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Genetic diversity and phylogenetic study of Ongole Grade cattle population in Central Java based on blood protein polymorphism

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Abstract. *Lestari DA, Sutopo, Setiaji A, Kurnianto E. 2023. Genetic diversity and phylogenetic study of Ongole Grade cattle population in Central Java based on blood protein polymorphism. Biodiversitas 24: 617-624.* This research provides useful information to reflect the genetic structure and to determine appropriate breeding strategies for the Ongole Grade cattle population in Central Java. The objectives of this research were to investigate the genetic diversity and phylogenetic relationship of the Ongole Grade cattle (*Bos indicus*) population in Central Java based on blood protein polymorphism. A total of 208 blood samples were collected, which belonged to 6 populations of Ongole Grade cattle in Central Java, namely Rembang, Blora, Kebumen, Semarang, Magelang, and Kudus regency. Blood protein analysis was conducted by Sodium Dodecyl Sulfate-Polyacrilamide Gel Electrophoresis (SDS-PAGE) methods to separate blood protein based on molecule weight. Results showed 4 blood protein locus were clearly identified, namely Albumin (Alb), Post Albumin (*Pa*), Ceruloplasmin (*Cp*), and Amylase-I (*Amy-I*), which formed 9 identified alleles and 15 genotypes. All populations had different allele and gene frequency distribution for each locus, which dominated by allele Alb^B (0.525), Pa^B (0.556), Cp^F (0.608), and $Amy\text{-}I^B$ (0.639) and by genotype BB (0.312) for *Alb* loci, BB (0.429) for *Pa* loci, FS (0.423) for *Cp* loci and BB (0.472) for *Amy-I*. Ongole Grade cattle population in this study showed deviation in Hardy-Weinberg Equilibrium (HWE) ($P<0.05$) for Blora, Kebumen, Semarang, and Kudus, while the rest populations showed in HWE condition. Average heterozygosity and genetic distance of the Ongole Grade cattle population in the recent study were range 0.328-0.529 (for Magelang and Kebumen populations, respectively) and 0.0057-0.0955 (for the Rembang-Kebumen population and Magelang-Kebumen population), respectively. In conclusion, Ongole Grade cattle population in Central Java had high genetic diversity and was divided into two major clusters.

Keywords: Allele, genetic distance, genotype, heterozygosity, phylogeny

INTRODUCTION

Ongole Grade cattle (*Bos indicus*) is one of the local cattle and genetic resources of Indonesia that belongs to Zebu group (Sutarno and Setyawan 2015). Evenly scattered in almost all Indonesian regions, Ongole Grade cattle are easy can be found on Java Island, especially in Central Java Province. Since 1904, Ongole Grade cattle have been raised and developed by local farmers from generation to generation in Central and East Java. So then, almost about 90% of the Ongole Grade cattle population in Indonesia were on Java Island. Previous researchers reported that in Kebumen (Central Java), long before the Ongolisation program in the 1930s, efforts had been made to genetically improve zebu cattle originating from Madras (East India) and have continued to grow, so that recently Ongole Grade cattle in Kebumen were also called as Madras cattle and reported had quality like pure Ongole cattle (Utomo et al. 2015).

Based on the decree No 2841/Kpts/LB.430/8/2012, Ongole Grade cattle are designated as one of the local cattle families and genetic resources of Indonesian local livestock that must be protected and conserved well (Ministry of Agriculture 2012). Having uniformity in physical form and good reproductive performance, Ongole Grade cattle have the ability to adapt, survive and grow under poor feeding conditions (high crude fiber content), high resistance to local diseases and parasites, high tolerance to the hot tropical climate and good meat quality (Rohyan et al. 2016; Sumadi et al. 2017; Affandy et al. 2018; Suyadi et al. 2020; Suhendro et al. 2021). Moreover, Ongole Grade cattle in particular populations have been certified free from zoonosis and deserve to be used as livestock germs (Sutiyono et al. 2018). Previous researchers revealed that Ongole Grade cattle is a crossbreed result of Javanese cattle (*Bos javanicus*), an indigenous Java cattle, and Ongole cattle (*Bos indicus*), an indigenous India cattle, it has unique characteristics which are having a hump, white-gray skin, long tail, short horns, and head, black fur around the eyes, long straight ears and a rather large belly (Sutarno and Setyawan 2016; Hartatik et al. 2018). Another study by Suyadi et al. (2014) reported that Ongole Grade cattle or Benggala is a crossbreed result of uncontrolled mating of Sumba Ongole cattle and Java cattle. Moreover, as reported by previous studies, Ongole Grade cattle had 31.88±3.78 kg of average birth weight, 94.46±2.12 kg of average weaning weight, and 358±24.6 kg of average mature weight with 0.70 kg of average daily weight gain (Winarti and Widyastuti 2016; Maharani et al. 2017; Maharani et al. 2018).

Since the decree concerning Ongole Grade cattle as one of the Indonesian local cattle families released, there are not much-structured efforts that have been made to maintain and improve their genetic quality. Even by the

years, the superiority of Ongole Grade cattle has begun to be displaced by other exotic cattle and their crosses. So, there are concerns that Ongole Grade pure breed will eventually become extinct in Indonesia (Aryogi and Romjali 2006). Preventing the decline of the genetic quality of Ongole Grade cattle in the breeding center area, particular efforts must be made in order to maintain and improve genetic quality and also to develop its population. Traditional breeding and selection systems still need to be improved through consideration of the proper breeding system, which refers to animal genetic diversity based on genotype traits as a first step in animal breeding. In addition, as stated by Sutarno et al. (2018), in an effort to increase the quality and quantity of breeds, information on genetic diversity can be used as a starting point for breeding and selection.

Identification of animal genetic diversity can be made through blood protein analysis by looking at the differences in specific loci by using the electrophoresis method. The results will provide information about the allele frequency of a population, the level of heterozygosity, homozygosity, genetic distance, and phylogenetic relationships of a species, genus, or population (Siparyanto et al. 2002). So, it will greatly assist in designing livestock breeding programs. Blood protein analysis also can be used for animal physiological characteristic status (Utomo et al. 2017), immunogenetic markers (Hrinca 2015; Tothova et al. 2016), and identification of phylogenetical relationships (Nigussie et al. 2016; Safran et al. 2017; Sutopo et al. 2021). Research on the genetic diversity of Ongole Grade cattle has been done by Sutopo et al. (2001) in Java; Hartatik et al. (2018) in Kebumen; Sutiyono et al. (2018) in Rembang; Sutarno et al. (2019) in Wonogiri. However, research on genetic diversity and phylogenetic study of Ongole Grade cattle, specifically in breeding center areas in Central Java Province, has not been done much. Therefore, in order to support government policy in the context of preserving local livestock breeding, comprehensive research on Ongole Grade cattle is necessary, especially in Rembang, Blora, and Kebumen as breeding center areas in Central Java Province. Information about genetic diversity and phylogenetic studies is important to support conservation and breeding programs. Because it can provide information about domestication events, relationships among breeds, the loss of within-breed genetic diversity, and breed structure (Toro et al. 2009). So that breeders nor policy maker can set conservation priorities and determine the next steps to be taken as breeding strategies. Therefore, the objectives of this research were to investigate the genetic diversity and phylogenetic relationship of the Ongole Grade cattle population in Central Java based on blood protein polymorphism.

MATERIALS AND METHODS

Ethical approval

The experimental protocol and animal treatment in this study were approved by the Animal Research Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 59-01/A-03/KEP-FPP).

Sample determination

A total of 208 blood samples were collected, which belonged to 6 populations of Ongole Grade cattle in Central Java, namely Rembang, Blora, Kebumen, Semarang, Magelang, and Kudus regency (Figure 1, Table 1).

Table 1. Sample list

Population	Number of cattle
Rembang	34
Blora	34
Kebumen	35
Semarang	34
Magelang	36
Kudus	35

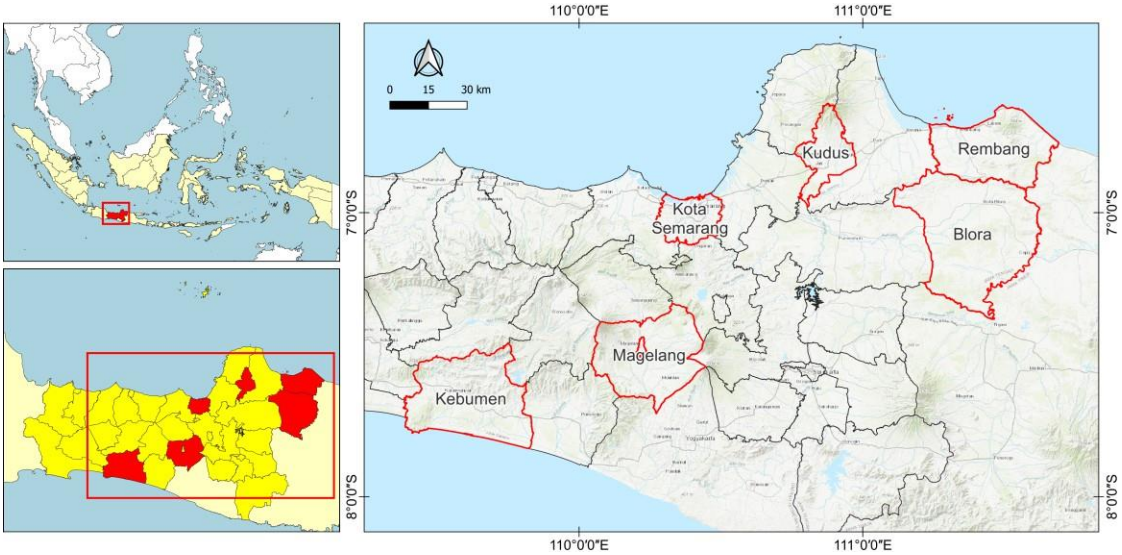

Figure 1. Rembang, Blora, Kebumen, Semarang, Magelang and Kudus regency map as sampling location of 6 populations of Ongole Grade cattle in Central Java

Samples were selected by purposive sampling methods, specifically: samples were bull rearing by a farm group, samples were unrelated to one another and samples had criteria that were noted in the decree of Ministry of Agriculture no. 358/Kpts/PK.040/6/2015 for the Kebumen population, 404/Kpts/PK.010/7/2017 for the Rembang population, and 2841/Kpts/LB.430/8/2012 for the other's population, including has grayish-white body color with black nose and tail; rectangle bodyform; convex forehead and flat nose; medium hump for female and large hump for male; thick wattle extend from the chin to the navel; short spiky horns that curved backward and the tail extends below the knees with whip hair.

Blood collection and separation

Blood was taken through a Jugular vein by using 5 cc disposable syringe. Then, the blood was placed into a vacutainer tube containing anticoagulant Ethylene Diamine Tetraacetic Acid (EDTA) and was stored in a cooling box containing ice gel. The blood was centrifuged at 5000 rpm for 5 minutes to separate blood plasma and blood cells. The separated blood plasma was then used to identify blood protein. Blood protein analysis was taken place in the Laboratory of Biochemistry, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Blood protein analysis

Analysis was conducted by Sodium Dodecyl Sulfate-Polyacrilamide Gel Electrophoresis (SDS-PAGE) methods to separate blood protein based on molecule weight (Gahne et al. 1977). The marker used in this study was ExactPro Broad Range (10-245 kDa). Buffer electrode (3 gram Tris pH 10, 14.4 gram SDS 10% (Dodecylsulfat Natrium salt), 1-Liter aquadest), 3% stacking gel (1.89 gram Acrylamide, 0.048 gram Bis AA (N-N methylene bisacrylamide), 5.04 ml Tris HCl pH 8.8, 0.6 mL SDS 10%, 34.36 mL aquadest) and 12.5% gradient gel (12.5 gram Acrylamide, 0.03 gram Bis AA, 3.13 mL Tris HCl pH 8.8, 0.13 mL SDS 10%, 9.25 aquadest) were prepared. A total 10 µL of blood plasma was diluted into 190 µL aquadest. Then, the mixture was mixed with 50 µL blue juice. The mixture was then heated at 100°C for 3 minutes by using water bath and was cooled immediately on the ice water. The gel that was prepared was set in the electrophoresis tank and was soaked with a buffer electrode. 10 µL of the mixture was loaded into the gel and was electrophoresed at 120 V for 2 hours. Afterward, the gel was stained with a staining solution for 1 hour on a shaker and de-stained with a de-staining solution as much as 2 times for 45 minutes. Observed bands were used to perform allele interpretation of four locus based on marker consisting of Albumin (*Alb*) (~69 kDa), Post-albumin (*Pa*) (~73 kDa), Ceruloplasmin (*Cp*) (~75 kDa), and Amylase-1 (*Amy-1*) (~110 kDa) (Wyne et al 1995; Keren 2003).

Data analysis

Frequency of allele was quantified based on Warwick et al. (1995):

$$FAi = \frac{\sum \text{Allele Ai}}{\sum \text{Allele Ai} + \sum \text{Allele Bi} + ... + \sum \text{Allele Ni}}$$

Where, F_{Ai} is allele frequency of A allele on i loci.

Frequency of expected genotype was quantified based on Hardy-Weinberg Equilibrium (HWE) theory (Falconer and Mackay 1996) by following equation:

$p^2 + 2pq + q^2 = 1$ (for *Po-Alb*, *Cp* and *Amy-1* loci) and
$p^2 + 2pq + 2pr + q^2 + 2qr + r^2 = 1$ (for *Alb* loci)

Where, p is allele frequency of 1st allele; q is allele frequency of 2nd allele; r is allele frequency of 3rd allele.

HWE was calculated using the Chi-square test that was applied to compare the heterozygosity value between the expected and observed genotype value (Hartl and Clark 1997):

$$x^2 = \sum_{i=1}^{k} \frac{(oi - ei)^2}{ei}$$

Where, X2 is Chi-square value; oi is an observed genotype frequency value; ei is an expected genotype frequency value. X2 table using 5% significance level for HWE test.

Average heterozygosity (H) was quantified to determine the genetic diversity of the Ongole Grade cattle population by using a formula of Nei (1987):

$$H = \frac{1 - \sum_{i}^{m} qi^2}{r}$$

Where, qi is allele frequency in each loci; m is number of allele; r is number of loci.

Allele frequency data were then used to analyze genetic distance (Nei 1972) and phylogeny tree constructed using the Neighbor-joining method (Saitou and Nei 1987) by using DISPAN: Genetic Distance and Phylogenetic Analysis program (Institute of Molecular Evolutionary Genetic 1993).

RESULTS AND DISCUSSION

Electrophoresis result of blood protein plasma clearly showed 4 blood protein locus, namely Albumin (*Alb*), Post Albumin (*Pa*), Ceruloplasmin (*Cp*), and Amylase-I (*Amy-I*) that comprised of 9 identified alleles (Figure 2). Results showed different protein band patterns indicating the diversity of individual genotypes and generated the differences of gene frequency of the Ongole Grade cattle population. Johari et al. (2007) stated that blood protein polymorphism could describe the genes and genotype frequency of a population based on the observed protein locus. Estimated allele and genotype frequency are presented in Tables 2 and 3.

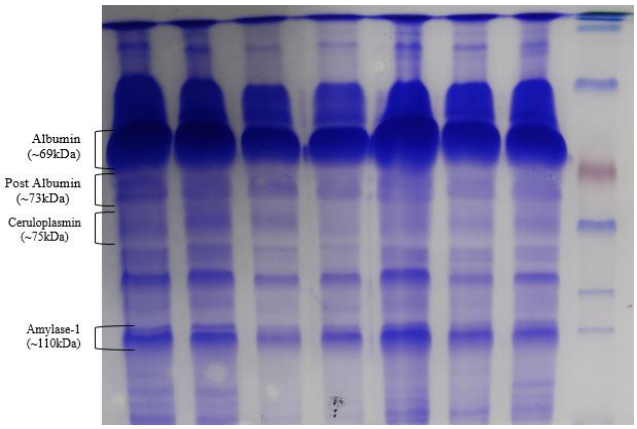

Figure 2. Electrophoresis result of blood protein of Ongole Grade cattle showing protein locus of Albumin, Post Albumin, Ceruloplasmin and Amylase-1

The result of the protein band electrophoresis of Alb loci showed polymorphically and had 3 alleles, namely Alb^A, Alb^B, and Alb^C. Alb^A allele moved faster than the other alleles, while the Alb^C allele was the slowest-moving allele compared to other alleles. The Alb loci showed clearer and thicker bands than the other loci because albumin protein had the largest portion in blood plasma, as much as 35-50% of the total plasma protein, with a molecular weight of 69 kDa (Kajal and Pathania 2021). So, it showed a thicker band than other protein loci bands. *Alb* molecules that had a small size and large electrical charge showed the fastest migration rates, so the band appeared thicker. The highest frequency of Alb^A, Alb^B, and Alb^C alleles was identified in Kudus (0.400), Semarang (0.750), and Kebumen (0.386) populations, respectively. Conversely, the lowest frequency of those alleles was observed in Magelang (0.208), Kebumen (0.357), and Semarang (0.029), respectively. Evenly, Alb^B allele was the highest allele that was found throughout the Ongole Grade cattle population in Central Java. Similar result was reported by Sutiyono et al. (2018) that allele frequency in *Alb* locus of Ongole Grade cattle was 50% Alb^B; 29,4% Alb^C, and 20.6% Alb^A. However, a different fact was reported by Yuwono et al. (2016) that Alb^A allele dominated Alb locus compared to Alb^B allele, while Alb^C was not found in Ongole Grade cattle.

Table 2. Allele Frequency of six population of Ongole Grade cattle in Central Java, Indonesia

Locus	Allele	Frequency						Overall mean
		Rembang	Blora	Kebumen	Semarang	Magelang	Kudus	
Alb	Alb^A	0.279	0.382	0.257	0.221	0.208	0.400	0.291
	Alb^B	0.368	0.412	0.357	0.750	0.736	0.529	0.525
	Alb^C	0.353	0.206	0.386	0.029	0.056	0.071	0.184
Pa	Pa^A	0.309	0.426	0.486	0.456	0.333	0.657	0.444
	Pa^B	0.691	0.574	0.514	0.544	0.667	0.343	0.556
Cp	Cp^F	0.438	0.606	0.357	0.765	0.750	0.729	0.608
	Cp^S	0.563	0.394	0.643	0.235	0.250	0.271	0.392
Amy-I	$Amy\text{-}I^B$	0.397	0.353	0.471	0.868	0.958	0.786	0.639
	$Amy\text{-}I^C$	0.603	0.647	0.529	0.132	0.042	0.214	0.361

Table 3. Genotype Frequency of six population of Ongole Grade cattle in Central Java, Indonesia

Locus	Genotype	Frequency						
		Rembang	Blora	Kebumen	Semarang	Magelang	Kudus	Overall mean
Alb	AA	0	0.059	0	0.059	0.055	0.228	0.067
	AB	0.235	0.235	0.200	0.323	0.306	0.343	0.273
	AC	0.324	0.412	0.314	0	0	0	0.175
	BB	0.088	0.294	0.114	0.559	0.528	0.286	0.312
	BC	0.324	0	0.286	0.059	0.111	0.143	0.154
	CC	0.029	0	0.086	0	0	0	0.019
Pa	AA	0.176	0.353	0.371	0.323	0.167	0.514	0.317
	AB	0.265	0.147	0.229	0.265	0.333	0.286	0.254
	BB	0.559	0.500	0.400	0.412	0.500	0.200	0.429
Cp	FF	0.156	0.364	0.029	0.618	0.639	0.572	0.396
	FS	0.563	0.485	0.657	0.294	0.222	0.314	0.423
	SS	0.281	0.151	0.314	0.088	0.139	0.114	0.181
Amy-I	BB	0.118	0.176	0.114	0.794	0.917	0.714	0.472
	BC	0.324	0.353	0.714	0.147	0.083	0.143	0.294
	CC	0.558	0.471	0.172	0.059	0	0.143	0.234

The 3 alleles of *Alb* loci in this study formed 6 genotypes comprising 3 homozygous genotypes (AA, BB, CC) and 3 heterozygous genotypes (AB, AC, BC). However, not all of them were identified in each population in this study. AA allele was not found in Rembang and Blora populations, AC allele was not found in Semarang, Magelang and Kudus populations, BC allele was not found in the Blora population, and CC allele was not found in Blora, Semarang, Magelang, Kudus population. Only AB and BB genotypes were observed in all Ongole Grade cattle populations in Central Java, and BB genotype was identified as the dominant genotype in this study. The absence of a specific genotype in a particular population in this study lead to a meaningful difference in the genotype frequency distribution, probably the effect of selection leading to certain preferable traits by farmers.

In this study, 2 alleles were found in *Pa* loci, namely Pa^A and Pa^B alleles. Pa^A allele migrated more rapidly to the positive pole than Pa^B allele. Pa^B allele was the dominant allele in this study, same as stated by Sutiyono et al. (2018). Estimated frequency of Pa^B allele was higher than Pa^A in Rembang (0.691), Blora (0.574), Kebumen (0.514), Semarang (0.544), and Magelang (0.667). Otherwise, Kudus population had the highest Pa^A allele frequency (0.657). Pa^A and Pa^B were forming 3 genotypes, which were homozygous AA and BB genotypes and heterozygous AB genotypes. All of them were found in all over Ongole Grade cattle population in this study and predominate by BB genotype with frequency 0.559; 0.500; 0.400; 0.412; and 0.500 in Rembang, Blora, Kebumen, Semarang, and Magelang, respectively. On the other hand, BB and AA genotype was the lowest and the highest frequency in the Kudus population (0.200; 0.514), respectively. AA genotype was the lowest frequency in Rembang (0.176) and Magelang (0.167), whereas AB genotype was observed as the lowest frequency in Blora (0.147), Kebumen (0.229), and Semarang (0.265). On the contrary, Yuwono et al. (2016) reported that most *Pa* locus of Ongole Grade cattle and Simmental Grade cattle were dominated by the Pa^A allele (72.8%; 73.5%) and AA genotype (0.615; 0.583) while Zitny et al. (2007) claimed that 3 alleles (A, B, C) forming 3 homozygous genotypes (AA, BB, CC) and 3 heterozygous genotypes (AB, BC, AC) were discovered in Pa locus of Slovak Spotted dairy cow breed.

Two alleles were discovered in *Cp* loci of the Ongole Grade cattle population in this study, which was Cp^F and Cp^S allele. Cp^F allele knew as the fastest one that moved to the positive pole, while Cp^S allele moved slower to the positive pole than Cp^F allele in *Cp* loci. Estimated allele frequency of Cp^F and Cp^S in this study was 0.438 and 0.563; 0.606 and 0.394; 0.357 and 0.643; 0.765 and 0.235; 0.750 and 0.250; 0.729 and 0.271 for Rembang, Blora, Kebumen, Semarang, Magelang and Kudus Ongole Grade cattle population, respectively. So, in this study, Cp^F allele was the dominant allele in these loci and was in parallel with a report by Sutopo et al. (2001). On the other hand, Cp^S allele was dominant, with a frequency 58.8% (Sutiyono et al. 2018). Both homozygous and heterozygous genotypes were observed in *Cp* loci, which were FF, SS and FS. The highest frequency in Rembang, Blora, and Kebumen populations was FS genotype (0.563; 0.485; 0.657), while in Semarang, Magelang and Kudus were FF genotype (0.618; 0.639; 0.572). Based on estimated genotype frequency, FS genotype was dominating genotype in Cp loci of the Ongole Grade cattle population in this study. While as a physiological function, aside from preserving cell integrity through cytoprotective and various antioxidative activities, ceruloplasmin facilitates the binding of Fe to Transferrin protein as well (Demir and Mert 2015). In addition, ceruloplasmin has a role as a copper-binding protein and an acute-phase protein, which is synthesized in the liver. It acts as an enzyme carrier for most of the copper in plasma (Lopez et al. 2021).

Ongole Grade cattle in this study had 2 alleles distributed in *Amy-I* loci, namely $Amy\text{-}I^B$, which was the fastest moving to the positive pole, and $Amy\text{-}I^C$ allele, which was the slowest moving to the positive pole. Otherwise, Yuwono et al. (2016) found 3 alleles in Ongole Grade and Simmental cattle, namely $Amy\text{-}I^C$, $Amy\text{-}I^B$, and $Amy\text{-}I^C$ allele. In the current study, the $Amy\text{-}I^B$ allele, the fastest one, was frequently found in this study compared to $Amy\text{-}I^C$. $Amy\text{-}I^B$ allele frequency of Rembang, Blora, Kebumen, Semarang, Magelang, and Kudus populations was 0.397; 0.353; 0.471; 0.868; 0.958; 0.786, respectively. While Amy-IC allele frequency was 0.603; 0.647; 0.529; 0.132; 0.042; 0.213, respectively. Three genotypes were observed in *Amy-I* loci, namely BB, BC and CC genotypes. BB genotype was predominant in Semarang, Magelang, and Kudus populations (0.794; 0.917; 0.714); CC genotype was predominant in Rembang and Blora populations with frequencies 0.558 and 0.471, while BC genotype was predominant in Kebumen population (0.714). Furthermore, in the overall population, *Amy-I* loci were dominated by BB genotype with a frequency 0.472. Prior study by Sutiyono et al. (2018) reported CC genotype dominated the Ongole Grade cattle population in Rembang, Central Java, as much as 41.1%.

Based on the Chi-square test, the Ongole Grade cattle population in this study showed deviation in HWE ($P<0.05$), especially at a specific locus in Blora, Kebumen, Semarang, and Kudus population (Table 4). This condition indicates that the population was changing in allele and genotype frequencies, which could be an effect of intensive selection by the breeder. Moreover, other effects like evolution, mutation, selection, inbreeding, and genetic drift, could possibly be one of the causes. Conversely, the Ongole Grade cattle population in Rembang and Magelang were in HWE condition. Both populations were suspected of having no significant evolution and were randomly mating. Moreover, they presumably were in an isolated condition, so there was no migration inside or outside the population leading to their allele and genotype frequencies remaining constant (Andrews 2010).

The average heterozygosity of the Ongole Grade cattle population in the recent study was a range of 0.328-0.529 (Table 5). Polymorphism was observed in the whole locus. The Ongole Grade cattle population in Rembang, Blora, and Kebumen had a heterozygosity value >50%, while the rest population had a heterozygosity value <44%. The highest heterozygosity value was in the Kebumen

population (0.529), while contrary the lowest heterozygosity value was in the Magelang population (0.328). Among overall locus, *Alb* locus had the highest individual heterozygosity value of another locus with an average 0.553. According to Nei (1987), heterozygosity values ranged from 0 to 1, where a value range of <0.2 means low genetic diversity; =< 0.2 - 0.3 means moderate genetic diversity, and => 0.3 means high genetic diversity. The result of this study showed that Ongole Grade cattle in Rembang, Blora, and Kebumen populations had high genetic diversity. This such a good condition since Rembang and Kebumen were known as Ongole Grade cattle breeding center areas in Central Java refers to Decree of the Ministry of Agriculture No. 47/Kpts/SR.120/1/2015 for Kebumen and 404/Kpts/PK.010/7/2017 for Rembang (Ministry of Agriculture 2015; Ministry of Agriculture 2017). The higher the heterozygosity value of a population, presumably the higher the incidence of outbreeding which has an effect on increasing the proportion of heterozygous genotypes. Moreover, low heterozygosity values will endanger the sustainability of a species or population due to the high inbreeding rate. In some species that undergo frequent inbreeding, males are hypothesized to play a key role in maintaining genetic heterozygosity (Yashiro et al. 2021). Besides, Iversen et al. (2019) stated that heterozygosity may be a useful indicator to predict the ability of purebreds to produce good crossbreed offspring.

The genetic distance of the Ongole Grade cattle population in the study was determined based on polymorphism of four blood protein locus (Table 6). The result showed the genetic distance of Ongole Grade cattle among the population ranged from 0.0057 to 0.0955. The closest relationship was between Kebumen and Rembang populations (0.0057), while the furthest relationship was between Kebumen and Magelang populations (0.0955).

This fact was also interpreted by the phylogenetic tree, as shown in Figure 3. Results showed that the phylogenic tree of the Ongole Grade population in Central Java based on blood protein polymorphism was divided into 2 major clusters. Cluster I consisted of the Blora population and sub-cluster occupied by the Rembang and Kebumen populations (0.0092 and 0.0057). While Cluster II consisted of Kudus population and sub-cluster that was occupied by Semarang and Magelang populations (0.0135 and 0.0288). Interestingly, based on Sudaryanto et al. (2018), Ongole Grade cattle in the Rembang population had a closer morphological relationship to the Blora population than the Kebumen population refers to discriminant Mahalanobis value analysis using morphometric data. They added that phenotypic diversity among Ongole Grade cattle subpopulations is suspected to be caused by various factors, including relatively diverse environmental influences, including lifespan, maintenance management, and the amount and type of feed that also affects performance. While in this study, Ongole Grade cattle of the Rembang population had a slightly closer genetic relationship with the Kebumen population than the Blora population (0.0057 vs 0.0092).

Table 4. Hardy-Weinberg equilibrium of six population of Ongole Grade cattle in Central Java, Indonesia

Locus	Population					
	Rembang	**Blora**	**Kebumen**	**Semarang**	**Magelang**	**Kudus**
Alb	9.114	26.745*	5.733	0.716	1.459	5.844
Pa	4.908	16.631*	10.300*	7.397*	2.250	4.687
Cp	0.653	0.008	6.505*	1.135	5.975	1.476
Amy-I	0.950	1.756	6.570*	4.400	0.068	11.602*

Note: *p<0.05

Table 5. Heterozygosity of six population of Ongole Grade cattle in Central Java, Indonesia

Population	Individual Heterozygosity				Heterozygosity
	Alb	***Pa***	***Cp***	***Amy-I***	
Rembang	0.662	0.427	0.492	0.479	0.515
Blora	0.642	0.490	0.478	0.457	0.516
Kebumen	0.658	0.500	0.459	0.498	0.529
Semarang	0.388	0.496	0.360	0.230	0.368
Magelang	0.412	0.444	0.375	0.080	0.328
Kudus	0.556	0.451	0.396	0.337	0.435

Table 6. Genetic distance estimation matrix of Ongole Grade cattle population in Central Java, Indonesia based on Nei (1972) and Nei (1978)

Population	1 Rembang	2 Blora	3 Kebumen	4 Semarang	5 Magelang
Rembang	-	-	-	-	-
Blora	0.0092 ± 0.0111	-	-	-	-
Kebumen	0.0057 ± 0.0171	0.0154 ± 0.0283	-	-	-
Semarang	0.0812 ± 0.0777	0.0614 ± 0.1169	0.0810 ± 0.0763	-	-
Magelang	0.0936 ± 0.1205	0.0826 ± 0.1576	0.0955 ± 0.0762	0.0060 ± 0.0054	-
Kudus	0.0707 ± 0.0301	0.0435 ± 0.0822	0.0636 ± 0.0698	0.0135 ± 0.0173	0.0288 ± 0.0394

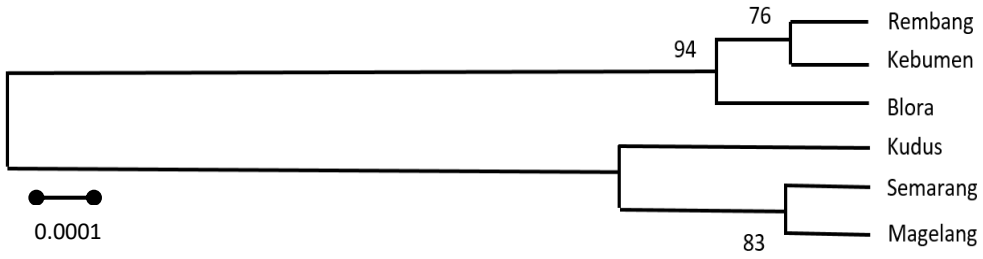

Figure 3. Phylogenetic tree of six population of Ongole Grade cattle in Central Java, Indonesia

The closeness of Ongole Grade cattle of the Rembang population with the Kebumen population is possibly caused by the fact that frozen semen of Ongole Grade sire from Kebumen is widely used in the implementation of artificial insemination in the Rembang region. Whereas the closeness of Ongole Grade cattle of the Rembang population with the Blora population may be due to geography, it is an adjacent area that allows for easy movement of livestock traffic. The same thing also happened to Ongole Grade cattle of Semarang, Kudus and Magelang populations, they probably originated from the same ancestor, so they had close similarity based on blood protein polymorphism. In another study using microsatellite markers, among breed cattle in Indonesia, Ongole Grade cattle position was in the second cluster together with Sumba Ongole cattle, Madura cattle, Pasundan cattle, and Pesisir cattle as Bos Indicus cluster (Agung et al. 2019).

In conclusion, this study identified that Albumin, Post-albumin, Ceruloplasmin, and Amylase-I locus in six populations of Ongole Grade cattle in Central Java were polymorphic. The Ongole Grade cattle population in Central Java showed high genetic diversity and was divided into two major clusters, each comprising three populations. The closest relationship between Kebumen and Rembang populations indicated that the distance between locations of the Ongole Grade population was not proportionately affected by the genetic distance based on blood protein polymorphism.

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