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Allele Diversity of Diiti Cattle Using Microsatellite Loci

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Abstract. Diiti cattle, also known as Gorontalo local cattle, exhibit phenotypic characteristics similar to those of Bali and PO cattle. To better understand the genetic status of Diiti cattle, this study assessed allelic diversity using the microsatellite loci ILSTS017, HEL13, and BM1818. The primary objective was to evaluate the genetic variation of Gorontalo local cattle through microsatellite marker analysis. A total of 117 Diiti cattle sampled from Gorontalo, Bone Bolango, and North Gorontalo were included in the study. Allelic variation was analyzed using Cervus version 3.0.7, focusing on the number of alleles, observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC), and Hardy–Weinberg equilibrium. Results revealed that all microsatellite loci were highly polymorphic within the population. A total of 26 alleles were detected, ranging from 6 alleles at HEL13 to 11 at ILSTS017, with a mean allele count of 6.667. The He and Ho values for ILSTS017, HEL13, and BM1818 were 0.686 and 0.512; 0.610 and 0.351; and 0.818 and 0.721, respectively. The corresponding PIC values were 0.645 (ILSTS017), 0.540 (HEL13), and 0.791 (BM1818), indicating a high degree of polymorphism and informativeness. In summary, the microsatellite markers employed in this study proved to be effective for analyzing genetic diversity in Gorontalo local cattle. These findings provide a foundational genetic dataset for future conservation and development efforts related to the Diiti cattle as a valuable genetic resource in Gorontalo.

Keywords: Diiti cattle, microsatellite, allele diversity, heterozygosity, polymorphic

Abstrak. Sapi Diiti atau sapi lokal Gorontalo merupakan sapi yang memiliki ciri eksterior yang sama dengan sapi Bali dan PO. Evaluasi keragaman alel menggunakan lokus mikrosatelit ILSTS017, HEL13 dan BM1818 dilakukan untuk memberikan informasi mengenai keadaan sapi Diiti yang tidak diketahui. Sebanyak 117 ekor sapi Diti dari Gorontalo, Bonebolango dan Gorontalo Utara digunakan dalam penelitian ini. Analisis keragaman alel dilakukan menggunakan aplikasi Cervus versi 3.0.7. Hasil penelitian menunjukkan bahwa semua lokus mikrosatelit sangat polimorfik dalam populasi. Sebanyak 26 alel ditemukan dalam penelitian ini, berkisar antara 6 alel (HEL13) dan 11 (ILSTS017). Rata-rata total alel sebesar 6,667. Nilai Heterozigositas yang diharapkan dan heterozigositas yang diamati masing-masing adalah 0,686, 0,610, 0,818 dan 0,512, 0,351, 0,721 pada ILSTS017, HEL13 dan BM1818. Nilai Polymorphism Information Content (PIC) yaitu 0,540 untuk HEL13, 0,645 untuk ILSTS017 dan 0,791 untuk BM1818. Kesimpulannya semua lokus mikrosatelit yang digunakan dalam penelitian ini bersifat sangat polimorfik dan informatif, serta semua alel yang diidentifikasi dalam penelitian ini mampu menganalisis keragaman alel pada sapi lokal Gorontalo. Hasil penelitian ini dapat digunakan sebagai informasi dasar untuk pengembangan sapi Diiti yang belum diketahui statusnya sebagai sumber daya genetik asal Gorontalo.

Kata kunci: sapi diiti, mikrosatelit, keragaman alel, heterozigositas, polimorfisme

Introduction

Gorontalo is the second smallest province in Indonesia after West Papua, covering a total area of 11,257 km², and was officially designated as the country's 32nd province in the year 2000. It is bordered by Central Sulawesi to the west, North Sulawesi to the east, the Sulawesi Sea to the north, and the Gulf of Tomini to the south. Administratively, Gorontalo comprises five regencies—Bone Bolango, Boalemo, Gorontalo, North Gorontalo, and Pohuwato—as well as Gorontalo City. The province's economy is predominantly supported by agriculture. Livestock farming, particularly cattle rearing, is one of the most common agricultural activities. In 2022, the cattle population reached 263,023, reflecting steady growth in the livestock sector. Local cattle breeds offer significant potential for further development and can contribute meaningfully to the region's agricultural advancement.

Gorontalo local cattle (Diiti) exhibit phenotypic characteristics resembling PO cattle

(*Bos indicus*), Bali cattle (*Bos sondaicus*), (Dako et al., 2024; Domili et al., 2021; Gobel et al., 2021; Laya et al., 2024) or a combination of both (Dako et al. 2023). Within local farming communities, these cattle are commonly referred to as "Ditti cows" or "small cows." The current population status and genetic structure of these cattle remain largely undocumented due to the absence of prior research. To better characterize these animals, it is essential to assess both phenotypic and genotypic diversity. One critical component of genetic diversity is allelic variation, which provides valuable complementary insights. An allele—or allelomorph—is one of two or more alternative forms of a gene located at a specific locus on a chromosome that can influence phenotypic traits. Measures of allelic diversity, typically based on the number of different alleles segregating within a population, are widely utilized in conservation genetics as they are correlated with long-term evolutionary potential and overall genetic responsiveness to selection. As such, allelic diversity serves as a strong predictor of adaptive capacity over time. This form of diversity can be effectively assessed using microsatellite loci analysis.

Microsatellite DNA loci are short tandem repeats consisting of two to six nucleotide motifs—such as di-, tri-, tetra-, or penta-nucleotide sequences—repeated multiple times (Selkoe and Toonen 2006). These loci are widely regarded as the most effective molecular markers in genetic studies, particularly those involving genetic diversity, relationships, gene flow, genetic distance, mating systems, and conservation demography (Kostro-Ambroziak et al. 2020; Coelho et al. 2018; Manlik et al. 2019; Nowicki et al. 2019; Seo et al. 2017). Intraspecific allele frequency variation is a key feature of microsatellite loci, making them highly informative (Symonds and Lloyd. 2003). The utility of these markers is attributed to their high polymorphism, co-dominant inheritance, and widespread distribution across the genome (Selkoe and Toonen. 2006). Given these properties, this study focuses on analyzing the allelic diversity of Gorontalo local cattle (Diiti) using microsatellite markers, as these cattle represent a distinct cluster likely derived from the genetic dilution of PO and Bali cattle.

Materials and Methods

Animal and Blood sample collection

A total of 117 Diiti cattle were sampled for this study, originating from three distinct regions: North Gorontalo (n = 28), Bone Bolango (n = 37), and Gorontalo (n = 52). Blood samples were obtained via the *vena coxygealis* and collected in tubes containing EDTA as an anticoagulant. All procedures involving animal handling and sampling were conducted in accordance with ethical guidelines and were approved by the Animal Ethics Committee of Brawijaya University (Approval No. 145-KEP-UB-2022).

DNA Isolation, Primer and DNA Amplification

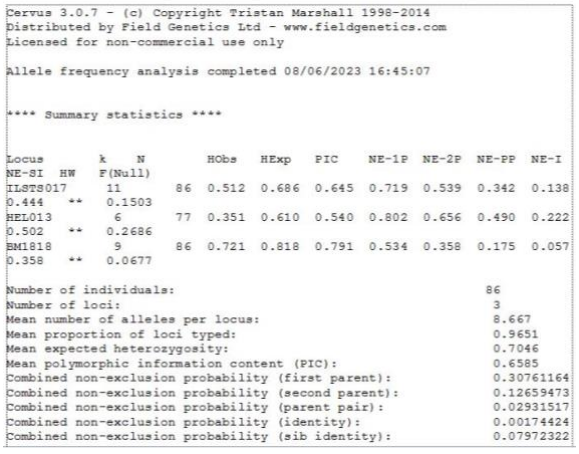
DNA isolation was performed at the Biotechnology Laboratory of the Faculty of Animal Science, Brawijaya University. Genomic DNA was extracted using a Genomic DNA Mini Kit, following the manufacturer's protocol. Three bovine-specific microsatellite primers—ILSTS017, HEL13, and BM1818—were employed in the PCR amplification process (Table 1). The thermal cycling conditions consisted of 35 cycles with an initial denaturation at 95 °C for 30 seconds, annealing at 58 °C for 45 seconds, and extension at 72 °C for 1 minute. DNA amplification products were visualized using the method described by Agung et al. (2018) for Indonesian cattle breeds. Fragment analysis of the microsatellite markers was conducted at First BASE Laboratories, Selangor, Malaysia.

Table 1. Microsatellite primer used in study

Primer	Chromosome	Sequence	Reference
ILSTS017	X, DXS11	F: 5'GCATCTCTATAACCTGTTCC 3' R: 5'AAGGAACTTTCAACCTGAGG 3'	Dako et al. (2023); Mukherjee et al. (2022); Shatalina et al. (2021)
HEL13	11	F: 5'TAAGGACTTGAGATAAGGAG 3' R: 5'CCATCTACCTCCATCTTAAC 3'	
BM1818	23	F: 5'AGCTGGGAATATAACCAAAGG 3' F: 5'AGTGCTTTCAAGGTCCATGC 3'	

Data Analysis

Allelic diversity in this study was assessed based on converted data obtained from microsatellite fragment analysis. The processed data were analyzed using Cervus version 3.0.7 to determine the number of alleles, observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC), and Hardy–Weinberg equilibrium (Susilorini et al., 2022; Dako et al., 2023). Microsatellite data analysis using Cervus followed several steps: first, the fragment analysis output was transferred to Microsoft Excel using a format compatible with Cervus, then saved as a .txt file. The file was converted into Genepop format using a separate converter tool. Within Cervus, the Genepop file was imported via *Tools > Convert Genepop to Cervus*, where users specified the three-digit format, saved the file as a Cervus project, and proceeded by selecting *OK*. The analysis was performed by navigating to *Analysis > Allele Frequency Analysis*, ensuring that the individual ID occupied column 2, the first allele was in column 3, and the number of loci was correctly defined. The output was saved through *Save > OK*. The resulting report included data on heterozygosity, PIC values, allele frequency per locus, Hardy–Weinberg equilibrium, and null allele estimates.



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Cervus 3.0.7 - (c) Copyright Tristan Marshall 1998-2014
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Allele frequency analysis completed 08/06/2023 16:45:07


**** Summary statistics ****


Locus        k    N        HObs   HExp   PIC    NE-1P  NE-2P  NE-PP  NE-I
NE-SI  HW   F(Null)
ILSTS017     11      86  0.512  0.686  0.645  0.719  0.539  0.342  0.138
0.444   **   0.1503
HEL013        6      77  0.351  0.610  0.540  0.802  0.656  0.490  0.222
0.502   **   0.2686
BM1818        9      86  0.721  0.818  0.791  0.534  0.358  0.175  0.057
0.358   **   0.0677

Number of individuals:                                    86
Number of loci:                                            3
Mean number of alleles per locus:                          8.667
Mean proportion of loci typed:                             0.9651
Mean expected heterozygosity:                              0.7046
Mean polymorphic information content (PIC):                0.6585
Combined non-exclusion probability (first parent):         0.30761164
Combined non-exclusion probability (second parent):        0.12659473
Combined non-exclusion probability (parent pair):          0.02931517
Combined non-exclusion probability (identity):             0.00174424
Combined non-exclusion probability (sib identity):         0.07972322
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Figure 1. Analysis result from Cervus application

Results and Discussion

Evaluating genetic variation in livestock provides essential insights that support breeding strategies and long-term development within the agricultural sector. The Diiti cattle examined in this study exhibited phenotypic traits consistent with both Bali and PO cattle (Figure 1). A total of 26 alleles were identified across the three microsatellite loci analyzed, with ILSTS017 displaying 11 alleles, HEL13 showing 6 alleles, and BM1818 yielding 9 alleles. Detailed information regarding the number of alleles, observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC), and Hardy–Weinberg (HW) equilibrium for each locus is presented in Table 2.

In Diiti cattle, the observed heterozygosity (Ho) for the ILSTS017 microsatellite locus was 0.512, while the expected heterozygosity (He) was 0.686, with a polymorphism information content (PIC) value of 0.645. For the HEL13 locus, Ho was 0.351 and He was 0.610, with a PIC of 0.540. The BM1818 locus exhibited the highest genetic variability, with Ho at 0.721, He at 0.818, and a PIC value of 0.791. The observed heterozygosity values in this study, ranging from 0.351 to 0.721, were within the acceptable range for assessing genetic variation. According to Takezaki et al. (1996), a genetic marker is considered informative when the average heterozygosity exceeds 0.3 in a population. In all

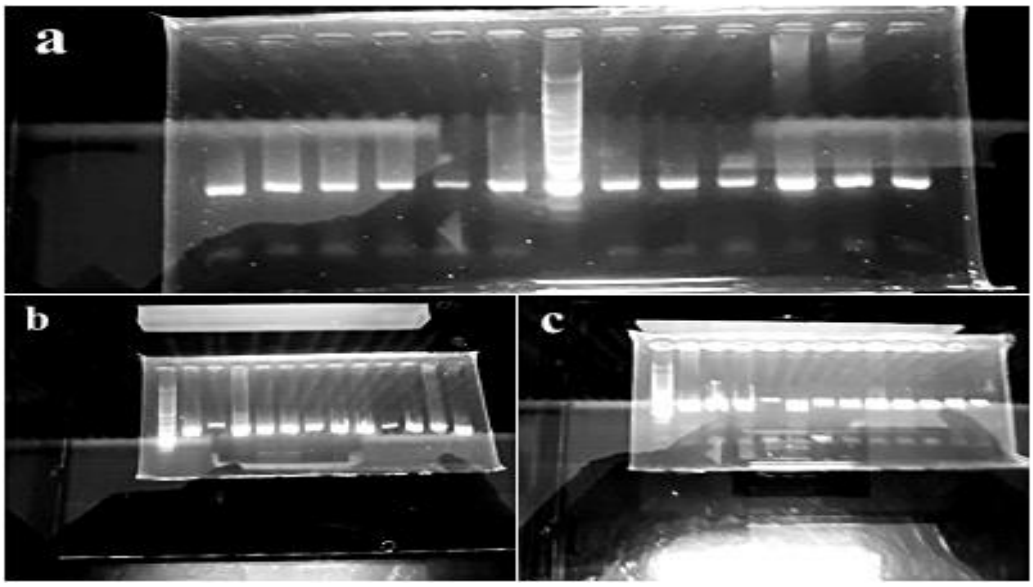

Figure 2. Visualization of resulted DNA amplification (a) ILSTS017 (b) HEL13 (c) BM1818

loci analyzed, observed heterozygosity was consistently lower than the expected values. Heterozygosity (H) is a key indicator of genetic diversity in natural populations, providing insights into population structure and historical dynamics. It represents the probability that an individual will possess different alleles at a given locus and is influenced by the number and frequency of alleles in the population. Heterozygosity ranges from zero (no variation) to one (maximum variation with equally frequent alleles). In individuals, it reflects the average proportion of heterozygous loci, while at the population level, it can be measured using both observed and expected heterozygosity. Expected heterozygosity (He), also known as Nei's gene diversity (D), estimates the likelihood of heterozygosity at a locus across the population, serving as a robust metric less affected by sample size. In contrast, observed heterozygosity (Ho) is calculated by dividing the number of heterozygotes by the total number of individuals sampled. Comparing Ho with He under Hardy–Weinberg equilibrium conditions helps reveal potential inbreeding or population substructure. The number of alleles detected at the ILSTS017 locus in this study was higher than reported by Saputra et al. (2020). For the HEL13 locus, the allele count was lower than that observed by Sun et al. (2008) but higher than findings by Zulkharnain et al. (2023). At the BM1818 locus, the number of alleles exceeded those reported by Stevanov-Pavlović et al. (2015), Gupta et al. (2016), and El-Sayed et al. (2016).

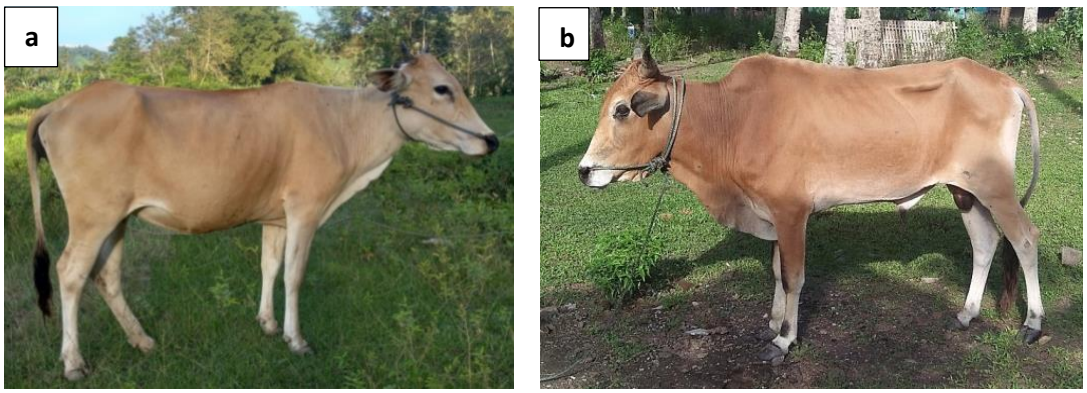

Figure 3. Diiti cattle (a. Female, and b. Male)
Source: Personal documentation

The heterozygosity results (Ho < He) indicate a deviation from Hardy–Weinberg equilibrium (HWE). The Hardy–Weinberg principle posits that genetic variation within a population remains constant across generations in the absence of evolutionary forces. However, various factors—including natural selection, inbreeding, artificial selection, the Wahlund effect, the presence of null alleles, mutations, nonrandom mating, gene flow, and genetic drift—can disrupt this equilibrium. Among these, natural selection and nonrandom mating alter gene frequencies by favoring or disfavoring specific alleles, thus influencing reproductive success. Mutations introduce new alleles into the gene pool, thereby altering allele frequencies. Gene flow introduces alleles from other populations, while genetic drift—especially in small populations—results in random fluctuations in allele frequencies. Inbreeding can reduce genetic diversity and lead to inbreeding depression, characterized by diminished fitness and performance. Because these forces occur naturally, true Hardy–Weinberg equilibrium is seldom observed in real populations. Instead, it serves as a theoretical baseline against which deviations can be measured to assess genetic structure and evolutionary influences (Barczak et al., 2009; Pan & Jinzeng, 2010; Rahal et al., 2021; Nguyen et al., 2007; Neamatzadeh et al., 2024). Evaluating genetic markers for HWE is also a valuable tool for identifying potential genotyping errors. Significant deviations — particularly heterozygote excess (HetExc)—may arise from such errors. Alternatively, HetExc may reflect selective pressures, whereby recessive alleles associated with advantageous traits reach high frequencies in the heterozygous state while remaining rare in homozygotes (Abramovs et al., 2020).

In this study, the estimated frequency of null alleles (F(null)) ranged from 0.068 to 0.269. Null alleles are defined as alleles that fail to amplify during genotyping, often due to polymorphisms at primer-binding sites, deletion events encompassing the marker, or the presence of triallelic single nucleotide polymorphisms (SNPs). This phenomenon, commonly referred to as “allele drop-out,” can lead to misclassification, where heterozygous individuals are incorrectly genotyped as homozygous. Despite their technical challenges, null alleles may also indicate underlying functional genetic variation (Crooks et al., 2013). The prevalence of null alleles varies widely among studies and has been reported across diverse taxa, including those with relatively small effective population sizes (Dakin & Avise, 2004). Notably, the presence of null alleles can result in the overestimation of population differentiation indices such as FST and genetic distance, particularly in contexts of pronounced population structure (Chapuis & Estoup, 2007).

The polymorphism information content (PIC) values obtained in this study ranged from 0.540 for HEL13 to 0.791 for BM1818. According to

Table 2. Number of alleles (Na), observed heterozygosity (OH), expected heterozygosity (EH), Polymorphism Information Content (PIC) and Hardy–Weinberg (HW) equilibrium in each microsatellite loci.

Microsatellite loci	Na	Allele size (bp)	PIC	Heterozygosity		HW	F(Null)
				Observed (OH)	Expected (EH)		
ILSTS017	11	102-127	0.645	0.512	0.686	**	0.150
HEL13	6	181-191	0.540	0.351	0.610	**	0.269
BM1818	9	251-269	0.791	0.721	0.818	**	0.068
	6.667±2.516						

Botstein et al. (1980), PIC serves as an indicator of marker informativeness and is classified into three categories: slightly informative (PIC < 0.25), reasonably informative (PIC = 0.25–0.50), and highly informative (PIC > 0.50). PIC reflects a genetic marker's capacity to detect polymorphism within a population, with higher values indicating greater discriminatory power. A marker is considered qualitatively polymorphic if it possesses at least two alleles, with the most common allele having a frequency below 99%. Heterozygosity and PIC are both quantitative indicators of polymorphism levels, and PIC is frequently employed in genetic studies to evaluate marker quality. For codominant markers, PIC values range from zero (monomorphic) to one (extremely polymorphic, with alleles of equal frequency) (Botstein et al., 1980; Shete et al., 2000). All three microsatellite loci evaluated in this study had PIC values greater than 0.5, indicating that they are highly informative and suitable for genetic characterization of Diiti cattle. Previous studies have reported PIC values ranging from 0.61 to 0.91 in Algerian cattle (Rahal et al., 2021), 0.752 in European cattle (Illie et al., 2015), and 0.536 in Taro White cattle (Heryani et al., 2019). The high PIC values observed in this study support the conclusion that the microsatellite loci used were highly polymorphic within the population. These findings provide a valuable genetic baseline for the further development and conservation of Diiti cattle as a unique genetic resource in Gorontalo.

Conclusions

The microsatellite loci ILSTS017, HEL13, and BM1818 demonstrated high levels of polymorphism and informativeness in assessing allelic diversity in Diiti cattle. These findings provide foundational genetic data that will support future efforts to characterize and develop Diiti cattle as a distinct local breed within Gorontalo Province, Indonesia.

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