH in Germany. PCR amplifications were performed on Biometra T Gradient thermal cycle from Promega. Coctail for running PCR total reaction of 20 µL, with 2 µL of 10x buffer, 13,8 µL ddH2O, 1 µL dnTP, 0,5 µL F primer, 0,5 µL R primer, 0,2 µL Tag Polymerase and 2 µL DNA sample that approximately 50 ng of genomic DNA. The reaction was carried out by denaturing at 94^oC for 1 min, annealing at the temperature optimized for each primer for 1 min and extending at 94^oC for 1 min for 30 cycles, followed by an extra extention step at 72^oC for 10 min. The optimized annealing temperature of different primer pairs are also listed in Table 1. The amplification product were separated by electrophoresis on 2% gel agarose along with DNA ladder (GeneRuler^{TM} 100bp) and visualized by Gel Doc. Table 1 also described primer sequence and annealing temperature for each locus of DNA microsattelitte. There are variation of alelle number from 3 until 8 alelle, and alelle size in base paire units.

Table 1. Microsatellite primer sequences and annealing temperature (^{0}C)

Locus	Primer Sequence	Tm (°C)	Allele no.	Allele size (bp)
AY283	GACCACAACATCGTGCAGAG GATAATGGCTGGCTCCTTGA	62,0	6	211 – 371
AY287	TGCAGGTAGGTCTTCTGTTCTG GCCAGTCCTTTGCTTCGTAA	60,0	8	154 – 294
AY295	GGCTTCTGTGCTCCTCAGAT GGACAAGTGGCATGTGTCAT	56,0	8	253 – 403
AY264	GCAGACTTTTACTTATGACTC CTTAGCCCAGTGAAGCATG	58,0	6	114 – 284
AY285	TCCCACCCCAAACCCTGC TGTGTAACCCGATAGACTGA	52,0	4	251 – 341
AJ581	ATTAGAGCAGGAGTTAGGAGAC GCAAGAAGTGGCTTTTTTC	58,0	3	159 – 289
AY314	CTCATTCCAATTCCTCTGTA CAGCATTATTATTTCAGAAGG	58,0	4	117 – 317
AY294	TGTAGTTTAGTTGCTGGATA TTAGTAAACTCTTGCCATCT	52,0	4	200 – 310
AY301	GCTTTAGTTTTTCAATTAGGTA TGGTGCGATGAGCTGAGAT	52,0	4	117 – 477

Statistical analysis

Analysis of microsatellite DNA polymorphism data at this stage of research includes: mean and standard deviation of heterozygosity also cluster analysis diagrams. Analysis genetic distance. For phylogenetic analysis, its use software Genepop (Version 3.3) to got data Genetic distance. The genetic distance between the breeds will be calculated based on the frequencies of alleles of the microsatellite. Furthermore, they will be classified into some groups, based on the genetic diversity by counting the heterozygocity of alleles and dendogram is using MEGA 5 program.

RESULTS AND DISCUSSION

Genetic variation

As heterozygosity value ($\hat{h}$) is the most accurate way to measure genetic variation, it is also called as gene variation (Nei & Kumar, 2000). From eight loci used for local ducks of West Sumatera, the highest mean heterozygosity (0.5971) was found in AY283 locus and the lowest (0.4137) was found in AY264 locus (Table 5). The highest *h* value (0.6316) for AY283 locus was found in Kamang duck group and the lowest (0.5714) was found in Pitalah duck group. Similarly, for AY287 locus, the highest *h* value (0.5789) was found in Kamang duck group and the lowest (0. 4286) was found in Pitalah duck group. For AY295 locus, the highest *h* value (0.6316) was found in Kamang duck group and the lowest (0.4118) was found in Bayang duck group. Kamang ducks had the highest *h* value in three loci, namely AY283, AY287, and AY295.

Five loci consisting of AY264, AY285, AY314, AY301, and AY294 were found to have high DNA heterogeneity in Bayang ducks. The highest *h* value (0.5294) of AY264 locus was found in Bayang duck group followed by the *h* values in Kamang duck group (0.4737) and Pitalah duck group (0.2381). AY285 locus had the highest *h* value (0.7059) in Bayang duck group and the lowest (0.3158) in Kamang duck group. The same pattern was found in AY314 locus where the highest *h* value (0.6429) was found in Bayang duck group and the lowest (0,4667) was found in Kamang duck group. In AY301 locus, the highest *h* value (0.7143) was found in Bayang duck group and the lowest (0.4167) was found in Kamang duck group. In AY294 locus, Bayang duck group was found to have the highest *h* value (0.4375) and Pitalah duck group had the lowest *h* value (0.2500). The availability of heterozygosity values ($\hat{h}$) is important in the analysis of population variation. (Muladno, 2002) stated that high $\hat{h}$ value indicated inbreeding (endogamy degree) as a result of intensive selection. (Moioli et al., 2004) stated that, in general, $\hat{h}$ value is a good indicator to describe the genetic variation of an animal population.

Pitalah duck group was not shown to have the highest $\hat{h}$ value in all loci. As explained by (Frankham et al., 2002), mean of heterozygosity is a parameter showing the genetic variation of a species or population. It was shown in this study that Pitalah ducks had less genetic variations compared to Kamang and Bayang ducks. This also indicated that breeding with other duck types was rare.

Bayang ducks group was found to have $\hat{h} > 0.60$ in 3 loci (AY285, AY314, and AY301) indicating that this group of ducks had high allelic variation. The $\hat{h}$ value higher than 0.60 was found in 2 loci (AY283 and AY295) in Kamang duck group and only in 1 locus (AY314) in Pitalah duck group. However, in Kamang and Pitalah duck groups, this $\hat{h}$ value was not the highest. These findings suggested that Bayang ducks had dominantly high gene variation which might be caused by several factors including migration, mutation, and breeding (Bourdon, 2000). This is reflected in the mean $\hat{H}$ values of each group as shown in Table 3. (Nei & Kumar, 2000) stated that heterozygosity of a population is measured by using mean $\hat{H}$ values if the observed loci are more than one.

Table 2. Heterozygosity value ($\hat{h}$) and mean heterozygosity ($\hat{H}$) of each microsatellite locus.

No	Locus	Pitalah	Kamang	Bayang	$\hat{H}$
1	AY283	0,5714	0,6316	0,5882	0,5971
2	AY287	0,4286	0,5789	0,4706	0,4927
3	AY295	0,5238	0,6316	0,4118	0,5224
4	AY264	0,2381	0,4737	0,5294	0,4137
5	AY285	0,3333	0,3158	0,7059	0,4517
6	AY314	0,6190	0,4667	0,6429	0,5762
7	AY301	0,5882	0,4167	0,7143	0,5731
8	AY294	0,2500	0,4118	0,4375	0,4698
$\hat{H}$		0,4440	0,4908	0,5626	

The genotypes revealed from PCR results of DNA templates for each microsatellite marker were analyzed based on their appearance frequency as listed in Table 2. An example of ribbon (fragment) pattern of DNA formed in AY264 locus in depicted in Figure 1.

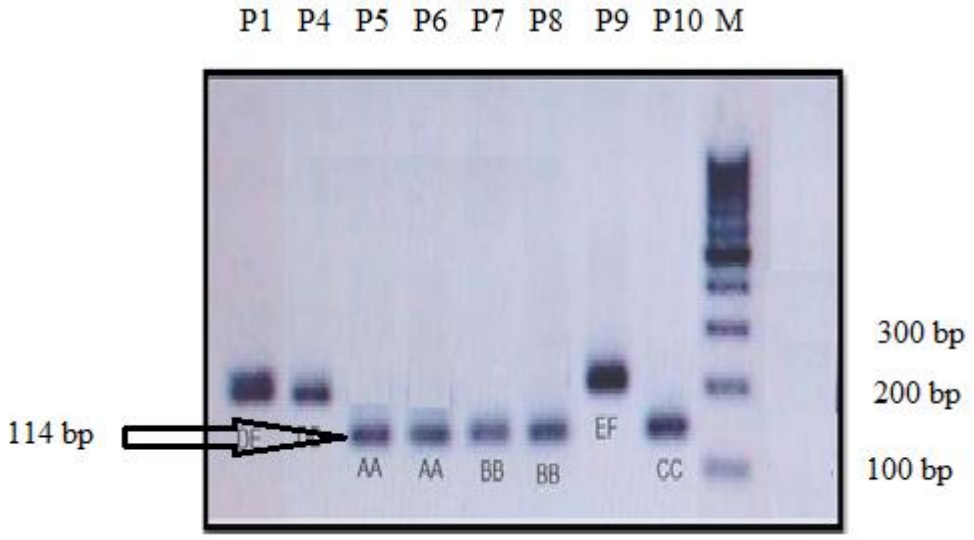

Figure 1. Fragment pattern of DNA formed in AY264 locus (1,5% Gel Agarose).

Genetic Distance

The genetic relationships between the breeds of domestic ducks, which are well known in West Sumatera, will be analyzed. *Pitalah* Duck, *Kamang* Duck and *Bayang* Duck are local duck in West Sumatera. Beside that three population, for compare the genetic relationship, it used other island duck from Java namely Mojosari Duck. There are many methods to measure the genetic distance, (Nei & Kumar, 2000) show that average codon margin of each locus can be estimated by the gene frequency of abundant loci.

Table 3. Genetic distance (Gd) local duck in West Sumatera.

Population	Pitalah	Kamang	Bayang	Mojosari
Pitalah	****			
Kamang	0,1649	****		
Bayang	0,2590	0,1393	****	
Java duck	0,2821	0,2097	0,2617	****

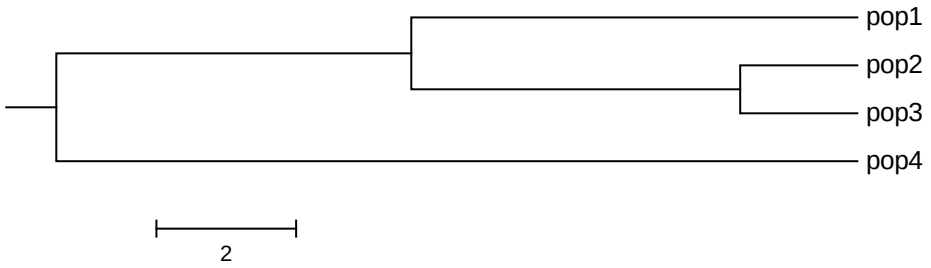

Figure 2. Dendogram genetic distance from Pitalah duck (Pop 1), Kamang duck (Pop 2), Bayang duck (Pop3) and Java (Mojosari) duck (Pop 4).

The dendrogram of populations in Figure 2 reflected the genetic relationships of populations and genetic distance (Table 3). The four populations clustered into three groups: one group only included Pitalah duck, while Kamang and Bayang duck was clustered into another group, and Mojosari duck clustered into another one. Kamang duck had the shortest genetic distance with Bayang duck, followed by Pitalah duck and Mojosari duck. The result of this cluster was consistent with breeding history and geographical locations. This particular important for conservation of duck genetic diversity on farm management or on-farm conservation, because the combination of farmers' diverse needs together with the breeds in different ecosystems has created and accumulated wide genetic variation. This study revealed a high genetic diversity between Java duck (local duck in Java), Pitalah Duck and other Local duck in West Sumatera. But Kamang duck and Bayang duck have less genetic diversity, it may tend to have experienced crossbreeding with other breeds such as Pegagan duck, Tegal, Alabio or Mojosari ducks (from Java).

CONCLUSION

The genetic distances analyses showed that Kamang had relatively closer distance with Bayang (0,1393) when compared to Pitalah (0,1649). In the other hand, genetic distances analyses between Bayang and Pitalah was further distance (0,2590). This experiment suggest that Pitalah had more differences characteristics, but Kamang and Bayang had more similarity.

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