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Identification of Genetic Diversity of Taro White Cattle Using Microsatellite DNA Markers

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ABSTRACT

Taro white cattle have a small population found in Taro Village, Tegalalang, and Gianyar, Bali, which must be protected as one of the most valuable biological resources for the Hindu community in Bali. White cattle require conservation efforts, with morphometric characterization and genetics providing the first phases of an animal conservation program. The purpose of this study was to identify the genetic diversity of Taro white cattle based on allele frequency, heterozygosity, and fixation index (FIT) using different microsatellite DNA loci so that it can be used as a study and reference in determining policies for the conservation of Taro white cattle in Indonesia, particularly in Bali. This cross-sectional observational study utilized *ETH 185*, *INRA 035*, and *INRA 037* microsatellite DNA markers. Polymerase chain reaction amplified a total of 22 Taro white cattle blood samples with an average of 4.33 alleles; the average observed heterozygosity and expected heterozygosity were 0.288 and 0.637, respectively, with an average fixation index value of 0.55. In conclusion, there is a significant deviation from the Hardy-Weinberg equilibrium with the likelihood of inbreeding, as indicated by the Hardy-Weinberg balance. The microsatellite loci used in this study can be further used to evaluate the genetic diversity of Taro white cattle.

Keywords: Conservation, Genetic diversity, Microsatellite DNA, Taro white cattle

INTRODUCTION

In the woodlands of Taro Village, Tegallalang District, Gianyar Regency, a very small cattle population is known as white cows native to Taro Village. The uniqueness of these white cattle is that they are holy, sacred, and respected by the community. The cattle may only be utilized in holy sites, including Merkur (Ngasti Widana), Tri Buana, and Eka Dasa Rudra. The extremely small population of Taro cattle will result in inbreeding. This can result in a loss of genetic diversity and a decline in environmental adaptability. Referring to the population risk threshold, white cattle are classified as critical (critical breed). According to FAO (2007), a population of less than 100 individuals is thought to be in an area critical to extinction. Provided that left uncontrolled, the amount of biological wealth not cared for will increase, resulting in the loss of genetic wealth. To prevent biodiversity loss in Indonesia, particularly the white cattle as germplasm and since they play a crucial role in religious events for the Hindu community in Bali, it is necessary to conserve white cattle.

This conservation initiative is linked to the Provincial Government of Bali's commitment and program to accelerate the achievement of the Millennium Development Goals (MDGs) in accordance with the Bali MDGs Roadmap, one of the eight Millennium Development Goals being environmental sustainability (Tanner et al., 2020). Therefore, support for the preservation of white cattle is essential. Reduced biological resource loss is one of the actions centered on conserving the environment (Allendorf, 2017). In conservation efforts, it is essential to understand the population's features; genetic characterization is a crucial stage in animal conservation projects. The genetic features can be used as a guide for determining the distinctness of the animal race (Abdelmanova et al., 2020). Microsatellite deoxyribonucleic acid (DNA) is now commonly used to determine genetic distances through molecular analysis (Moniruzzaman et al., 2015). Since it is not affected by the environment, microsatellites are ideally suited for identifying an order (Abdelkader et al., 2018). Microsatellites are valuable for genome mapping due to their very random distribution within the genome (Aguirre-Liguori et al., 2020). Microsatellites are currently the most prevalent genetic markers utilized in molecular testing (Bora et al., 2023).

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Microsatellites exhibit significant rates of polymorphism and mutation (Glazko et al., 2023). Variation in the number of repeat units accounts for the high microsatellite polymorphism (Bhargava and Fuentes, 2010). Primarily due to the existence of a slippage replication mechanism, mutations occur (Richard, 2021). The rapid mutation rate of microsatellite DNA is mainly attributable to variations in the number of repeating bases added or removed relative to variations in the base sequence (Lei et al., 2021). The reported microsatellite mutation rate is roughly 10-3 to 10-6 (Zhang et al., 2003; Svishcheva et al., 2020). Therefore, the present study aimed to identify the genetic diversity of Taro White cattle using microsatellite DNA loci.

MATERIALS AND METHODS

The present study was a cross-sectional observational study that involved collecting primary data and descriptive analysis of microsatellite DNA polymorphisms to establish the genetic profile of Taro White cattle to use in conservation efforts (Figure 1). A total of 22 adult White Taro cattle (8 males and 14 females) aged 2-8 years and weighing 200-350 kg were investigated in the present study. Three milliliters of blood samples were taken aseptically through the jugular vein and then charged with three distinct microsatellite DNA markers, *ETH 185*, *INRA 035*, and *INRA 037*, which are found on chromosome 17 (*ETH 185*), chromosome 16 (*INRA 035*), and chromosome 10 (*INRA 037*), respectively. Food and Agriculture Organization-International Society for Animal Genetics (FAO-ISAG) suggested that microsatellite DNA markers successfully extracted from diverse breeds of cattle in Indonesia were used to select the primers in the following order (FAO, 2011).

a. *ETH 185*: TGCATGGACAGAGCAGCCTGGC
   GCACCCCAACGAAAGCTCCCA
b. *INRA 035*: TTGTGCTTTATGACACTATCCG
   ATCCTTTGCAGCCTCCACATTG
c. *INRA 037*: GATCCTGCTTATATTTAACCAC
   AAAATTCCATGGAGAGAGAAAC

Figure 1. Taro White Cattle in Taro village, Tegalalang district, Gianyar Regency, Bali, Indonesia

The amplification using the applied biosystem thermal cycler was performed for 35 cycles, with the first cycle at 95°C for five minutes, the next 33 cycles (denaturation at 94°C for thirty seconds, annealing at 58°C for eighty seconds, extension at 72°C for ninety seconds), and the last cycle was elongation at 72°C for five minutes.

Analysis of genetic polymorphism

Allele frequency

The frequency of each allele at each microsatellite locus was estimated using the following formula (Chen et al., 2016).

$$X1 = \frac{(2N1.1 + N1.2)}{2N}$$

X1 is the allele frequency of a first locus, N1.1 is the number of individuals that are homozygous for allele 1, N1.2 is the number of individuals that are homozygous for allele 1, and N is the total number of individuals.

Heterozygosity

The level of heterozygosity was computed via the following formula.

$$H = \frac{2\,N\,(1 - \sum X1^2)}{2N - 1}$$

H is locus heterozygosity, X is allele frequency, and N is the number of individuals.

The microsatellite Toolkit V.3.1 program was used to calculate allele frequencies, allele counts, observed and anticipated heterozygosities (Ho and He), and total heterozygosity (Ht) (Meglécz et al., 2014). To determine random mating in the local population, Hardy-Weinberg equilibrium analysis used the fixation index (FIT), and to determine the deviation of the FIT value from zero (inbreeding occurred), it was evaluated with one degree of freedom, as indicated in the following formula.

$$F_{IT} = 1 - \frac{H_o}{H_T}\; X^2 = n\, {F_{IT}}^2$$

Ethical approval

The current study has received approval from the Animal Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, Indonesia, with Number B/70/UN14.2.9/PT.01.04/2024, and was carried out at the Biomedical Laboratory of the same institution from January to March 2024.

Data analysis

The observed number of alleles (na), allele frequency, observed Ho, expected He, and fixation index value or the inbreeding coefficient (F) from each locus of microsatellite DNA were analyzed using GenAlex, version 6.5 (Genetic analysis in Excel 6.5 version) (Peakall and Smouse, 2012). The GENEPOP, version 4.7.x was used to generate the P-value of Hardy-Weinberg equilibrium (HWE) and the P-value of Linkage disequilibrium (LD) (Rousset, 2008). Hardy-Weinberg disequilibrium is a model for predicting genotype and allele frequencies in a non-evolving population (Abramovs et al., 2020). Linkage disequilibrium is a measure of non-random association between segments of DNA (alleles) at different positions on the chromosome (loci) in a population (Slatkin, 2008). Loci are considered independent or in linkage disequilibrium when the association frequency of different alleles is higher than expected. Loci are said to be randomly associated when the association frequency of different alleles is lower than expected and lower than expected if the loci were associated randomly (Slatkin, 2008). The p-value significant deviation from the HWE test was $p < 0.05$. The p-value significance associated randomly with the LD test was $p < 0.05$.

RESULTS AND DISCUSSION

The results indicated that the three markers had been amplified successfully at the microsatellite locus of Taro cattle. All samples had 13 alleles, with the number of alleles at each location ranging from 3 at the *INRA 037* locus to 5 at the *ETH 185* and *INRA 035* loci, with an average allele diversity of 4.33. The average allele frequency was 0.231. The range of allele diversity was from 110 bp (*INRA 035*) to 250 bp (*ETH 185*, Table 1).

The *INRA 035* locus contained five alleles with the following product sizes: 110 bp, 114 bp, 118 bp, 120 bp, and 128 bp. The frequency of the most dominant 110 bp allele was 0.364%. The mean observed Ho and anticipated He were 0.18 and 0.74, respectively, and the fixation index was 0.755. The *INRA 037* locus had three distinct alleles with 144 bp, 148 bp, and 150 bp product length. The frequency of the most dominant 150-bp allele was 0.614. Mean observed Ho and anticipated He were 0.318 and 0.505, respectively, with a fixation index of 0.370.

The average fixation index (F) or inbreeding coefficient was 0.526, which means close to one. The *ETH 185*, *INRA 035*, and *INRA 037* locus suggested the likelihood of inbreeding in the Taro cattle population ($p < 0.05$, Table 2).

The p-value linkage disequilibrium test from three loci *ETH 185*, *INRA 035*, and *INRA 037* were 0.071, 0.114, and 0.194, respectively. These three loci that were analyzed stated that the loci were independent (Table 3, $p > 0.05$).

Table 1. The number, size, and frequency of alleles of locus *ETH 185*, *INRA 035*, and *INRA 037* in Taro white cattle

Breed	Locus	Number of alleles	Allele size (bp)	Allele frequency
Taro White cattle	*ETH 185*	5	230	0.523
			236	0.114
			240	0.068
			248	0.159
			250	0.136
Taro White cattle	*INRA 035*	5	110	0.364
			114	**0.023**
			118	0.182
			120	0.227
			128	0.205
Taro White cattle	*INRA 037*	3	144	0.045
			148	0.341
			150	**0.614**
Total allele		13		
Average Allele diversity		4.33		
Average allele frequency				0.231

Table 2. Observed heterozygosity, expected heterozygosity, fixation index, and Hardy-Weinberg equilibrium analysis among three microsatellite loci in adult Taro White Cattle

Locus	Number of alleles	Allele size	Ho	He	F	p-value*	HWE
ETH 185	5	230-250	0.364	0.665	0.453	0.0000	*
INRA 035	5	110-128	0.182	0.741	0.755	0.0000	*
INRA 037	3	144-150	0.318	0.505	0.370	0.0367	*
Average	4.33		0.288	0.637	0.526		

Ho: Observed heterozygosity, He: Expected heterozygosity, F: Fixation index, HWE: Hardy Weinberg equilibrium. *p less than 0.05 is Significant

Table 3. Linkage disequilibrium test among three microsatellite loci in adult Taro White Cattle

Pop	Loci 1	Loci 2	p-value*	LD
Taro Village	*ETH 185*	*INRA 035*	0.071	NS
Taro Village	*ETH 185*	*INRA 037*	0.114	NS
Taro Village	*INRA 035*	*INRA 037*	0.194	NS

Pop: Population, LD: Linkage disequilibrium, NS: Not significant; *p less than 0.05 is Significant

DISCUSSION

The development of several molecular markers (DNA markers) has made it possible to identify the genetic changes in a cross and their relationship to changes in cattle's quantitative and qualitative traits (Glazko et al., 2023). In addition, molecular markers can be utilized to distinguish between cattle breeds, particularly in connection with attempts to conserve and maintain the breed's purity (Yaro et al., 2017). Microsatellite markers can be used to examine the genetic diversity of a population and determine the genetic relationship between two populations (Machmoum et al., 2020).

The average heterozygosity within a breed quantifies its genetic variety (Abdelmanova et al., 2020). Heterozygosity is a parameter that can be used to determine a population's genetic diversity (Bora et al., 2023). The heterozygosity value runs from zero to one; provided that the value is close to zero, the heterozygosity is low, which can threaten the survival of a population or species (Misrianti et al., 2022). In case the value is close to one, the heterozygosity is high; the higher the heterozygosity value of a population, the greater the frequency of inbreeding (Alvarez et al., 2011). Low genetic variability can result in animal populations with high inbreeding coefficients (White et al., 2021). Additionally, a high level of inbreeding in specific animal groups might reduce hybrid vigor (Sharma et al., 2020).

At the *INRA 035* gene, five alleles with various sizes ranging from 110 to 128 bp were identified. The allele size with the highest frequency was 110 bp, with a frequency of 0.364. In contrast to the research of Kuantan 1 cattle, Kuantan 2 cattle, Pesisir cattle, and Madura cattle at Riau province, Indonesia, the number of alleles and allele sizes observed in the current study at the *INRA 035* locus are 17, 11, 5, and 98-120 bp, respectively (Misrianti et al., 2022). Indicative of the genetic variability of a population, the number of alleles is proportional to sample size. Increasing the number of populations and samples can increase the number of detectable alleles (Allendorf, 2017). A large number of alleles indicates greater genetic diversity (Sheriff and Alemayehu, 2017).

The Ho, the fraction of Ho in a population, and He, and the proportion of heterozygous loci per individual are the parameters most frequently employed to evaluate genetic diversity in a population (Toro et al., 2009; Yaro et al., 2017). The current study observed that the Ho was less than the expected He, indicating that the Taro cattle population was genetically homogeneous. The Ho values ranged from 0.182 to 0.364 with an average value of 0.288, whereas expected He values ranged from 0.505 to 0.741 with an average value of 0.637. Small sample sizes, inbreeding, and a lack of migration of new genetic material can all contribute to low genetic diversity (Cañón et al., 2006; Peixoto et al., 2021). The *ETH 185* and *INRA 037* have greater heterozygosity compared to *INRA 035*. The difference between the observed Ho and the predicted He can be utilized as a measure of genotype imbalance in the Taro cattle population, indicating that an acceptable selection activity has been carried out and that random mating has not occurred (Tambasco et al., 2003; Labroo et al., 2021). According to Allendorf (2017), a population is considered to be in equilibrium if the genotype and allele frequency remain constant from generation to generation due to random mating in a large population. The Hardy-Weinberg equilibrium model is based on many key assumptions that must be met for a population to be in genetic equilibrium. These key assumptions include no mutation, no gene flow, a high population size, random mating, and no natural selection (Cayuela et al., 2018). Inbreeding is a common cause of non-random mating, as it increases homozygosity across all genes (Alvarez et al., 2011; Abramovs et al., 2020).

According to the heterozygosity value, the *ETH 185* microsatellite locus with a Ho value of 0.364 was the most informative in this study. In contrast, the *INRA 035* microsatellite locus with a Ho value of 0.182 was the least informative locus, following the statement of Sharma et al. (2009), which asserts that a Ho value below 0.5 implies a low gene variation in a population. The average Ho of Taro cattle in this study was 0.288, indicating a very low level of variation in Taro cattle. A deviation from the Hardy-Weinberg equilibrium is shown by a significant difference between the Ho mean (0.288) and expected He (0.637) for the microsatellite loci employed in this study and the low Ho value. Tree loci have been analyzed, and the deviation of HWE with significant p-values was shown, indicating that inbreeding has occurred. The linkage disequilibrium test from three loci that have been analyzed was not significant, stating that the loci are independent. The meaning-independent loci used in this study did not cause deviations in the HWE test (Slatkin, 2008). The inbreeding coefficient or the fixation index (F) values also supported the deviation HWE results with a fixation index (F) value close to 1, which indicates low heterozygosity. The homozygosity of Taro cattle from the *ETH 185*, *INRA 035*, and *INRA 037* loci was greater than anticipated. According to (Alvarez et al., 2011), the reported genetic effects of inbreeding in a small population of animals can decrease heterozygosity, or gene variety, and increase the frequency of recessive disorders. According to Kardos (2021), inbreeding has negative effects on a small population, including a decrease in diversity and their ability to evolve or adapt to the environment. The population decrease of animals can also be attributed to habitat fragmentation, which disturbs gene flow and increases genetic drift and inbreeding (Bora et al., 2023).

CONCLUSION

According to the obtained results of the study, the average number of alleles was 4.33, with allele sizes at loci *ETH 185* (230-250), *INRA 035* (110-128), and *INRA 037* (144-150). The *INRA 037* locus had the highest allele frequency at 150 bp, while the INRA35 locus had the lowest frequency at 114 bp. *ETH 185* was the most informative microsatellite locus, whereas *INRA 035* was the least informative. In the present study, the Taro cattle population had low heterozygosity, confirmed by observed Ho, which was less than He. The loci did not cause low genetic diversity in the study, but it was caused by inbreeding. Inbreeding has caused low genetic diversity, as confirmed by the fixation index (F) or the inbreeding coefficient value close to one. The significant p-value of the HWE test also indicated that inbreeding occurred in this Taro cattle population. Further research is recommended to develop a breeding program that can avoid inbreeding and prevent loss of genetic diversity.

DECLARATIONS

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Availability of data and materials

The presented data is available and can be reasonably requested by correspondence.

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Authors' contribution

Luh Gde Sri Surya Heryani conceptualized and designed the research. Ni Nyoman Werdi Susari and I Made Merdana conducted research, collected samples, and literacy. Ni Luh Astria Yusmalinda carried out laboratory examinations. Luh Gde Sri Surya Heryani and Ni Nyoman Werdi Susari wrote the paper. All authors contributed proportionally and confirmed this article.

Competing interests

All authors state no conflict of interest in publishing this study.

Ethical considerations

Ethical issues, such as data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct, have been checked by all the authors before publication in this journal.

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