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DNA variants and population structure of Magelang ducks across generation

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ABSTRACT

Magelang ducks (MD) are Indonesian local ducks and are known as dual-purpose type ducks which have high egg production, Duck Day Production (DDP), and body weight size. Nowadays, the MD is selected to establish the pure line to advance the egg production trait in the Breeding and Rearing Center of Non-Ruminant Animals Banyubiru, Central Java. The mitochondrial DNA (mtDNA) D-loop region, is highly polymorphic and could be used to analyze the population genetics. The present research aims to examine the impacts of continuous selection on population structure and genetic mutations on MD across two generations using mtDNA D-loop region. Thirty six blood samples from the second (G2) and third (G3) generations were examined using the sequencing method. The MEGA X and DnaSP software were applied to calculate the genetic diversity, genetic distance, and to generate a phylogenetic tree. The number of haplotypes (H), haplotype diversity (Hd), and Tajima's D are 26, 0.9746, and -1.46, respectively. The Fst value of MD is 0.156. The genetic distance among populations ranges from 0.0000 – 2.097. The UPGMA analysis constructs one clade in a phylogenetic tree between MD, Indonesian local Ducks, Indian Ducks, Vietnamese Ducks, and Chinese Ducks. The study found that the genetic variation and population structure did not significantly change between the second and third generations.

Keywords: D-loop, Ducks, Genetic diversity, MtDNA, Phylogenetic tree

INTRODUCTION

Local ducks are of paramount importance as a cheap source of protein (De *et al.*, 2021) and could be developed potentially as a strategic animal-based food source (Hidayati *et al.*, 2016). During 2018-2022, the duck population and duck meat production decreased 1,29% and 3.48% respectively. On the contrary, the duck's egg production has elevated 5.24% (Ministry of Agriculture, 2022). This situation indicated that a breeding selection program is needed to enhance the local duck's productive performance genetically. If the correlation between alleles and the production traits could be established, the gene variants might be useful for the advancement of

the production traits through genetic selection (Moazeni *et al.*, 2016).

The distribution of duck population is mostly found on the Java islands (48,49%), especially in Central Java Province, namely Tegal Ducks, Magelang Ducks, and Pengging Ducks (Wulandari *et al.*, 2015; Ministry of Agriculture, 2022). Magelang Ducks (MD) are Indonesian local ducks (Ministry of Agriculture, 2013) and are known as dual-purpose type ducks (Yuniarinda *et al.*, 2019). Previous research showed that MD has higher Duck Day Production (DDP), body weight, and high egg production than two other breeds (Purwantini, 2013[a]; Mahfudz *et al.*, 2005; Muryanto, 2015; Sulistyawan *et al.*, 2018), although those three breeds have similar body size (Wulandari *et al.*, 2015).

Mitochondrial DNA (mtDNA) has been used in diversity analysis and phylogenetic studies because of its rapid evolutionary rates (Sato *et al.*, 2004). Mitochondrial DNA diversity is a useful molecular tool in establishing phylogenetic relationships among breeds and at the species level (Zhao *et al.*, 2011). The D-loop region,which is a single noncoding segment in mtDNA, is highly polymorphic and, thus, is used in the analysis of population genetics (Song *et al.*, 2020, Peng *et al.*, 2021, Pan *et al.*, 2021, Rendón-Hernández *et al.*, 2021), further mitochondrial DNA (mtDNA) has emerged as a very powerful and reliable marker in animal population and evolutionary biology (Rissler, 2016; Ladoukakis and Zouros, 2017; Duong *et al.*, 2018). In the prior research, the mtDNA has been applied to identify the population structure, genetic and morphological diversity of MD (Purwantini *et al.*, 2013[a]; Purwantini *et al.*, 2013[b]).

The Magelang Ducks are selected to establish the pure line to advance the egg production trait in the Breeding and Rearing Center of Non-Ruminant Animals Banyubiru, Central Java (Kurnianto, 2017). Since the MD-selected lines are still under the selection program, it is important to observe the genetic of MD between generations using the mtDNA D-loop region of the ducks. The present research aims to examine the impacts of continuous selection on population structure and genetic mutations on MD across two generations using mtDNA D-loop region The current result could provide a potent fundamental to explore MD as a local prominent genetic resource.

MATERIALS AND METHODS

Ethical Clearance

The appropriate standard regulations and guidelines of animal treatment were referred to the Republic of Indonesia's law, number 41, 2014, and adhere to during the observation.

Sample Collection and DNA extraction

The ducks used were clustered as selected lines from the second (G2) and third (G3) generations. A total of 36 blood samples, both male and female unrelated ducks. The ducks are kept in the Breeding and Rearing Center of Non-Ruminant Animals Banyubiru, Central Java.
Three milliliters of whole blood was collected from the brachial vein of each duck using syringes and stored in EDTA vacutainer tubes. The genomic DNA was extracted from whole blood using the standard protocol (Sambrook *et al.*, 1989). The DNA concentration was quantified and evaluated using a spectrophotometer and agarose gel electrophoresis.

Amplification and Sequencing

The polymerase chain reaction (PCR) protocol following the sequences of the forward and reverse primers were 5'- GTTATTT-GGTTATGCATATCGTG-3' and 5'-CCATATACGCCAACCGTCTC-3', respectively (Sultana *et al.,* 2016). 50 µl volume containing 4 µl DNA extraction (20-30 ng/ µl), 1 µl for each primer (10 pmol/ µl), 19 µl ddH2O, and 25 µl of MyTaq Red Mix Bioline (1st BASE) on Thermal Cycler (Bio-Rad, USA). PCR cycling program contains pre-denaturation at 94°C (5 min), followed by 35 cycles of denaturation at 94°C (30 s), annealing at 48°C (45 s), extension at 72 °C (1,5 min), and post extension at 72 °C (5 min). The amplicon was verified using electrophoresis

Table 1. The Reference of mitochondrial DNA sequences in several duck breeds

Accession number	Species	Breed	Location
HM010684.1	Anas platyrhynchos	Shaoxing	China
GQ922102.1	Anas platyrhynchos	Unknown	Uttar Pradesh, India
GQ922081.1	Anas platyrhynchos	Unknown	Kerala, India
MW911844.1	Anas platyrhynchos	Vit Troi Co xanh	Vietnam
MW911842.1	Anas platyrhynchos	Minhhuong	Vietnam
MW911843.1	Anas platyrhynchos	Sincheng	Vietnam
KX756172.1	Anas platyrhynchos	Unknown	Indonesia

in 2 % agarose gel (Invitrogen, Life Technologies Co, CA) at 100V for 30 min. The isolated DNA samples were kept at −20°C. The PCR products were sequenced by direct sequencing method in both directions using commercial service (1st BASE). The DNA sequence length was approximately 707 bp for G2 and G3.

Data Analysis

The MEGA X software was applied to evaluate the multiple nucleotide sequence alignments to identify the singleton variable, genetic distance, and parsimony sites within and between duck generations, and to generate a phylogenetic tree. The present investigation used MD and several D-loop region sequences from other duck isolates from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/) as comparisons for MD's in the present research. All of the DNA sequences used are listed in Table 1. The phylogenetic tree was constructed using the UPGMA method with the 1000 × bootstrap and Kimura-2-parameter method. The genetic diversity, haplotype diversity, nucleotide diversity, number of nucleotide differences, Fst, number of haplotypes, and Tajima's D were computed with the DnaSP 5.10 software.

RESULTS AND DISCUSSION

Sequence Variation and Polymorphic Sites

Mitochondrial DNA was utilized in this investigation due to the sequence stability across species and the enormous number of copies in each cell. On the other hand, single-nucleotide alterations across different breeds within a species have been found in duck genome studies (Natonek-Wiśniewska *et al.*, 2021). In the current investigation, several samples were excluded from sequencing analysis due to the quality of the band pattern which was presumed to have a less reliable nucleotide sequence. The amplification fragment sizes were found to comply with the target area, and results showed high specificity and obvious band patterns (Figure 1). Moreover, the samples could be directly analyzed by sequencing method. Ou *et al.* (2015) stated that sharp and reproducible DNA extraction bands in the PCR method could be used for further analysis because a single clear gel band showed a highly folded monomeric structure (Schnitzbauer *et al.*, 2017).

There were 36 samples acquired from two generations of selected ducks, Generation 2 (G2) and Generation 3 (G3), 18 samples respectively. The 707 bp fragment of the mtDNA D-loop region of MD was analyzed and amplified by a pair of primers (Figure 1). The partial mtDNA D-loop region analyzed in this study corresponds to nucleotide positions 64 to 770 of the *Anas platyrhynchos* sequence (GenBank Accession Number HM010684.1).

The alignment of mtDNA D-loop nucleotide sequences of MD in Central Java enabled polymorphic site data to be obtained and is presented in Table 2. Several mutations in the nucleotide sequence of MD consist of deletion and substitution (transition and transversion). The polymorphisms were determined by the few numbers of base changes compared to the reference sequence. In the D-loop sequence, substitution, transitions, and transversion frequently occur (Putri *et al.*, 2019).

There are two categories of base mutations

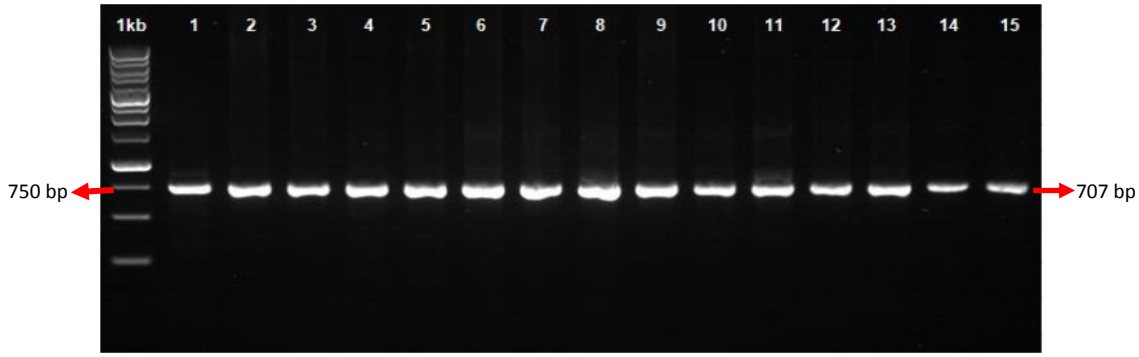

Figure 1. 707 bp Fragment of the mtDNA D -loop region of the Magelang Ducks. 1,2,..15: number of samples; 1kb: marker size.

called parsimony bases and singleton bases. A total number of 69 polymorphic nucleotide sites were discovered which consisted of 21 singleton variable sites (S), 48 parsimony informative sites (P), and one indel (insertion or deletion) as shown in Tabel 2. MD has higher polymorphic sites than other duck breeds (Table 4), demonstrating that MDs were highly different in genetics. The average of nucleotide compositions in MD samples from both generations consisted of 24.9 T base, 33.9% C base, 25.1% A base, and 16.0% G base, respectively. The ratio between the A-T base pair and the C-G base pair is similar. In the present study, Zhang *et al.* (2023) found that A and T bases reach 50.06% and C-G bases with 49.94% in Chinese local ducks. This condition indicated that the sequences have no base bias.

Genetic Diversity and Haplotypes of mtDNA D-loop region in Magelang Ducks

A basic and crucial tool for the selection and development of domestic animals is genetic diversity (Islam *et al.*, 2019). The genetic diversity was estimated by assessing the number of polymorphic sites (S), nucleotide diversity (π), haplotype diversity (Hd), the number of haplotypes (H), Tajima's D test statistic (D), and the average number of nucleotide differences (K) in MD populations. The major indicators for assessing the mtDNA polymorphism and genetic diversity of a breed or population were haplotype diversity (Hd) and nucleotide diversity (π) (Li *et al.*, 2010). Moreover, nucleotide diversity is more accurate in evaluating the genetic diversity in populations than haplotype diversity (Islam *et al.*, 2019). From 36 samples, there were 26 haplotypes (Hap). The genetic diversity of MD is shown in Table 3.

The research revealed high haplotype diversity (0.9746) and the Tajima's D for a whole population is -1.46. Tajima's D for neutrality test shows a non-significant negative value ($p>0.10$) for G2 and G3. This condition indicated that the duck population is not under evolutionary pressures and is more balanced (Tajima,1989). The negative Tajima's D demonstrated that the population confirmed the hypothesis that MD mtDNA mutations are selectively neutral in current collection sites (Nwafili and Gao, 2016), the possibility of demographic expansion and indicated that the nucleotide variants between generations under a neutral model of evolution (Chan *et al.*, 2016). Thus, the findings showed that the investigated population is under equilibrium (Phromnoi *et al.*, 2022). The indigenous poultry was raised in free-range backyards, which allowed random mating among the population without men's intervention even in various environments. Thus, the effects of selection pressures both natural and artificial are lessened on these populations which promotes the equilibrium (Teinlek *et al.*, 2018). The high genetic diversity in line with the negative Tajima's D value might influenced the equilibrium. This condition could be led by the introgression of a new breed in the population.

Based on the sequences analysis, nucleotide

Table 2. Identified nucleotide variants of mtDNA D-loop region in Magelang Ducks

Base Sequence	Polymorphic Site																																																																						Haplo type
	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	5	
	1	8	0	0	1	2	4	4	4	4	4	4	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7	7	8	8	8	8	9	9	0	0	0	0	0	1	1	1	1	1	1	2	2	2	3	3	3	4	4	4	4	5	6	7	9	9	0	1	3	3	6	9	4	
	3	2	0	1	9	8	2	3	4	5	6	7	0	2	3	4	5	6	8	9	1	2	4	5	6	8	9	0	1	3	4	1	6	8	9	7	9	0	1	2	4	6	0	1	3	5	7	9	2	7	9	0	5	9	1	5	7	8	0	6	7	4	5	9	7	0	4	7	9	0	
A. platyrinchos	A	C	T	A	C	A	T	A	C	C	C	A	A	C	T	C	A	A	A	C	A	C	T	A	A	A	T	G	C	C	C	G	A	G	C	T	A	T	G	A	T	C	A	C	G	C	T	C	T	A	A	C	A	C	T	C	C	C	A	G	G	T	A	A	T	T	C	C	C	C	1
G2 A	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
G2 B	-	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
G2 C	-	T	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	C	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
G2 D	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
G2 E	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	4
G2 F	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
G2 G	-	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
G2 H	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6
G2 I	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7
G2 J	-	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8
G2 K	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
G2 L	-	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9
G2 M	-	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10
G2 N	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	4
G2 O	-	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	A	.	.	.	G	.	.	.	.	11
G2 P	-	T	C	G	.	.	.	.	.	T	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	G	G	T	.	.	.	.	C	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12
G2 Q	-	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9
G2 R	-	T	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	C	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
G3 A	-	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	13
G3 B	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	14
G3 C	-	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	15
G3 D	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16
G3 E	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	T	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	17
G3 F	-	T	C	.	.	.	.	.	.	.	G	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	T	G	A	T	.	.	.	T	.	.	.	.	C	.	.	.	G	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	18
G3 G	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	19
G3 H	-	.	.	.	.	G	.	.	A	.	T	.	T	.	C	.	.	T	.	A	G	.	C	.	C	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	T	C	.	G	T	T	.	20
G3 I	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	T	.	C	.	.	.	.	.	.	.	.	.	.	.	21
G3 J	-	T	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	C	A	.	T	.	C	.	.	C	C	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	22
G3 K	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	A	.	T	.	C	.	.	C	C	.	.	.	.	C	.	.	G	C	.	.	.	.	.	.	T	.	T	C	.	C	.	.	.	.	T	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	23
G3 L	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
G3 N	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16
G3 M	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	14
G3 O	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	T	.	C	.	.	.	.	.	.	.	.	.	.	.	21
G3 P	-	T	C	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	24
G3 Q	-	.	.	.	.	G	A	G	A	.	T	.	T	T	C	.	.	T	.	T	G	.	C	.	C	C	C	A	A	T	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	T	C	.	G	T	T	T	25
G3 R	-	T	C	.	.	.	.	.	.	.	G	.	.	T	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	T	G	A	T	.	.	.	T	.	.	.	.	C	.	.	.	G	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	26

Vertical numbers denote the location of the variable site. The similar nucleotide base was represented by the dots (.) based on the reference sequence (GenBank accession number: HM010684.1), while substitution was indicated by different nucleotides.

Table 3. Genetic diversity of Magelang Ducks across generations

Group	N	S	P	π	Hd	K	Tajima's D	H	Fst
G2	18	25	13	0.00914	0.9412	5.6013	0.91023 (NS)	12	0,156
G3	18	55	44	0.02432	0.980	14.908	-0.7724 (NS)	15	
Total Population	36	69	48	0.01833	0.9746	11.236	-1.46 (NS)	26	

N: number of sequences; S: Polymorphic site; P: Parsimony site; π: Nucleotide diversity; K: the average number of nucleotide differences; π: nucleotide diversity; H: Number of Haplotype; Hd: haplotype diversity; NS: Not Significant

Table 4. The genetic diversity of various duck breeds

Breed/Location	S	H	Hd	π	Reference
Indian local ducks	7	25	0.71	0.92	Gaur *et al.* (2017)
Nigerian local ducks	70	7	0.381	0.315	Adebambo *et al.* (2017)
Iraqi local ducks	7	7	0.667	0.00388	Abdulkareem (2020)
Andaman Local Ducks	19	13	0.00897	0.881	De *et al.* (2021)

S: Polymorphic site; H: Number of Haplotype; Hd: haplotype diversity; π: nucleotide diversity.

Table 5. Genetic distance for Magelang Ducks and ducks from several countries

Duck breed	1	2	3	4	5	6	7	8	9
1									
2	0.027								
3	0.006	0.024							
4	0.008	0.025	0.002						
5	0.006	0.023	0.000	0.002					
6	1.111	1.123	1.110	1.114	1.092				
7	1.118	1.131	1.117	1.121	1.098	0.023			
8	1.115	1.127	1.114	1.118	1.095	0.018	0.002		
9	1.402	1.443	1.415	1.354	1.375	2.027	c	2.017	

1: Generation 2; 2: Generation 3; 3: Anas_KX756172.1; 4: Anas_GQ922102.1; 5:Anas_ GQ922081.1; 6: Minhuong Ducks; 7: Sincheng Ducks; 8: Vit Troi Co xanh Ducks; 9: Shaoxing Ducks

diversity can be described as the average number of nucleotide variations per site in pairwise comparisons across DNA sequences, whereas haplotype diversity is a possibility that two randomly selected alleles are dissimilar (Canales Vergara *et al.*, 2019). Further, the previous research revealed the genetic diversity of different duck breeds/countries as shown in Table 4.

The Fst value of MD is 0.156 and is categorized as moderate differentiation (Sultana *et al.*, 2017). Table 4 proved that MDs have a higher genetic diversity than other duck breeds in several countries. De *et al.* (2021) stated that the introduction of genetic material from different breeds is shown by the high genetic diversity. In accordance, Purwantini *et al.* (2013[b]) stated that local duck crossbreeding may have generated the high variability in the mtDNA D-loop region as seen in the Indonesian duck population. Further, high genetic diversity indicates that the population hasn't experienced either population expansion or selection, as well as human exploration activities in the particular area, thus the population still preserves its genetic uniqueness (Adebambo *et al.*, 2017). On the contrary, the π value is denoted as low (Table 3). This situation is frequently related to an expansion process that begins with a fast growth phase that is followed by a phase of low effective population size and a bottleneck (Grant and Bowen, 1998).

D Loop mtDNA was used in this analysis due to the large number of copies in every cell and the sequence stability within the species. However, genome research in ducks indicates that single-nucleotide substitutions occur between individual breeds within a species. This phenomenon has been observed in duck populations from Europe, Asia, and America, and it concerns diverse mtDNA fragments (Wiśniewska *et al.*, 2021).

Phylogenetic Study

A phylogenetic tree and its genetic distance describe the relationship both within and between species. The genetic distance among the duck population is presented in Table 5. Among the duck breeds, the genetic distance ranged from 0.0000 – 2.097. The result showed that the highest genetic distance between the Sincheng Ducks and Shaoxing Ducks was 2.097, while the lowest genetic distance between Indonesian local Ducks and Kerala Ducks was 0.000, as shown in Table 5. This study showed that the genetic distances among breeds were classified into low and high genetic distances.

Further, MD performed phylogenetic analysis alongside those from the reference sequences displayed in Table 1. The Magelang Ducks' position was determined using the reference sequences. A total of forty-three sequences were

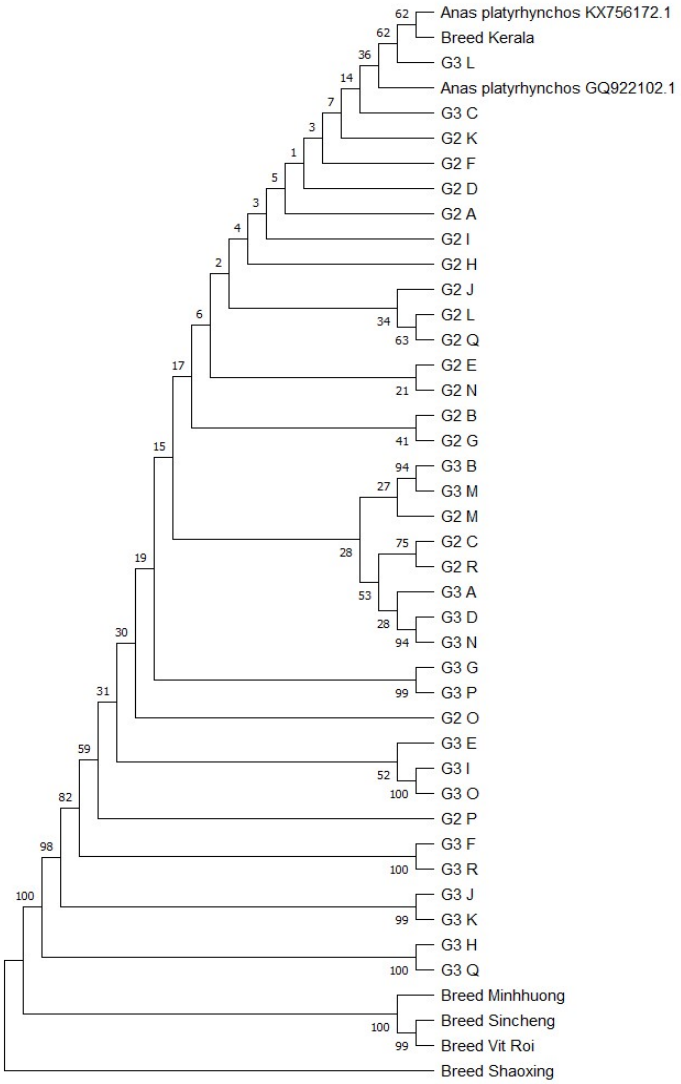

Figure 2. Phylogenetic tree of Magelang ducks

used to build a phylogenetic tree and determine the genetic distance within and between MD populations and those from reference sequences. This showed that every sequence in the current research belonged to a single clade (Figure 2).

The UPGMA tree showed a close resemblance between MD, a local Indonesian duck, and two local ducks in India. The Vit Troi Co xanh and Minhhuong joined with Sincheng Ducks as the ducks belong to Vietnam. Likewise, previous research found that Indonesian Ducks and Vietnamese Ducks have far genetic distances and different genetically (Okabayashi *et al.*, 1998). On the other hand, Shaoxing Ducks from China are the farthest ducks from others. The ducks in Vietnam were clustered in one branch, due to the same geographical area (Hariyono *et al.*, 2019).

According to the previous study, the Indonesian duck breed is in the same clade as the Chinese duck (Abdulkareem, 2020) and Indian duck (Islam *et al.*, 2019). The phylogenetic tree also indicated that MD was domesticated from Anas platyrhynchos. In accordance, Purwantini *et al.* (2013[b]) discovered that the maternal line of local duck populations in Indonesia originated from *Anas zonorhyncha* and *Anas platyrhynchos*. De *et al.* (2021) confirm that Indonesian ducks are grouped in the same cluster as Chinese Ducks. On the contrary, the Indian Ducks are in a distinct clade from Indonesian ducks. Okabayashi *et al.* (1998) stated that the ducks that were first domesticated from mallards (*Anas platyrhynchos*) in China had been brought to Indonesia by men. Purwantini *et al.* (2013b) confirmed that MD originated from *Anas platyrhynchos*. On the contrary, Indian ducks which had been migrated from Southeast Asia, locally evolved by natural selection pressure forced by environmental conditions (De *et al.*, 2021).

As a result, it's important to track how genetic diversity evolves. Rotational mating systems or increasing the number of drakes can be applied if necessary to maintain genetic heterozygosity. This breeding and mating method is now can serve as a model for other duck conservation and breeding programs.

CONCLUSION

The genetic diversity in MD is high. The study found that the genetic variation and structure did not significantly change between the second and third generations. Further, to preserve the germplasm, genetic monitoring should be carried out on frequent and longer time intervals. Future conservation methods, breeding, and selection programs could be applied with the use of mitochondrial D-loop sequences that reveal the genetic variety of local ducks.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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