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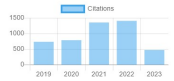


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# Genetic Diversity of Kedu Chicken Based on Phenotypic Characteristics and Microsatellite Loci

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**Abstract:** This study was aimed to evaluate the genetic diversity based on phenotype and the genetic relationship between four kinds of Kedu chicken, using 4 microsatellite markers. The result from the phenotype observations showed that the four chicken breeds have qualitatively different plumage, skin, comb and shank colours. Cemani chickens' have lower egg production than others. The results from the isolation and identification of DNA using microsatellite primers showed that the 4 primers were polymorphics. The highest polymorphic information contain values based on locus derived from the entire population was LEI 0147 (0.643), while the average polymorphic information contain value on each population were 0.362, 0.531, 0.482 and 0.568 for Cemani, white Kedu, red Kedu and black Kedu chickens, respectively. Estimation of the heterozygosity value on loci of different populations of Kedu chickens showed a large variation (0.618-0.743). Genetic distance analysis showed that among Kedu chickens had a genetic relationship ranging from 0.018 to 0.236. The conclusion was that the genetic diversity based on chicken phenotypes and based on microsatellite markers in the population of Kedu chickens indicated a high diversity and had a relatively distant genetic relationship.

**Key words:** Genetic distance, heterozygosity, Kedu chickens, microsatellite

## INTRODUCTION

Domesticated chicken have long history, both in the genetic research and as human food source. Chicken is a species with large genetic diversity and it is the first species used for heredity experiments by Mendel. Chicken also the first animal that have genome sequenced and have been used for genetic experiments (Siegel *et al*., 2006). The population of chicken is big which is about 11 billion heads (Dohner, 2001). Daikwo *et al*. (2011) divided animal farming system in the developing countries into: (1) commercial and (2) traditional in which both serve as human food source. Traditional animal farming system consists of local chicken that cannot be distinguished based upon their breeds despite their variability is high.

In Indonesia, some indigenous chicken are found, one of them is Kedu chicken. There are different kinds of Kedu chicken i.e., Cemani, Black Kedu, Red Kedu, White Kedu and Lurik Kedu that have specific characteristics and are potential to be conserved and developed as indigenous germ plasma. Identification and characterization of Kedu chicken are really needed to study the existence for the genetic improvement program in Indonesia. The chicken identification can be done mainly on the phenotype both qualitatively (feather colour, skin, shank and comb size) and quantitatively (morphometric, productivity and resistance to diseases or parasites). Descriptive phenotype identification is needed to know the specific characteristics of Kedu chicken that visually-clearly can be differentiated from other kinds of local chicken. Identification for chicken can also be done through biomolecular to know the genetic diversity. The study of genetic diversity can be used to analyze the population structure of a breed in a country for conservation purposes (Zanetti *et al*., 2010).

Genetic diversity and genetic distance studies can be done through some methods. One of the methods is analysis of molecular genetic diversity rapidly developing through DNA techniques. On the development field, genetic characteristics are needed to maintain animal breed integrity and is a prerequisite for genetic management. Among the so many molecular markers available today, microsatelites are frequently used to determine genetic variability within and between breeds because the polymorphic information obtained are abundance, many genotypes are obtained and the PCR amplification is simple (Rosenberg *et al*., 2002). Microsatelites showing DNA polymorphism are easily transferred to separate genetic sources in the population related with QTL and almost the segregation of DNA polymorphism can be used as genetic marker. DNA location in the genome can be used to map the genetic relationship in chicken within the same species (comparative mapping) (Siegel *et al*., 2006). SNP or microsatellite markers are very limited to the specific target enzyme (Emara and Kim, 2003). Recently, some studies using microsatelite markers aimed to evaluate genetic diversity and genetic relationship within and

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between various kinds of local chicken (indigenous) as well as commercial ones included in the jungle fowl (Wimmers *et al*., 2000; Romanov and Weigend, 2001; Zhang *et al*., 2002; Hillel *et al*., 2003; Osman *et al*., 2006; Tadano *et al*., 2007). This study assessed the genetic diversity of Kedu chicken based on both their phenotypic and genetic based on microsatelite loci.

## MATERIALS AND METHODS

This research included Kedu chicken raised in Makukuhan Mandiri Farmer Groups in Kedu district, Temanggung regency, Central Java province, Indonesia. The genetic diversity assessment based on microsatelite markers included 24 adult male Kedu chicken and 24 adult female Kedu chicken. As many as 133 Kedu chicken male and female of 54 weeks age were involved in observation of the phenotypic characteristics including feather, skin, comb, wattles and shank. Chemical reagent used to isolate DNA and PCR as well as electrophoresis were EDTA functioning as anticoagulant, kit for DNA isolation, 4 microsatelite primers (Table 1), PCR core kit, agarose, TBE solution and ethidium bromide. Equipments used in the study included digital scale, measure tape, analytic scale, measure cup, syringes, efendorf, sterile tubes, micropipette, blue type, yellow type, centrifuge, PCR machine, horizontal electrophoresis and DNA documenting tools.

Kedu chicken sampled in the study had the same age. As many as 48 chicken were used for genetic diversity characterization. The Kedu chicken studied consisted of Black Kedu, Cemani Kedu, White Kedu and Red Kedu. Blood samples were taken by syringe on the intra-axillaries vein as much as 3 ml and the samples were then put into tubes filled with EDTA for DNA analysis. DNA extraction was done by DNA purification kit (Dneasy Blood and Tissue Kit Qiagen).

PCR was done by mixing 15 μl green mix, 11 μl $H_2O$, 1 μl forward primer and 1 μl reverse primer added with 2 μl DNA. PCR was done in Amplitron® II Thermolyne with the following cycle: 5 minutes predenaturation of 94°C, 30 seconds denaturation of 94°C, 45 seconds annealing of 50°C, one minute Ellongation of 72°C, 5 minutes post elongation of 72°C. The PCR was done in 35 cycles. The individual PCR product was then separated by electrophoresis through 2% agarose gel.

Table 1: Primary microsatellites that were used in this study

| Microsatellites | Primary | Base sequence |
|---|---|---|
| ADL0022 | F | 5'-GCATCAGAGGAAGAAGGAAA-3' |
| | R | 5'-GGTCAAGGAAATCATAGAAA-3' |
| ADL217 | F | 5'-TCTACTTCGTTGGAGTGTCA-3' |
| | R | 5'-GGAAAACAGAGGAGAAATGG-3' |
| LEI0147 | F | 5'-TCTGACAATTGGAAGGGATGGC-3' |
| | R | 5'-ATGGCAGTGTGCATGTGTGG-3' |
| ADL0273 | F | 5'-GCCATACATGACAATAGAGG-3' |
| | R | 5'-TGGTAGATGCTGAGAGGTGT-3' |

Qualitative characteristics data were then tabulated and analyzed descriptively while quantitative characteristics data were analyzed with analysis of variance. If the kind of Kedu chicken had significant effect on the observed phenotypes then the Honesty Significant Test was done. The total genetic diversity of different kinds of Kedu chicken and the average genetic diversity was measured based on DNA polymorphism of the microsatelites loci and average Heterozygosity (H). Gene frequencies were calculated following Pirchner (1981):

$$FA_n = \frac{\sum \text{locus } A_n}{\sum \text{locus } A_1 + \sum \text{locus } A_2 + .... + \sum \text{locus } A_n}$$

Fan: Gene frequency of A of $n^{th}$ locus

The genetic variation of the population was computed by Heterozygosity formula after Nei (1987):

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

Where:

h : Heterozygosity
m : Number of alleles
xi : Gene frequency of ith allele

The average Heterozygosity (H) is defined as the average of H of all loci included in the study:

$$H = \frac{1 - \sum_{i=1}^{m} x_i^2}{R} = \frac{h}{r}$$

where, r = number of loci observed

The genetic Distance (D) measures were computed from gene frequency data of all loci using formula of Nei (1987):

$$D = \log_e I$$

where, I = genetic similarity between two populations, computed following the formula:

$$I = \frac{\sum X_{ij} Y_{ij}}{\left(X_{ij}^2\right)\left(Y_{ij}^2\right)}$$

$X_{ij}$ : ith allele frequency, jth locus of population X
$Y_{ij}$ : ith allele frequency, jth locus of population Y

Polymorphism Information Content (PIC) is a value showing the polymorphism within a population based on a molecular marker. PIC value depends on the number and distribution of the detected alleles:

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}$$

$P_{ij}$ : allele frequency of marker I

## RESULTS AND DISCUSSION

**Phenotypic characteristics of kedu chicken:** The results of phenotypic characteristics observation of four kinds of Kedu chicken are presented in Table 2. The phenotypic characteristics observation was done on 30 heads of male Cemani Kedu, 50 heads of female Cemani Kedu, 9 heads of male White Kedu, 14 heads of female White Kedu, 7 heads of male Black Kedu and 12 heads of female Black Kedu.

Cemani chicken have completely black phenotypic characteristics including feather, skin, comb, wattles and shank. They have buttercup and pea comb (2.5%) versus single comb (97.5%) of the population. Other type of Kedu chicken have cushion and pea comb (6%) versus single comb (94%). Black Kedu and Red Kedu chicken have blackish red comb and wattles. White Kedu chicken have the same colour of comb and wattles as Red Kedu chicken. Shank colour of Kedu chicken varies from black, grey, blackish green and yellow. Daikwo *et al*. (2011) reported the distinction of phenotypic characteristics of Dekina local chicken including plumage colour, shank colour, type of comb. Plumage colour varies ranging from black-brown, brown, white, black-white, black and brown-black-white. The chicken have shank colour of black, greenish black and white. The shape of comb varies from pea, single and rose with the majority of single comb.

Different kinds of Kedu chicken have similar body weight being males have more body weight than females. Cemani chicken have lowest egg production compared with other kinds of Kedu chicken. Sulandari *et al*. (2006) reported the average body weight of Cemani chicken of 2.33+0.5 kg (males) and 1.91+0.35 kg (females). Egg production were reported to be 56-77 eggs per year in both extensive and semi-intensive management. In an intensive management, the egg production achieved 215 eggs per year (Iskandar, 2005). White Kedu chicken have body weights of 1.73+0.67 and 1.28+0.27 kg (Sulandari *et al*. 2006) with 197 egg production per year (Sulandari *et al*., 2007). Black Kedu chicken is somehow good layer with egg production of 71 per year for a period of 20 week observation (Nataamijaya and Sitorus, 1992).

**Genetic analysis of Kedu chicken by microsatelites characteristics:** The DNA isolation and identification based on four microsatelite primers showed polymorphism (Table 3). Allele sizes varied from ranging 100-250 bp with the biggest allele size of 150-250 bp (LEI 0147 allele). The number of alleles of each locus varied from 2 to 3. The total number of genotypes of the four microsatelite primers in the population was 18. The highest PIC value based on all loci was 0.643 (LEI 0147 locus/allele) and the lowest PIC value was 0.385 (ADL 0273). Rosenberg *et al*. (2001) reported 98 % of 27 microsatelites of 20 chicken breeds was polymorphic while 12-15 markers showed high variability on 15-20 individuals of each chicken breed. Nasiri *et al*. (2007) reported polymorphism on Isfahan chicken analyzed with 9 microsatelites with the number of alleles identified of 2-5 (3.9 alleles in average).

Genetic variation is needed by organisms to adapt with the environment which tends to be inconstant. However, the most important about genetic variation is the allelic

Table 2: Phenotypic characteristics of various type of Kedu chicken in Kedu region of Temanggung district

| Characteristics | Cemani | White Kedu | Red Kedu | Black Kedu |
|---|---|---|---|---|
| Plumage colour | black | white | red | black |
| Skin colour | black | white | white | white |
| Comb colour | black | red | red | Blackish red |
| Wattles colour | black | red | red | Blackish red |
| Comb shape | Single buttercup, pea | Single, pea, cushion | single Singe, cushion | |
| Shank colour | black | black, yellow, grey | black, blackish green, yellow, grey | black |
| **Body weight (g)** | | | | |
| Males | 1907.50±221.66$^{a}$ | 1676.67±169.09$^{a}$ | 1840.00±293.60$^{a}$ | 1806.20±220.99$^{b}$ |
| Females | 2119.00±285.90$^{b}$ | 1731.80±170.76$^{ab}$ | 2116.60±407.4$^{b}$ | 1737.60±280.16$^{ab}$ |
| Egg production(egg/period) | 21.83±7.91$^{a}$ | 29.40±3.13$^{b}$ | 29.00±3.00$^{b}$ | 24.00±3.61$^{a}$ |

Note: Different superscripts of the same line indicate significant different on HSD test (P<0.05)

Table 3: Output of the four microsatelites analysis of Kedu chicken

| Locus | Allele size (bp) | No. of observation | No. of alleles | Genotypes | Expected heterozygosity (He) | PIC |
|---|---|---|---|---|---|---|
| ADL 0022 | 100-200 | 39 | 3 | 6 | 0.859 | 0.578 |
| ADL 0273 | 100-200 | 41 | 2 | 3 | 0.692 | 0.385 |
| LEI 0147 | 150-250 | 42 | 3 | 6 | 0.661 | 0.643 |
| ADL 0217 | 150-200 | 41 | 2 | 3 | 0.706 | 0.412 |
| Means | | | 2.500 | 4.500 | 0.730 | 0.504 |
| Standard of deviation | | | 0.577 | 1.732 | 0.089 | 0.126 |

variation. Allele is defined as the variation of the same gene expressed as different phenotypes. New alleles appears in the population due to random process and mutation and the allele frequencies occurs regularly as a result of mutation, selection and genetic drift.

**Genetic variability of kedu chicken:** The estimated of expected heterozygosity (He) and the Polymorphic Information Content (PIC) were obtained from data of Kedu chicken on each loci. The value He was quite high ranging from 0.661 (LEI 0147) to 0.859 (ADL 0022) with the average of 0.73+0.089 (Table 3). The estimated He value on all loci showed big variability i.e., 0.665 (Cemani), 0.743 (White Kedu), 0.723 (Red Kedu) and 0.618 (Black Kedu) (Tabel 4). The He value obtained from this study was higher than those of Riztyan *et al*. (2011) reporting He values between 0.224 and 0.0263 from a study done in various Indonesian local chicken. The observation result of moleculer genetic diversity on syria chicken indicated that the black phenotype individuals had higher genetic diversity than grey phenotype individuals (Al-Jallad *et al*., 2012).

The He value obtained from this study showed that the genetic variability of White Kedu was the highest and that of Black Kedu was the lowest. High He value was caused by low inbreeding and low pressure of selection as well as caused by the number of alleles detected. The average of the identified number of alleles on Kedu chicken population was 2.500+0.577. This allele number was smaller compared with the one reported by Tadano *et al*. (2007) reporting the number of alleles of 12 kinds of chicken in Japan to be 3-16 alleles with PIC of 0.241-0.0585 and He value of 0.290-0.646. Nasiri *et al*. (2007) reported PIC value of Isfahan chicken of 0.375-0.697 per locus. The more number of alleles identified the more was the genetic variability. Low He value of a population shows low inbreeding level, selection pressure and few number of alleles identified. High He value shows that outbreeding has occurred in the population and the population has been derived from a wide region (Zhang *et al*., 2002; Hillel *et al*., 2003; Osman *et al*., 2006; Tadano *et al*., 2007).

PIC indicates value of a marker used to detect polymorphism in a population. PIC depends on the number of identified alleles and distribution of the allele frequencies. PIC of Cemani, White Kedu, Red Kedu and Black Kedu chicken were 0.362, 0.516, 0.482 and 0.568, respectively. PIC value of Indonesian local chicken ranges from 0.765 to 0.878 analyzed with Single Nucleotide Polymorphism (SNP) marker. Microsatelite markers used in this study was cross species markers for chicken and other poultry species. Huang *et al*. (2005) explained that microsatelite for conserving poultry DNA can be used in other poultry species and within chicken and ducks species there are 20.42% fixed microsatelite loci.

Hardy Weinberg Equilibrium (HWE) test showed that all loci were not in equilibrium state. The deviation from HWE can be caused by selection and migration in Kedu chicken population. Selection is inevitably practiced by farmers in keeping Kedu chicken either at DOC period or when selecting roaster and hen for future keeping. Migration occurs when farmers introduce individuals from other population for improving the performance of the existing population. Vanhala *et al*. (1998) pointed out that selection and migration cause lost of alleles. The deviation from HWE could also be caused by miss genotyping. This study observed some alleles with 0 value causing that some homozygous genotypes did not exist. Non random mating could also cause the population to deviate from the HWE (Tadano *et al*., 2007).

**Genetic distance:** Genetic distance computed following Nei (1987) showed that White Kedu and Red Kedu had the closest genetic relationship (0.018) and Cemani and Black Kedu had the farthest genetic relationship (0.236) (Table 5). Riztyan *et al*. (2011) stated that Indonesian local chicken can be grouped into 3 cluster based on SNP analysis: (1) Black Kedu cluster, Arab cluster and local cluster (Kampung and Kedu chicken). Kampung chicken is closely genetically related to Kedu chicken while Black Kedu chicken has a far distant genetic relationship with Arab chicken.

Genetic distance was measured based upon gene frequencies showing genetic variability within a breed. Close genetic relationship shows that both White Kedu and Redu chicken have similarity as meat producing type chicken. This is backed up by Tadano *et al*. (2007) stating that the same purpose of animal keeping results in selection for the economically important trait causing the genetic variability to diminish. Cemani chicken was derived from Black Kedu chicken which experienced selection and inbreeding making its genetic distance to be relatively far.

The background of breeding and distribution of poultry are positively correlated with the genetic distance. Population of a breed inhabiting different geographical regions is the influencing factor to its genetic relationship.

Some conclusions were drawn from this study. Kedu chicken phenotypically differed in plumage colour, skin colour, shank colour, comb and egg production. PIC values based on all loci analyzed ranged from 0.385 (ADL 0273 locus) to 0.643 (LEI 0147 locus). The biggest heterozygosity was observed on White Kedu chicken (0.806). White Kedu and Red Kedu chicken had the closest genetic distance (D = 0.02) and the farthest

Table 4: Microsatellite locus, Polymorphic Information Content (PIC) and heterozygosity on Kedu chicken

| Locus | Parameter | Cemani | White Kedu | Red Kedu | Black Kedu | Average |
|---|---|---|---|---|---|---|
| ADL 002 | N | 11 | 9 | 9 | 10 | |
| | PIC | 0.413 | 0.579 | 0.601 | 0.505 | 0.524 |
| | He | 0.804 | 0.860 | 0.867 | 0.498 | 0.757 |
| ADL 0273 | N | 12 | 11 | 9 | 9 | |
| | PIC | 0.219 | 0.435 | 0.493 | 0.558 | 0.426 |
| | He | 0.609 | 0.718 | 0.746 | 0.721 | 0.699 |
| LEI 0147 | N | 12 | 11 | 9 | 10 | |
| | PIC | 0.652 | 0.649 | 0.553 | 0.420 | 0.569 |
| | He | 0.663 | 0.662 | 0.638 | 0.645 | 0.652 |
| ADL 0217 | N | 11 | 11 | 9 | 10 | |
| | PIC | 0.16 | 0.46 | 0.28 | 0.79 | 0.42 |
| | He | 0.58 | 0.73 | 0.64 | 0.61 | 0.64 |
| | Average PIC | 0.362 | 0.531 | 0.482 | 0.568 | |
| | Average He | 0.665 | 0.743 | 0.723 | 0.618 | |

Note: N (No. of identified individuals), PIC (Polymorphic Information Content), He (expected heterozygosity)

Table 5: Genetic distance between various kinds of Kedu chicken based on microsatelite loci

| Locus | ADL 0022 | ADL 0273 | LEI 0147 | ADL 0217 | Average |
|---|---|---|---|---|---|
| Cemai vs White Kedu | 0.035 | 0.020 | 0.035 | 0.038 | 0.032 |
| Cemani vs Red Kedu | 0.124 | 0.063 | 0.126 | 0.002 | 0.079 |
| Cemani vs Black Kedu | 0.191 | 0.022 | 0.095 | 0.635 | 0.236 |
| White Kedu vs Red Kedu | 0.024 | 0.011 | 0.025 | 0.021 | 0.020 |
| White Kedu vs Black Kedu | 0.055 | 0.000 | 0.013 | 0.219 | 0.072 |
| Red Kedu vs Black Kedu | 0.009 | 0.009 | 0.007 | 0.480 | 0.126 |

genetic distance was between Cemani and Black Kedu (0.236). The introduction of other chicken breeds into the region caused the population of other type of Kedu chicken to diminish.

Educating the farmers about the importance of genetic variability conservation of Kedu chicken is required so that the farmers are actively involved in conserving the breed. The related authority (local or central government) should actively involve in controlling the type of mating of the breed on farmer level so that the breed will not loose its genetic potential and diminish its genetic variability.

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