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# Interspecies and intraspecies genetic diversity of Ongole Grade cattle and Madura cattle based on microsatellite DNA markers

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**Abstract.** *Sutarno, Kurnianingrum N, Herawati E, Setyawan AD. 2018. Interspecies and intraspecies genetic diversity of Ongole Grade cattle and Madura cattle based on microsatellite DNA markers. Biodiversitas 19: 1593-1600.* DNA microsatellite has been extensively employed for estimating the degree of kinship between genotypes and improving the quality of cattle products. Microsatellite markers are short-patterned DNA sequences and repeated tandem (sequentially) with 2-5 nucleotide units scattering the entire genome. The purpose of this study was to investigate the genetic characteristics of inter and intraspecies of Ongole Grade cattle and Madura cattle using microsatellite DNA markers. Blood samples from 20 individuals of each species were extracted by the method referring to Wizard Genomic DNA Purification Kit (Promega, USA) and PCR amplification was performed using 5 microsatellite loci, i.e., BM1824, ETH225, INRA005, MM12, and TGLA227. Results of the genetic characteristics of both species were calculated using the POPGENE program version 1.31. The data suggest that there is a genetic diversity of inter and intraspecies of Ongole Grade cattle and Madura cattle. The average value of Shannon's Information Index (I) at all microsatellite loci for Ongole Grade cattle was 0.76 and for Madura cattle was 1.12. Meanwhile, the average interspecies I value was 1.03. The mean intraspecies Polymorphic Information Content (PIC) of Ongole Grade and Madura cattle was 0.43, and 0.63, respectively, and the mean interspecies PIC value was 0.57. The data altogether suggest that all loci meet the standards as being informative markers in the assessment of genetic population because it has a PIC value> 0.5 especially for intraspecies of Madura cattle.

**Keywords:** Ongole Grade cattle, Madura cattle, genetic diversity, DNA microsatellite

## INTRODUCTION

Livestock such as cattle continues to make significant contributions to global food supply due to the high-value protein that the cattle products offer. The type of beef cattle available in Indonesia today are indigenous and imported breeds. The Indonesian native beef cattle have distinctive properties, both in their morphological (body size, coat color) or genetic (growth rate) features. Beef cattle that are raised in Indonesia for meat production include Bali, Ongole, Ongole Grade (in Indonesian called *Sapi Peranakan Ongole*), Madura, and Aceh (Sutarno and Setyawan 2015, 2016).

Genetic diversity is regarded as the basis of crossbreeding for livestock (Buis et al. 1994). Information on the genetic diversity may serve as a starting point for artificial selection in an attempt to increase the quality and quantity of breed. Knowledge of the pattern of genetic variability from each breed will help the development of crossbreeding programs and serve as a basis for the conservation of genetic resources. Thus, information on the genetic diversity and genetic kinship are critical to obtaining superior cattle breed.

Genetic diversity in cattle, as well as in other livestock has declined very rapidly (Hall dan Bradley 1995; Hammond dan Leitch 1995). The selection of a particular breed for consideration of economic benefits, e.g., for production, becomes the primary factor that potentially reduced its genetic diversity (Sutarno et al. 2002). Currently, breeders compete to crossbreed many cattle in Indonesia in the hope of getting the best quality of cattle product. The conventional breeding technique based on phenotypic selection has no longer perceived as an effective nor optimum method. Therefore, a more sophisticated approach based on genetic data is indispensable.

Recent technological advance such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Radom Amplified Polymorphic DNA (RAPD), and microsatellite have reached DNA polymorphism testing for genetic mapping, markers for breeding, and exploration of genetic relationships (Powell et al. 1996). Microsatellite markers share similar properties with RFLP markers; it is a simple motif of a sequence of nitrogen bases found on the chromosome of an organism. This sequence is repeated as a unique motif. Researchers have mapped such sequences; thus, microsatellite markers can detect a segregated population as accurately as RFLP (Panaud et al. 1995; McCouch et al. 1997). Besides, microsatellite markers can distinguish up to one base pair difference so that the use of microsatellite markers will greatly assist in accurate genome analysis. Especially for the study of the linkage between individuals differentiated only by one base pair,

microsatellite markers provide a higher level of confidence than other known markers (Cooper and Krawczak 1993).

Population and genetic links studies using microsatellite markers are often done with the assumption that the polymorphism of each locus is high and complex, with higher polymorphism when the replication unit is more than 10 fold (Weber 1990). The difference in the length of the microsatellite allele at the locus is usually due to the variation in the number of replicates, and the nucleotide pairing mismatches in the event of replication is considered the main mechanism causing the length of the allele variation, even the emergence of new alleles (Travis at al. 1996). Variations in microsatellite loci can be tested by Polymerase Chain Reaction (PCR) method using a complementary primer with a sequence of repeating nucleotide sequencing unit, followed by electrophoresis of the PCR product (Tauzt 1993). The data of allele frequency on microsatellites can be used for the study of genetic relationships within species as well as between populations with relatively close relationship (Takezaki and Nei 1996). The use of microsatellite markers is relatively easy because PCR method allows amplification on the entire chromosome. Accordingly, the chances of getting a marker that corresponds to a character are higher. Utilization of microsatellite-based linkage maps in the generation of new varieties can also save time, cost, and laboratory effort (Akagi et al. 1996)

In Indonesia, value and need for the characterization and conservation of different livestock genetic resources have been well realized. The current study aimed to assess the genetic diversity of Ongole Grade and Madura cattle in Java using microsatellite DNA markers. The results of this study may serve as basic information in breeding programs to improve livestock productivity, primarily because Ongole Grade and Madura cattle are commonly traded as beef cattle.

## MATERIALS AND METHODS

### Blood sampling and DNA isolation

Blood samples of Ongole Grade and Madura cattle were obtained from Wonogiri District, Central Java, Indonesia and Sampang District, Madura, East Java, Indonesia respectively. The blood samples were collected by venal puncture from jugular veins, using 10 mL of venoject containing 2.5 mL of 200 mM EDTA as an anticoagulant.

Genomic DNA was isolated from 20 blood samples from each species using Wizard Genomic DNA Purification Kit (Promega, USA) as per manufacturer instruction. Briefly, 450 µL Cell Lysis Solution was added into 300 µL total blood, mixed thoroughly by inverting the tube 5-6 times, then incubate for 10 minutes at room temperature (invert 2-3 times once during the incubation) to lyse the red blood cells. The mixture was then centrifuged at 14,000×*g* for 20 seconds at room temperature to obtain pellet containing white blood cells. Supernatant was removed and discarded, followed by vortexing the tube for 3-5 minutes to separate clumps of white blood cells. Nuclei Lysis Solution (150 µL) was added to the tube containing the resuspended cells and mixed well by pipetting the solution 5-6 times to lyse the white blood cells. RNase Solution (1.5 µL) was mixed with the nuclear lysate, and incubated at 37°C for 15 minutes, and then cool to room temperature. Protein Precipitation Solution (60 µL) was added to the nuclear lysate, vortex vigorously for 10-20 seconds, then centrifuged at 14,000×*g* for 3 minutes at room temperature. At this step, a dark brown protein pellet should be visible. The supernatant was transferred to a clean 1.5 mL microcentrifuge tube containing 150 µL of room-temperature isopropanol. The solution was gently mixed by inversion until the white thread-like strands of DNA form a visible mass. DNA was centrifuged at 14,000×*g* for 1 minute at room temperature. The DNA will be visible as a small white pellet. Supernatant was removed by decantation, added with one sample volume (300 µL) of room-temperature 70% ethanol to the DNA. The tube was inverted gently several times to wash the DNA pellet and the sides of the microcentrifuge tube, followed by centrifuge again at 14,000×*g* for 1 minute at room temperature. After removing the ethanol, the tube was inverted on clean absorbent paper to let the pellet air-dry, then the DNA was resuspended in Rehydration Solution (100 µL).

### Determining DNA concentration and purity

Determination of DNA concentration and purity is very crucial to obtain best result of DNA amplification by PCR. DNA concentration was measured on spectrophotometer at wavelength 260 nm with distilled water as a blank (Boesenberg-Smith et al. 2012). Because DNA absorbs light most strongly at 260 nm, the absorbance value at this wavelength ($A_{260}$) can be used to estimate the double stranded DNA concentration using the equation below. DNA sample purity was estimated by calculating the ratio at 260 nm and 280 nm.

$$\text{DNA concentration (µg/mL)} = \frac{A_{260} \text{ x dilution factor x 50 µg/mL}}{1.0}$$

Where:
$A_{260}$: DNA absorbance at wavelength 260 nm

### Primers and DNA amplification by PCR

A total of 5 microsatellite labelled primers (SIGMA), were used in the PCR process (primers sequence, range of PCR product size, and annealing temperature are shown in Table 1). The PCR reagent composition is as follows: Go Tag Green Master Mix 2X (Promega, USA) (12 µL), forward and reverse primers (200 ng/µL), DNA samples (5-30 ng/µL), and nuclease free-water meshed up to 25 µL per reaction. The program in the PCR machine (Gene Amplification PCR System 9700 Thermocycler, Applied Biosystem) was set as follows: 95°C; 5 min (1 cycle), 30 cycles consisting of three stages: i) 95°C; 90 s, ii) 55°C to 60°C; 90 s depends on the primers (55°C for BM1824, 59°C for ETH225, 60°C for TGLA227, 55°C for INRA005, and 55°C for MM12), and iii) 72°C; 30 s, followed by 1 cycle at 72°C; 5 min.

**Table 1.** Sequence of 5 microsatellite loci used in this study

| Locus | Primers sequence | Chrom. number | Size range (base pairs) | Annealing temp. (°C) |
|---|---|---|---|---|
| BM 1824 | F: 5'- GAG CAA GGT GTT TTT CCA ATC -3' R: 3'- CAT TCT CCA ACT GCT TCC TTG -5' | 1 | 180-192<br>Bishop et al. (1994) | 58<br>Bishop et al. (1994) |
| ETH 225 | F: 5'- GAT CAC CTT GCC ACT ATT TCC T -3' R: 3'- ACA TGA CAG CCA GCT GCT ACT -5' | 29 | 141-159<br>Bishop et al. (1994) | 58<br>Bishop et al. (1994) |
| INRA 005 | F: 5'- CAA TCT GCA TGA AGT ATA AAT AT -3' R: 3'- CTT CAG GCA TAC CCT ACA CC -5' | 29 | 240-246<br>Vaiman et al. (1992) | 58<br>Vaiman et al. (1992) |
| TGLA 227 | F: 5'- CCC TCC TCC AGG TAA ATC AGC -3' R: 3'- AAT CAC ATG GAA ATA AGT ACA TAC -5' | 21 | 64-115<br>Georges and Massey (1992) | 60-48<br>Crawford et al. (1995) |
| MM 12 | F: 5'- CAA GAC AGG TGT TTC AAT CT -3' R: 3'- ATC GAC TCT GGG GAT GAT GT -5' | 9 | 107-133<br>Mommens and Coppieters (1994) | 50<br>Mommens and Coppieters (1994) |

Note: F= Forward Primer dan R= Reverse Primer

**Electrophoresis**

The PCR products were visualized by electrophoresis using 12% polyacrylamide gel containing 7.2 g urea, 1.5 mL of 10X TBE buffer, 5.6 mL of 30% acrylamide: bis (19: 1), 75 uL 10% ammonium persulfate (APS), and 7.5 uL N,N,N',N'-Tetramethylethylenediamine (TEMED) (Tegelstrom 1986). Polyacrylamide gel 12 % provide good separation for small DNA fragments (40-200 bp) and only requires small amount of sample (Allen et al. 1984). Before loading into the wells, the PCR products were denatured at 95°C for two minutes, then cooled down on ice. Electrophoresis was performed at 125 volt/400 milliampere, followed by ethidium bromide (EtBr) staining by soaking the gel into EtBr solution (1 mg/mL) for 15-30 minutes. The DNA banding patterns were visualized on a UV transilluminator and documented using Gel Documentation System (Vilber Lourmat, France).

**Data analysis**

A heterozygous allele was confirmed when a sample yielded two bands, as such, a homozygous allele was confirmed when only one band was detected. For ease of scoring, the lowest band was identified as A, continued with B, C, D, and so on for upper band. The scoring is based on assumption that all bands that migrate at the same rate are homologs allele (Nei 1987).

The expected heterozygosity (gene diversity) was calculated from individual allele frequency according to Nei's equation (Nei 1987):

$$Xi = (2nii + \Sigma nij)/(2n)$$

Where:
Xi : frequency of allele -i
nij : number of individual with genotype ij
nii : number of individual with genotype ii
n : number of samples

Therefore, level of heterozygosity ($\hat{h}$) can be calculated through the following equation:

$$\hat{h} = 2n (1 - \Sigma Xi2)/(2n - 1)$$

Where:
Xi : frequency of allele -i
$\hat{h}$ : level of heterozygosity of each locus

Calculation of fixation index, allele frequency, number of alleles, effective allele number, observed homozygosity, expected homozygosity, observed heterozygosity, expected heterozygosity, and Shannon information index are done with POPGENE Program Version 1.31. Botstein et al. (1980) described that the value of Polymorphism Informations Content (PIC) for microsatellite loci is measured by equation:

$$PIC = 1 - \sum_{j=1}^{n} Pij^2$$

Where:
ij is the frequency of allele i and j

## RESULT AND DISCUSSION

**Intraspecies genetic diversity**

Genotype data of individuals from Ongole Grade and Madura cattle are presented in Table 2. All the microsatellite loci (TGLA227, ETH225, BM1824, INRA005, and MM12) showed a genotype variation except for the TGLA227 locus on the Ongole Grade cattle in which all samples were uniformly identified as AB. Variations of genotypes that appear in these loci indicate the presence of polymorphism. The highest variety was found at MM12 locus, with genotypes of an AD, CD, CE, AB, AC, and BC present in Ongole Grade cattle, whereas genotypes of BC, BE, AE, BD, AD, AC, and AB appears in Madura cattle. The TGLA227 locus in Ongole Grade cattle shows deficient levels of variation, where all individuals bearing AB. Low variation level was also found at ETH225 locus, in which, among the domination of genotype AA, only one individual showing different genotype (AB). Although displaying similar band pattern, TGLA227 locus differs from the ETH225 locus in the fact that the later locus is homozygous.

**Table 2.** Results of genotype of Ongole Grade and Madura cattle on 12% polyacrylamide gel with ethidium bromide staining.

| Ind. no. | Ongole Grade cattle | | | | | Madura cattle | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TGLA227 | ETH 225 | BM 1824 | INRA 005 | MM 12 | TGLA 227 | ETH 225 | BM 1824 | INRA 005 | MM 12 |
| 1 | AB | AA | AA | BC | AD | AB | AC | AC | AB | BC |
| 2 | AB | AA | AA | BC | AD | AA | AD | AC | AB | BC |
| 3 | AB | AA | AA | BC | AD | AB | AD | AC | AB | BE |
| 4 | AB | AA | AA | AB | AD | AD | AD | AC | AB | BC |
| 5 | AB | AB | AA | AB | AD | AD | AD | AC | AB | BC |
| 6 | AB | AA | AA | AB | AD | AC | AD | AC | AB | AE |
| 7 | AB | AA | AA | AB | AD | AB | AC | AB | AB | BD |
| 8 | AB | AA | AA | BC | AD | AB | AC | AB | AC | BE |
| 9 | AB | AA | AA | BC | CD | AC | AC | AB | AB | AD |
| 10 | AB | AA | AA | AB | CE | AA | AB | AB | AC | BC |
| 11 | AB | AA | AB | AB | AB | AB | AD | AB | AC | AE |
| 12 | AB | AA | AB | AB | AC | AB | AD | AB | AC | AE |
| 13 | AB | AA | AC | AC | BC | AA | AD | AC | AC | AE |
| 14 | AB | AA | AA | BC | BC | AA | AC | AB | AC | AB |
| 15 | AB | AA | AA | AB | BC | AB | AC | AB | AC | AC |
| 16 | AB | AA | AC | AB | BC | AB | AC | AB | AC | AB |
| 17 | AB | AA | AC | AB | BC | AA | AC | AB | AC | AB |
| 18 | AB | AA | AA | AB | BC | AB | AC | AB | AC | AB |
| 19 | AB | AA | AA | BC | BC | AB | AD | AA | AC | AB |
| 20 | AB | AA | AA | BC | BC | AB | AB | AA | AC | AB |

**Table 3.** Intraspecies variation and allele frequency of Ongole Grade and Madura cattle at 5 microsatelite loci.

| Alelle | Species | Locus | | | | |
|---|---|---|---|---|---|---|
| | | TGLA227 | ETH225 | BM1824 | INRA005 | MM12 |
| A | OG | 0.50 | 0.97 | 0.87 | 0.30 | 0.25 |
| | Madura | 0.62 | 0.50 | 0.55 | 0.50 | 0.30 |
| B | OG | 0.50 | 0.02 | 0.05 | 0.47 | 0.22 |
| | Madura | 0.27 | 0.05 | 0.27 | 0.20 | 0.35 |
| C | OG | - | - | 0.07 | 0.22 | 0.27 |
| | Madura | 0.05 | 0.15 | 0.17 | 0.30 | 0.15 |
| D | OG | - | - | - | - | 0.22 |
| | Madura | 0.05 | 0.22 | - | - | 0.05 |
| E | OG | - | - | - | - | 0.02 |
| | Madura | - | - | - | - | 0.15 |

Note: OG: Ongole Grade

**Table 4.** Parameters of intraspecies genetic diversity observed in Ongole Grade and Madura cattle at 5 microsatellite loci

| Locus | Species | Na | Ne | OHm | EHm | OHt | EHt | I | PIC |
|---|---|---|---|---|---|---|---|---|---|
| TGLA227 | OG | 2 | 2.00 | 0.00 | 0.49 | 1.00 | 0.51 | 0.69 | 0.50 |
| | Madura | 4 | 2.12 | 0.25 | 0.46 | 0.75 | 0.54 | 0.95 | 0.53 |
| ETH225 | OG | 2 | 1.05 | 0.95 | 0.95 | 0.05 | 0.05 | 0.12 | 0.05 |
| | Madura | 4 | 2.83 | 0.00 | 0.34 | 1.00 | 0.66 | 1.17 | 0.65 |
| BM1824 | OG | 3 | 1.29 | 0.75 | 0.77 | 0.25 | 0.23 | 0.46 | 0.23 |
| | Madura | 3 | 2.45 | 0.10 | 0.39 | 0.90 | 0.61 | 0.99 | 0.59 |
| INRA005 | OG | 3 | 2.73 | 0.00 | 0.35 | 1.00 | 0.65 | 1.05 | 0.63 |
| | Madura | 3 | 2.63 | 0.00 | 0.36 | 1.00 | 0.63 | 1.03 | 0.62 |
| MM12 | OG | 5 | 4.16 | 0.00 | 0.22 | 1.00 | 0.78 | 1.46 | 0.76 |
| | Madura | 5 | 3.85 | 0,00 | 0.24 | 1.00 | 0.76 | 1.44 | 0.74 |
| Average | OG | 3,00 | 2.25 | 0.34 | 0.55 | 0.66 | 0.44 | 0.76 | 0.43 |
| | Madura | 3,80 | 2,.77 | 0.07 | 0.64 | 0.93 | 0.64 | 1.12 | 0.63 |

Note: OG: Ongole Grade. Na.: Observed number of alleles; Ne.: Effective number of alleles [Kimura and Crow 1964]; I.: Shannon's Information index [Lewontin 1972]; OHm.: Observed Homozygosity; EHm.: Expected Homozygosity, EHt.: Expected Heterozygosity, OHt.: Observed Heterozygosity. PIC.: Polymorphism Information Content

Table 2 also indicates that some individuals from the Ongole Grade and Madura cattle are still pure breed, as evidence from the dominance of homozygous genotypes such as AA at ETH225 locus in Ongole Grade cattle. The pure breed can be obtained via inbreeding of animals after many years; thus, it is possible to obtain lines which produce uniform offspring.

The variation and allele frequency in each species for each locus are presented in Table 3. Because allele variation exists in all loci, this finding suggests that the percentage of polymorphism reaches 100%. High allele variations were found at the MM12 locus that extends until the E allele, whereas the lowest variation was located at the INRA005 and BM1824 loci, only reaching the variation of C allele.

In Ongole Grade cattle, ETH225 locus shows dominance in AB allele frequency (97%) and TGLA227 locus shows the balance of frequencies between allele A and allele B, each by 50%. The BM1824 locus also explains the dominance of the allele A by 87%, while for allele B and C by 5% and 7%, respectively. Nearly comparable allele frequency was shown at INRA005 locus, where A, B, and C allele account for 30%, 47%, and 22%, respectively. No dominance of a specific allele at the MM12 locus which has the greatest allele variation. The allele frequencies are almost equal between alleles A, B, C, D, i.e, 25%, 22%, 27%, and 22%, respectively, while the lowest value was found in allele E by 2%. In Madura cattle, the dominance of allele frequencies was observed in allele A (62% at TGLA227 locus, 50% at ETH225 and INRA005 loci, 55% at BM1824 locus). Except for the MM12 locus, the most considerable frequency was found in allele B by 35%. In all case, the highest combination was found in allele A, an indicator that this allele mostly controls the variation of the traits that appear on the Ongole Grade cattle and Madura cattle.

Table 4 shows the number of alleles, observed homozygosity, expected homozygosity, observed heterozygosity, expected heterozygosity, effective number of alleles, and Shannon's Information Index. All loci indicate polymorphism, and the number of alleles obtained varies between 2 (TGLA227 and ETH225) to 5 (MM12). The average number of alleles for Ongole Grade cattle is 3.00, while for Madura cattle is 3.80. It is crucial to know the number of alleles per locus to estimate the genetic distance between species populations. Multiple alleles generally yield more accurate estimation on the genetic distance than loci with few alleles, especially for closely related populations (Kalinowski 2002). For improving the quality of livestock, it is generally advisable to cross-breed between two species with distant kinship. Crossbreeding allows the flow of genes from the parent to the offspring to establish better genetic stability in the offspring.

The effective number of alleles (Ne) is the approximate number of alleles by adjusting their frequency to the PIC value. The lowest value was found in the Ongole Grade cattle (ETH225 locus; 1.05), whereas the highest value was measured in the Madura cattle (MM12 locus; 3.85). The average Ne score was 2.25 for Ongole Grade cattle and

2.77 for Madura cattle. The average Ne values obtained in this study were slightly lower than the average Ne values in Hariana cattle (2.87) and Hissar cattle (2.89), as reported by Rehman and Khan (2009). Also, it was lower than the studies on Kangayam cattle (*Bos indicus*) of Tamilnadu with an average Ne score of 2.90 (Karthickeyan et al. 2009). The effective number of alleles was smaller than the observed number of alleles in general. One of the reason might be due to fluctuation in the number of populations in the past. There are also contributions from mutations (deletions) occurring in the population resulting from balance of mutation-selection (Crow and Dove 2000).

The value of observed homozygosity (OHm) in Ongole Grade cattle ranged from 0.00 to 0.95, while for Madura cattle varied between 0.00-0.25. The expected value of homozygosity (EHm) in Ongole Grade cattle has a narrower range than the observed homozygosity value. The observed heterozygosity (OHt) per locus for Ongole Grade cattle ranged from 0.05 to 1.00 while for Madura cattle ranged from 0.75 to 0.90. The expected value of heterozygosity (EHt) in Ongole Grade cattle falls in the range of 0.05-0.77, while Madura cattle ranges from 0.54 to 0.76. The range of observed heterozygosity values obtained in this study is much higher than that of Sumantri et al. (2007) conducted on Friesian-Holstein dairy cow at the Dairy Cattle Breeding Center, Baturaden (0.6151-0.7303) and of five local cattle (Luxi, Nanyang, Jinnan, Qinchuan and Yanbian) in China that ranged from 0.63-0.86 (Zhou et al. 2005). The main differences can be caused by different genus and more microsatellite loci used in previous studies, i.e, between 8-10 loci. The average observed heterozygosity value for Ongole Grade cattle was 0.66 and for Madura cattle was 0.93. Sodhi et al. (2006) reported an average observed heterozygosity in Tharparkar cattle from India was 0.57, while in Indian Cow Red Kandhari and Deoni are 0.47 snf 5.47, respectively. Ibeagha-Awemu et al. (2005) argued that the low observed heterozygosity (0.117) in cattle in Cameroon and Nigeria might be due to inherited factor.

Table 4 shows that almost all loci for each species have an observed heterozygosity higher than expected heterozygosity, thereby indicating a decrease in heterozygosity. The lower expected heterozygosity may be an effect of gene flow from outside into the studied population, whereas the expected value of heterozygosity in the existing population depends on the application of artificial insemination techniques (Brotherstone and Goddard 2005). Inbreeding can increase the frequency of homozygous genes and decrease the proportion of heterozygosity (Khan and Sing 1990). The further the relationship between the two cattle, the less common their genes and the greater the degree of heterozygosity (Noor 2000).

Shannon Information Index (I) is the information of gene diversity measurements (Kimura and Crow 1964). The I in Ongole Grade cattle was 0.69 (TGLA227), 0.12 (ETH225), 0.46 (BM1824), 1.05 (INRA005), and 1.46 (MM12). Meanwhile, the value for Madura cattle was 0.95 (TGLA227), 1.17 (ETH225), 0.99 (BM1824), 1.03 (INRA005), and 1.44 (MM12). Madura cattle demonstrated higher I value at some loci, for example for the TGLA227, ETH225, BM1824, and MM12 loci which suggests that the genetic diversity of Madura cattle for the above four loci is higher than that of the Ongole Grade Cattle. On the other hand, for the INRA005 locus, genetic diversity is more prevalent in Ongole Grade cattle than in Madura cattle. The I average for Ongole Grade cattle was 0.76, whereas Madura cattle was 1.12. Nevertheless, the value of I in this study was much greater when compared with the findings from previous study, e.g, Red Chittagong cattle in Bangladesh, cattle from Anwara region (0.29) (Mufti et al. 2009), and Holstein's Cow (1.606) (Movahedin et al. 2010). Pashaei et al. (2009) suggest that low biodiversity in Holstein cattle may be due to an intensive selection program.

Polymorphism information content (PIC) is an important measurement of DNA polymorphism. The PIC illustrates the probability that a marker genotype of an offspring of a heterozygous parent affected with a dominant disease allows one to deduce which marker allele the offspring inherited from the parents (Botstein et al. 1980). The PIC value of Ongole Grade is 0.50 at TGLA227 locus, 0.05 at ETH225 locus, 0.24 at BM1824 locus, 0.63 at INRA005 locus, and 0.76 at MM12 locus. In Madura cattle, the PIC value is 0.53 at TGLA227 locus, 0.68 at ETH225 locus, 0.59 at BM1824 locus, 0.62 at INRA005 locus, and 0.74 at MM12 locus. According to Botstein et al. (1980), genetic markers with PIC values less than 0.25 (PIC <0.25) were categorized as less informative and those with values greater than 0.5 (PIC> 0.5) were considered informative markers in the assessment of genetic populations.

Based on the microsatellite locus selection standard (Barker 1994), the microsatellite locus should have at least four alleles to be considered for its use for the evaluation of genetic diversity. The results obtained in this study are not in line with the findings of Zhou et al. (2005) on five local livestock species in China where the ETH225 and BM1824 loci both exhibited PIC values> 0.5. Another study conducted by Karthickeyan et al. (2008) in Ongole cattle mentioned that the PIC values for the INRA005 and ETH225 loci are >0.5. However, Sodhi et al. (2006), claimed that Zebu cattle in India showed a PIC value <0.5 for locus ETH225. Based on the differences in these data, it can be concluded that the same microsatellite locus works differently for different types of livestock.

Fixation Index (Fis) or Wright's Fixation Index is a calculation of inbreeding estimates in the population (Table 5 and Table 8). Data on the calculation of the Fisher value (Table 5) shows that the Ongole Grade and Madura cattle have a broad genetic diversity and are also amongst cattle that breed naturally (Karthickeyan et al. 2009), as indicated by the emergence of negative physical values that reached 100% of all loci used.

The addition of a heterozygous allele ratio compared to a homozygous allele can be a useful deviation factor in Hardy-Weinberg equilibrium that can justify the emergence of negative values in the Wright's Fixation Index (F*is*). Fis presents an increase or decrease in the observed heterozygosity frequency rating against the expected heterozygosity of -1 to 1 and 0 which in Hardy-Weinberg

equilibrium occurs when both frequencies are equal. Negative physical values may also be caused by controlled breeding systems that occur in the studied population and unintentional breeding strategies (Movahedin et al. 2010).

**Interspecies genetic diversity**

The interspecies allele frequencies of Ongole Grade and Madura cattle are presented in Table 6. The data show a considerable diversity for the MM12 locus, i.e, there are variations ranging from alleles A to E, whereas, for the TGLA227 and ETH225 loci, the variation only extended to allele D. Meanwhile, BM1824 and INRA005 loci have the lowest allele diversity, showing only allele A, B, and C.

The parameters of interspecies genetic diversity of Ongole Grade and Madura cattle are presented in Table 7. The number of alleles ranges from 3 to 5 with an average number of alleles of 3.80. The lowest effective number of alleles was 1.75 for ETH225 locus, the highest was 4.35 for MM12 locus, and the average effective number of alleles of 2.59. The average value of the observed homozygosity is 0.20, while the expected average value of homozygosity is 0.42. The largest observed heterozygosity values were found at the INRA005 and MM12 loci at 1.00, while the greatest heterozygosity was found at the MM12 locus by 0.78.

The highest value of I was 1.53 for MM12 locus whereas the lowest value of I was 0.80 for BM1824 locus. The mean interspecies I value of Ongole Grade and Madura cattle was 1.03. The highest value of PIC was 0.77 for the MM12 locus whereas the lowest value was 0.43 for the ETH225 locus. The mean value of PIC is 0.57, meaning that the locus used in this study is generally considered informative in the genetic population assessment because it has a PIC value > 0.5.

The number of interspecies inbreeding between Ongole Grade and Madura cattle shows a negative value, suggesting there is no inbreeding between the population of Ongole Grade and Madura cattle. In the context of livestock breeding efforts, inbreeding also means degradation of the livestock quality, since the chance of accumulation of undesirable traits brought on from closely related species to their offspring is much higher than the accumulation of its good qualities.

The value of intraspecies genetic diversity of Madura cattle is higher than that of Ongole Grade cattle, whereas the interspecies genetic diversity of Ongole Grade-Madura is smaller than the value of intraspecies genetic diversity of Madura, albeit only 0.09 difference. This may be attributed to the considerable range of intraspecies Shannon's Information Index of Ongole Grade and Madura cattle, thus affecting the low interspecies genetic diversity.

This study is a small part of the great effort to improve the quality of livestock in Indonesia. To bring up certain traits in a species, the cooperation of genetic and environmental factors is needed. Therefore, to achieve the ultimate goal of this research, genetic data and other supporting data, such as phenotype, geographical conditions of the livestock environment, and the culture of local communities are needed. By knowing those supporting data, we can analyze the relationship between genetic data and phenotype data formed from the geographical condition of the environment and the treatment (culture) of the local community to the livestock concerned.

**Table 5.** Intraspecies Fixation Index (Fis) of Ongole Grade and Madura cattle at 5 microsatelite loci.

| Alelle | Species | TGLA 227 | ETH 225 | BM1824 | INRA005 | MM12 |
|---|---|---|---|---|---|---|
| A | OG | -1.00 | -0.02 | -0.14 | -0.42 | -0.33 |
| | Madura | -0.60 | -1.00 | -0.82 | -1.00 | -0.43 |
| B | OG | -1.00 | -0.02 | -0.05 | -0.90 | -0.29 |
| | Madura | -0.38 | -0.05 | -0.38 | -0.25 | -0.54 |
| C | OG | - | - | -0.08 | -0.29 | -0.38 |
| | Madura | -0.05 | -0.29 | -0.21 | -0.43 | -0.18 |
| D | OG | - | - | - | - | -0.29 |
| | Madura | -0.05 | -0.29 | | | -0.05 |
| E | OG | - | - | - | - | -0.02 |
| | Madura | | | | | -0.17 |
| Total | OG | -1.00 | -0.02 | -0.10 | -0.58 | -0.31 |
| | Madura | -0.42 | -0.55 | -0.52 | -0.61 | -0.35 |

Note: OG: Ongole Grade

**Table 6.** Interspecies variation and allele frequency of Ongole Grade and Madura cattle at 5 microsatelite loci.

| | Locus | | | | |
|---|---|---|---|---|---|
| Alelle | TGLA227 | ETH225 | BM1824 | INRA005 | MM12 |
| A | 0.56 | 0.74 | 0.71 | 0.40 | 0.27 |
| B | 0.39 | 0.04 | 0.16 | 0.34 | 0.29 |
| C | 0.02 | 0.11 | 0.12 | 0.26 | 0.21 |
| D | 0.02 | 0.11 | - | - | 0.18 |
| E | - | - | - | - | 0.09 |

**Table 7.** Parameters of interspecies genetic diversity in Ongole Grade and Madura cattle at 5 microsatelite loci

| Locus | Na | Ne | OHm | EHm | OHt | Eht | I | PIC |
|---|---|---|---|---|---|---|---|---|
| TGLA227 | 4 | 2.14 | 0.12 | 0.46 | 0.87 | 0.54 | 0.87 | 0.53 |
| ETH225 | 4 | 1.75 | 0.47 | 0.56 | 0.52 | 0.43 | 0.84 | 0.43 |
| BM1824 | 3 | 1.82 | 0.42 | 0.54 | 0.57 | 0.46 | 0.80 | 0.45 |
| INRA005 | 3 | 2.92 | 0.00 | 0.33 | 1.00 | 0.66 | 1.08 | 0.66 |
| MM12 | 5 | 4.35 | 0.00 | 0.22 | 1.00 | 0.78 | 1.53 | 0.77 |
| Mean | 3.80 | 2.59 | 0.20 | 0.42 | 0.79 | 0.57 | 1.03 | 0.57 |

Note: Na.: Observed number of alleles; Ne.: Effective number of alleles [Kimura and Crow 1964]; I.: Shannon's Information Index [Lewontin 1972]; OHm.: Observed Homozigocity; EHm.: Expected Homozigosity, EHt.: Expected Heterozigosity, OHt.: Observed Heterozigocity. PIC.: Polymorphism Information Content

**Table 8.** Interspecies Fixation Index (Fis) of Ongole Grade and Madura cattle at 5 microsatellite loci.

| | Locus | | | | |
|---|---|---|---|---|---|
| Allele | TGLA227 | ETH225 | BM1824 | INRA005 | MM12 |
| A | -0.78 | -0.35 | -0.40 | -0.67 | -0.38 |
| B | -0.63 | -0.04 | -0.19 | -0.51 | -0.40 |
| C | -0.02 | -0.13 | -0.14 | -0.35 | -0.27 |
| D | -0.02 | -0.13 | - | - | -0.16 |
| E | - | - | - | - | -0.09 |
| Total | -0.64 | -0.22 | -0.28 | -0.52 | -0.30 |

In conclusion, based on Shannon's Information Index, the intraspecies genetic diversity of Ongole Grade cattle was greater than that of Madura cattle. The average Shannon's Information Index of interspecies was 1.03. The average intraspecies PIC value of Ongole Grade and Madura cattle were 0.43 and 0.63, respectively, whereas the mean interspecies PIC value of Ongole-Madura cattle was 0.57. Therefore, all loci meet the standard to be considered as being an informative marker in the assessment of genetic populations due to having a PIC value of> 0.5 especially for intraspecies in Madura cattle.

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