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Dragon Chicken: Genetic diversity and interrelationship of rare indigenous Dong Tao chickens from Vietnam and Thailand, a unique resource for the management of a highly adaptable and productive local breed

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Abstract

Dong Tao (DT), also called Dragon Chicken, is a rare, unique and highly productive poultry breed introduced from Vietnam to Thailand ~30 years ago. They have a very peculiar appearance, including enormously enlarged feet with reddish scales and are considered both local and culturally significant in both countries. Their adaptability and distinct genetic traits have attracted global interest, underscoring their potential for breeding programs and a need for their thorough genetic makeup assessment. Using 28 microsatellite markers, therefore, the genetic diversity of three populations, DT-U and DT-L from Thailand and DT-HY from Vietnam, plus 54 other indigenous and local chicken and red junglefowl populations of Thailand, was analyzed. High genetic variability and low inbreeding levels were observed in these populations, indicating their effective management, despite historical bottlenecks. Genetic similarities between DT-U and DT-HY and indigenous breeds, as well as the closer alignment of DT-L with red junglefowl, highlighted existing introgression and adaptation processes. Two markers, *MCW0098* and *MCW0216*, showed a variation pattern due to potential impact of directional selection, possibly driven by environmental adaptation pressures. These findings emphasize the importance of DT chickens as genetic resources for breeding programs that focus on climate resilience and productivity enhancement. Our research paves the way for ongoing conservation efforts to preserve genetic diversity and conduct a further anticipated in-depth genomic exploration of this breed, ensuring the continued contribution of DT chickens to sustainable poultry production.

KEYWORDS: Dong Tao; indigenous chickens; local populations; genetic diversity; genetic differentiation; microsatellite genotyping

1. INTRODUCTION

Chickens (*Gallus gallus*) were domesticated from the red junglefowl approximately 8,000 years ago in Southeast Asia, particularly Thailand (West & Zhou,1988; Moiseyeva et al., 1996, 1997, 1999; Lawal et al., 2020; Abdulwahid & Zhao, 2022). Their ability to survive in basic housing with low nutrient intake, plus their resistance to numerous pathogens were among the many reasons why they have been domesticated globally as numerous breeds. In contemporary culture, chickens provide proteins for human nutrition in terms of meat and eggs, serve as companions, and fulfil sociocultural roles as ornamental, long-crowing, and game-fighting birds; they are also a model species for a range of academic disciplines including developmental biology and immunology (Ekarius, 2007; Choprakarn & Wongpichet, 2008; Alders & Pym, 2009; Komiyama et al., 2016; Bettridge et al., 2018; Desta, 2021).

Globally, a decline in genetic resources in farm animals has been observed, as low-productivity indigenous and local breeds have largely been replaced by highly productive commercial breeds over the last century. The rapid development of intensive livestock production systems has caused a decline in pure indigenous and local breeds, as well as the depletion of animal genetic resources (Besbes, 2009; Mel'nyk et al., 2009). The characterization and conservation of indigenous and local poultry breeds are thus required to preserve and monitor their genetic diversity and maintain adaptability to future needs (e.g., Romanov et al., 1995; Bondarenko & Podstreshny, 1996; Nikiforov et al., 1998; Ryabokon et al., 2005; Moiseyeva et al., 2007). Resilient commercial lines are likely to be developed through facilitated crossbreeding under systematic conditions, making preservation essential to maintain biodiversity and safeguard genetic resources. To date, DNA markers are broadly used for genetic diversity assessment as a *sine qua non* to elaborate strategies for preservation, breeding and exploitation of local gene pools (e.g., Romanov & Weigend, 1999; Semenova et al., 2002; Cong, 2016; Dementeva et al., 2017; Abdelmanova et al., 2021; Larkina et al., 2021b).

In Vietnam, indigenous, indigenous village, and local groups constitute more than 70% of the national poultry population (Phuong et al., 2015). Vietnam hosts 21 distinct indigenous chicken breeds, including Ri, Tau Vang, Mia, Dong Tao (DT; Vietnamese: gà Đông Tảo), and Ho, and each are specific to certain regions (Cong, 2016). DT, also called Dragon Chicken, is a rare and important indigenous breed that is characterized by large feet with reddish "dragon-like" scales, varying feather colors, and a comparatively high body weight (Ton et al., 2018; Figure 1). It takes eight months to one year for DT chickens to reach a slaughter weight of three–five kilograms, with males sometimes reaching six kg (Patowary, 2015). Consumers favor the DT chicken over other indigenous Vietnamese breeds because of its massive body weight, thick tarsus, and good meat quality (Phuong et al., 2015; Nguyen et al., 2015; Duy et al., 2016). The purebred DT population is highly valued by farmers because of its unique traits and premium market value (Duy, 2022). Intra-breed selection is prioritized to enhance the economic efficiency of farming systems and preserve this prized breed (Duy, 2022). Its unique characteristics have captured the interest of many countries, leading to its popularity in several regions of Asia, including Thailand (Johnson, 2024). DT chickens were imported from Vietnam by Thai farmers over 30 years ago and maintained there ever since. This led to their integration as local chickens in Thailand and, indeed, it has been suggested that many local Thai breeds may be derived from indigenous Vietnamese chickens that have adapted to the prevailing environmental conditions.

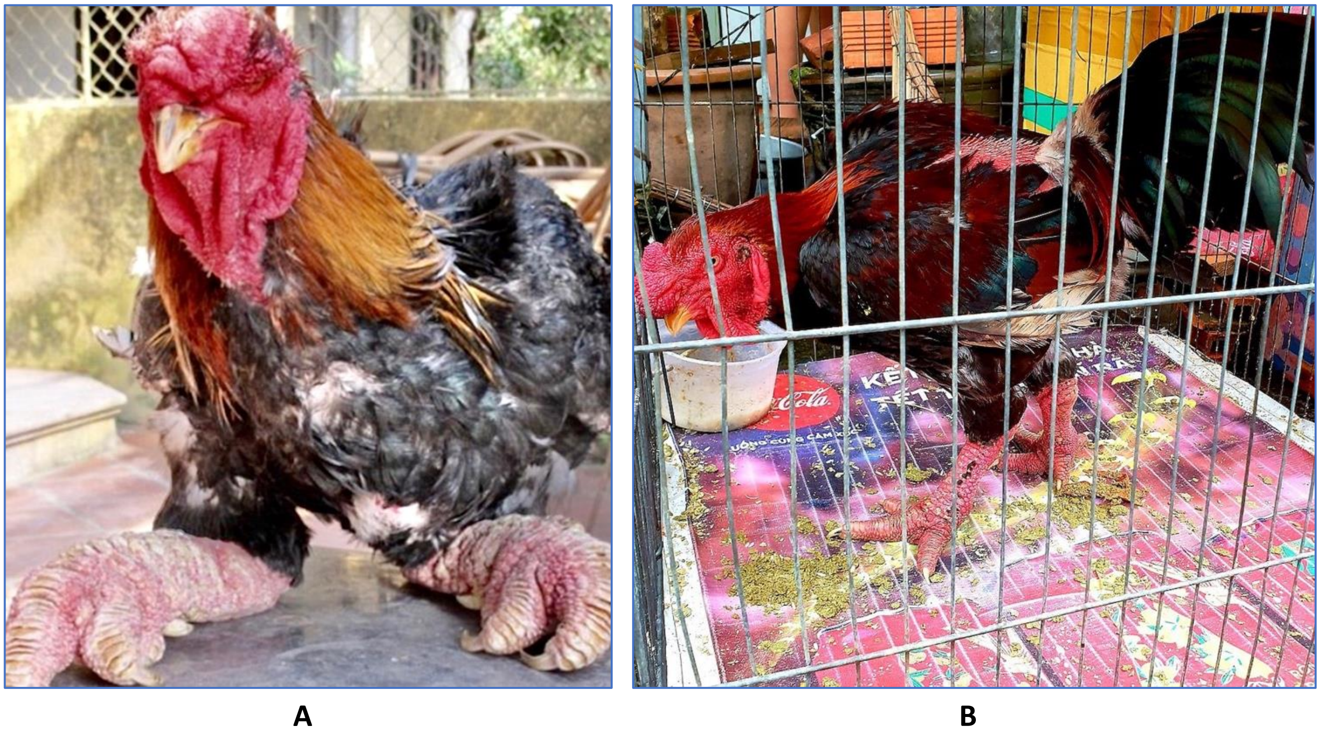


FIGURE 1. Roosters of the Dong Tao breed also known in Vietnam as Dragon Chicken: (**A**) in the backyard; (**B**) in a cage. Credit: (**A**) https://commons.wikimedia.org/wiki/File:Pouletdong.jpg (by Computer0001, CC-BY-SA-4.0); (**B**) https://commons.wikimedia.org/wiki/File:G%C3%A0_%C4%90%C3%B4ng_T%E1%BA%A3o_%E1%BB%9F_B%C3%ACnh_Long,_ng7th8n2022_(1).jpg (by Phương Huy, CC-BY-SA-4.0).

Although the exact timing and circumstances of such introductions are not always known accurately, the genetic introgression of indigenous and local populations in Thailand through crossbreeding is likely. This situation raises questions about the genetic relationships of DT chickens in Thailand and Vietnam and the purity of each. Determining this is the main purpose of the present study by testing two hypotheses: (i) significant genetic variation exists between DT chicken populations in Thailand and Vietnam; and (ii) the gene pools of Thai DT chickens may partially overlap with those of indigenous and local breeds and red junglefowl in Thailand, rather than with Vietnamese DT chickens. This research was thus conducted using 28 microsatellite markers and the DT genotyping results were compared with data from the extended gene pool library developed for the Siam Chicken Bioresource Project (SCBP; https://www.sci.ku.ac.th/scbp/) and 54 indigenous and local chicken and red junglefowl populations of Thailand. Valuable insights were provided for DT chicken populations, emphasizing the importance of maintaining their genetic diversity, and guiding regional breeding programs, potentially making them more effective.

2. MATERIALS AND METHODS

2.1. Sample collection and DNA extraction

The Vietnamese DT chicken specimens were collected from the Khoái Châu District (near Hanoi), Hưng Yên Province, Vietnam (DT-HY, *N* = 12; 20°49'28.6"N 105°58'18.1" E; Figure 2, site 1). The chickens were characterized by substantial size and sturdy legs adorned with reddish scales (Phuong et al., 2015) and are believed to represent a purebred population (Cao Van Thanh, personal communication). Permission was granted by the farm owner and all chickens were immediately released into the same area after sample collection. Further information regarding the samples used in this study is provided in Table S1. Blood samples were collected from the brachial wing veins of DT chickens. Genomic DNA was subsequently extracted and its quality and quantity were assessed following the methods outlined by Budi et al. (2023). The Thai samples used in this research were represented by those of the DT Lopburi (DT-L, *N* = 5) and DT Udon Thani (DT-U, *N* = 20) populations (Figure 2, sites 2 and 3, respectively) retrieved from the SCBP DNA bank (https://www.sci.ku.ac.th/scbp/dna-bank-with-legal-compliance/).

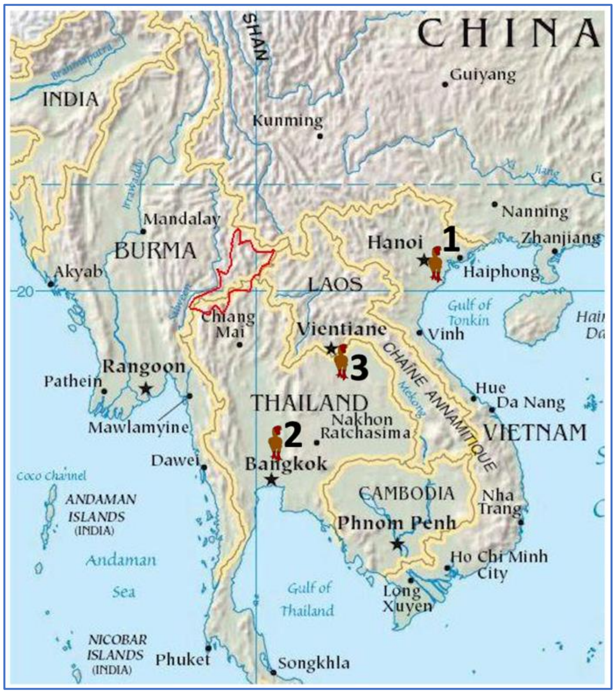


FIGURE 2. Sampling sites of the Dong Tao (DT) breed in Vietnam and Thailand. The sites are marked with a DT rooster silhouette: (**1**) Khoái Châu/Hưng Yên, Vietnam; (**2**) Lopburi, Thailand; (**3**) Udon Thani, Thailand.

2.2. Microsatellite genotyping and data analyses

2.2.1. Genotyping data

Twenty-eight microsatellite loci were employed to generate genotyping data (see Table S2 for the used microsatellite primer pairs as recommended by the Food and Agriculture Organization for chicken biodiversity research; FAO, 2011). A fluorescent dye (6-carboxyfluorescein, 6-FAM, or hexachloro–fluorescein, HEX, Macrogen Inc., Seoul, Korea) was utilized to label the 5'-end of the forward primer of each primer set. Polymerase chain reaction (PCR) amplification was performed as previously described within the framework of the SCBP (Wattanadilokcahtkun et al., 2023; Budi et al., 2023) using samples of the DT-HY, DT-L and DT-U populations. The DT genotyping data were additionally analyzed in comparison with those for 54 other indigenous and local chicken and red junglefowl populations of Thailand available from the SCBP and the Dryad dataset (https://datadryad.org/stash/share/x2qlPmboMgCROXO8).

2.2.2. Genetic diversity examination

Genetic diversity parameters, including allelic richness (*AR*), number of alleles per population, polymorphic information content (*PIC*), Shannon's information index (*I*), fixation index, heterozygosity (H_o and H_e), inbreeding coefficients (F_{IS}), pairwise genetic distances among populations (F_{ST} and R_{ST} values), and relatedness (*r*), were assessed as previously described by (Budi et al., 2023). Hardy-Weinberg equilibrium and linkage disequilibrium (LD) were evaluated using Arlequin software version 3.5.2.2 (Excoffier & Lischer, 2010). Arlequin version 3.5.2.2 was applied to analyze molecular variance (AMOVA) to identify group structures (Excoffier & Lischer, 2010).

2.2.3. Genetic relationship analysis

To investigate the relationships among chickens within breeds/populations, principal coordinates analysis (PCoA) was performed using GenAlEx version 6.5 (Peakall & Smouse, 2006). The ADEGENET package (Jombart, 2008) in R version 4.3.2 (R Core Team, 2023) was used to perform the discriminant analysis of principal components (DAPC). GENETIX version 4.05 was applied to perform factorial correspondence analysis (FCA) based on allelic frequency data (Belkhir et al., 2003; Tantia et al., 2006). Multidimensional scaling (MDS) analysis was conducted in Python (version 3.7.1) to analyze individual differences across samples using the MDS function from the sklearn.manifold submodule.

2.2.4. Population structure and origin determination

The population structure was identified using a model-based clustering approach implemented in STRUCTURE version 2.3.4 (Pritchard et al., 2000). The run duration was set to 100,000 Markov chain Monte Carlo repetitions, following a burn-in time of 100,000 generations, utilizing the correlated allelic frequencies under a straight admixture model. The number of clusters (*K*) ranged from 1 to 25 with 15 repetitions for each *K* value. The most probable number of clusters was identified by plotting the log-likelihood of information ($\ln \Pr(X|K)$) (Pritchard et al., 2000) over a range of *K* values before selecting the *K* value at which $\ln \Pr(X|K)$ stabilized. Structure Harvester (Earl & VonHoldt, 2012) was also used to implement the ΔK strategy. The possible origin of the DT chicken in Thailand was determined using Approximate Bayesian computation (ABC) analysis implemented in DIYABC version 2.1.0 (Cornuet et al., 2014).

2.2.5. Genetic bottleneck and selective sweep analyses

The allelic range for each locus was determined using Arlequin version 3.5.2.2 (Excoffier & Lischer, 2010) to calculate the relative long-term genetic bottleneck events based on the *M* ratio. A recent bottleneck event was investigated using both the Single Mutation Model (SMM) and Two-Phase Mutation (TPM) models, following the methodology outlined in Wattanadilokchatkun et al. (2024). Selective sweep analysis was performed by plotting the H_e and F_{IS} values for each of the 28 microsatellite loci in the chicken groups, following a previously described method (Budi et al., 2023). High F_{IS} and low H_e values indicate sweeping or purifying/negative selection, whereas low F_{IS} and high H_e values suggest neutral or balanced selection (Reddy et al., 2015). The neutrality of the microsatellite loci was evaluated using the Bayesian regression method in BAYESCAN (Foll & Gaggiotti, 2008), which calculates the Bayes factor to determine the probability of locus selection. Based on these data, this factor represents the ratio of the posterior probabilities of the selection and neutral models.

2.3. A broader investigation of the DT breed genetic origin

The genetic background of the DT breed was evaluated by analyzing microsatellite genotyping data from various 54 chicken populations, including red junglefowl, indigenous, and local Thai chicken breeds available in the SCBP DNA bank. Indigenous and local chicken populations were evaluated as distinct groups. Pairwise genetic distances between populations, clustering analyses based on PCoA, DAPC, FCA, and MDS, and the model-based clustering approach developed in STRUCTURE version 2.3.4, were used according to the steps specified by Budi et al. (2023).

3. RESULTS

3.1. Genetic variability based on combined genotype data of the DT breed derived from Thailand and Vietnam

Three populations of DT-L, DT-U, and DT-HY generated 266 alleles, averaging 4.679 ± 0.259 alleles per locus (Table 1 and Table S3). All populations of DT chickens tended to follow the Hardy-Weinberg equilibrium, with multiple evidence of linkage disequilibrium (Tables S4–S6). No null alleles were detected across the examined loci. The H_o was 0.638 ± 0.032, whereas H_e was 0.594 ± 0.020 (Tables 1 and S3). Welch's *t*-tests revealed no significant differences between H_o and H_e in the DT-L, DT-U, and DT-HY groups (Table S7). The mean *AR* values for the three DT groups were 4.488 ± 2.123. The *PIC* of all DT chicken groups ranged from 0.472 to 0.594, whereas the Shannon's Information Index (*I*) was from 0.950 to 1.289 with an average of 1.155 ± 0.052. The standard genetic diversity indices are presented in Table 1.

Table 1. Genetic diversity between three DongTao chicken populations of Thailand and Vietnam based on 28 microsatellite loci.

Population		**N_a**[1]	***AR***[2]	**N_{ea}**[3]	***I***[4]	**H_o**[5]	**H_e**[6]	***M* ratio**[7]	***PIC***[8]	***F***[9]
Lopburi	Mean	3.393	3.393	2.513	0.950	0.543	0.529	0.414	0.472	-0.017
	SD[10]	0.259	1.345	0.229	0.077	0.049	0.033	0.280	0.173	0.069
Udon Thani	Mean	5.286	5.131	3.208	1.227	0.701	0.616	0.375	0.568	-0.119
	SD	0.504	2.435	0.263	0.096	0.056	0.037	0.200	0.199	0.053
Hưng Yên	Mean	5.357	4.939	3.352	1.289	0.672	0.636	0.442	0.594	-0.059
	SD	0.452	1.993	0.288	0.088	0.056	0.033	0.254	0.173	0.065
Total	Mean	4.679	4.488	3.024	1.155	0.638	0.594	0.400	0.545	-0.065
	SD	0.259	2.123	0.154	0.052	0.032	0.020	0.147	0.190	0.036

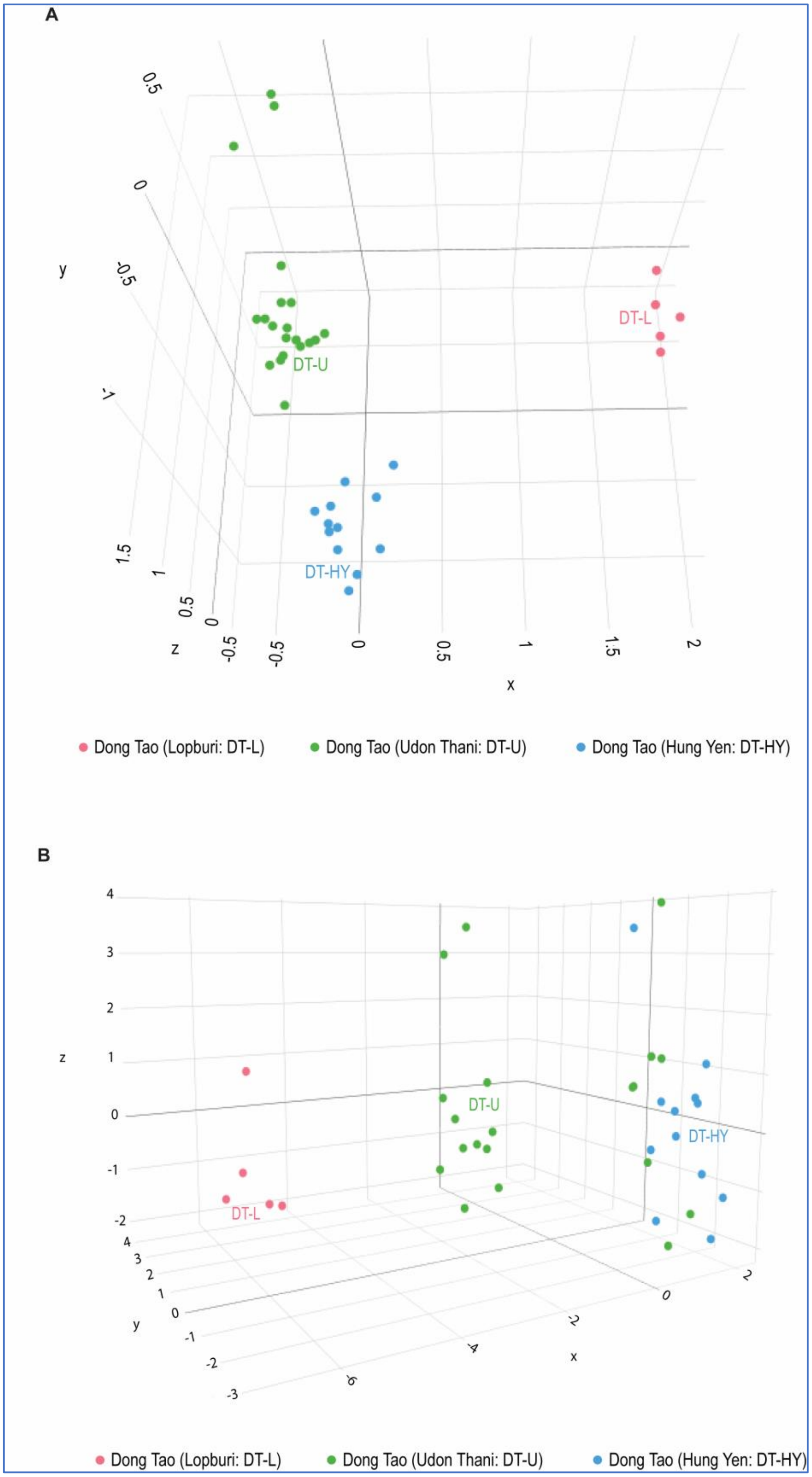
[1]N_a, number of alleles; [2]*AR*, allelic richness; [3]N_{ea}, number of effective alleles; [4]*I*, Shannon's information index; [5]H_o, observed heterozygosity; [6]H_e, expected heterozygosity; [7]*M* ratio; [8]*PIC*, polymorphic information content; [9]*F*, fixation index; [10]SD, standard deviation.

The average pairwise *r* values across all populations ranged from -0.116 to -0.025, whereas the F_{IS} value ranged from -0.079 to 0.008 (Table S8). F_{ST} showed significant differences ($p < 0.05$) between the population pairs, with values ranging from 0.102 to 0.268 (Table 2). R_{ST} values ranged from 0.130 to 0.393 (Table S9). AMOVA indicated that genetic variation accounted for 20% of the population and 1% of the chickens within the population (Table S10). The Nei's genetic distance between the DT populations varied from 0.430 to 1.402 (Table 2).

Table 2. Pairwise genetic differentiation between three Dong Tao (DT) chicken populations from Lopuri (DT-L), Udon Thani (DT-U), and Hưng Yên (DT-HY) based on 28 microsatellite loci and expressed via F_{ST} (below the diagonal and marked with *) and Nei's genetic distance (above the diagonal) values.

Population	DT-L	DT-U	DT-HY
DT-L	0.000	1.402	1.394
DT-U	0.268*	0.000	0.430
DT-HY	0.251*	0.102*	0.000

Population clustering analyses using PCoA, FCA, and MDS identified three distinct groups among chicken populations (Figures 3A, S1, and S2). However, the DAPC revealed that some individuals from the DT-U population clustered with those from the DT-HY population (Figure 3B).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● Dong Tao (Udon Thani: DT-U)
● Dong Tao (Hung Yen: DT-HY)

FIGURE 3. Principal coordinates analysis (PCoA) (**A**) and discriminant analysis of principal components (DAPC) (**B**) for Dong Tao chicken populations of Vietnam (DT-HY) and Thailand (DT-L and DT-U). Scatter plots illustrate genetic clusters based on different colors. Dots and colors represent different individuals and populations/breeds, respectively.

Various population structure patterns were observed using STRUCTURE with *K*-values ranging from 1 to 25 (Figure S3). Optimized population structure patterns were observed at *K* = 3 based on Evanno's ΔK and *K* = 4 based on the mean ln P(*K*) values in the DT-L, DT-U, and DT-HY chicken populations. Based on *K* = 3, the three chicken populations showed independent pooled patterns. At *K* = 4, all three DT chicken populations exhibited distinct gene pool patterns. Similarly, at a higher *K*-value (*K* = 23), all three populations showed separate gene pool patterns, with evidence of an admixture in the DT-U and DT-HY populations (Figure 4).

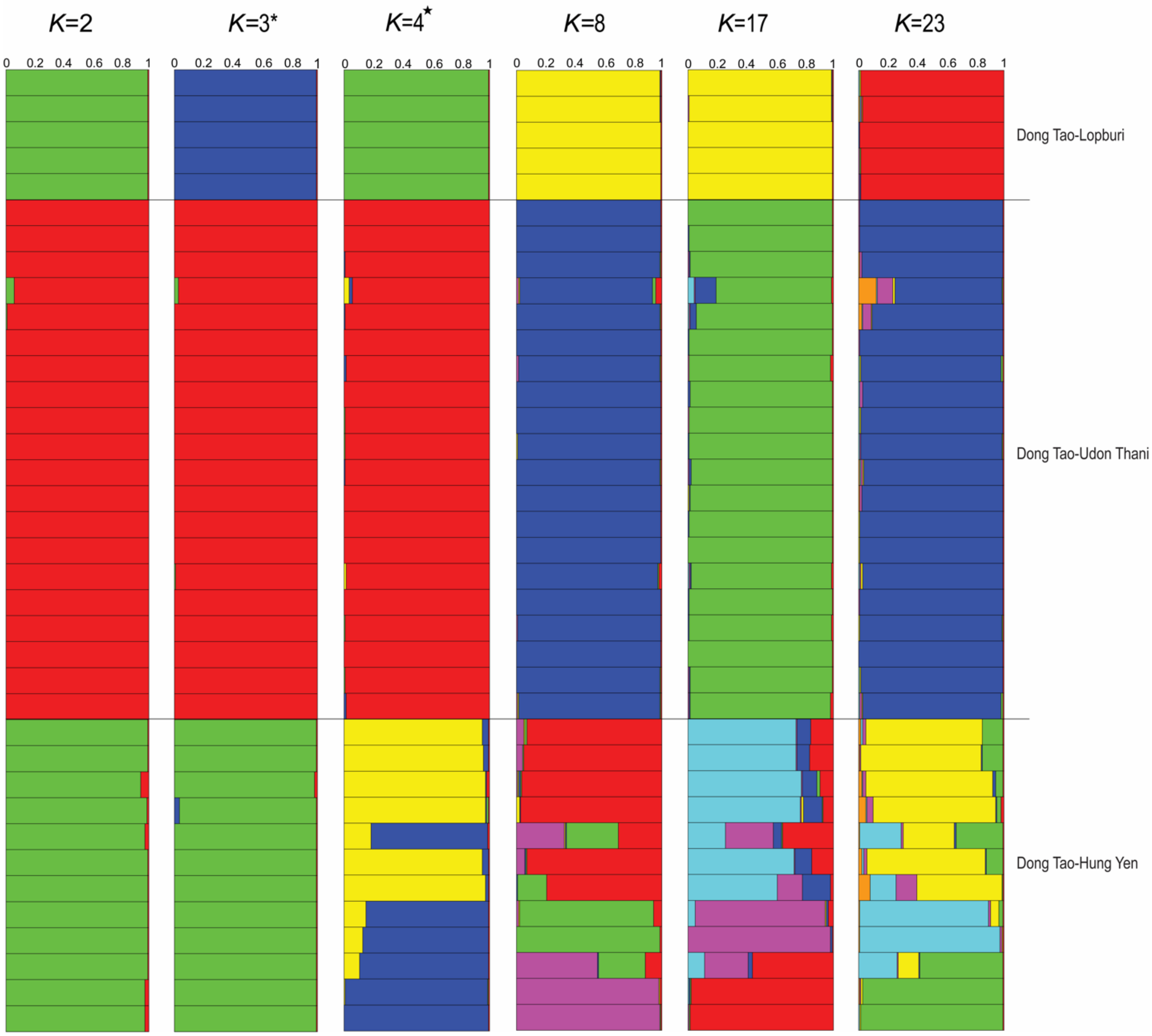

FIGURE 4. Population structure of the DT chicken breed of Thailand and Vietnam. The proportion of membership (posterior probability) per genetic cluster is represented by each vertical bar on the x-axis, while the y-axis shows individuals within each population. Black horizontal lines indicate the boundaries. *The optimal number of clusters estimated with the Δ*K* method of Evanno was in *K* = 3 and with the mean ln P(*K*) was K = 4.

ABC analysis demonstrated that the DT-HY and DT-L populations originated from the DT-U population in Scenario 2 (Pp = 0.920). In contrast, the results from scenarios 1 (Pp = 0.067) and 3 (Pp = 0.007) suggested that the DT-L population was an admixture of the DT-U and DT-HY populations (Figure 5). The genetic selective sweep plot revealed higher H_e values than F_{IS} in the 28 microsatellite loci and three DT chicken groups, indicating neutral or balanced selection (Figure S4). Using the Bayescan approach, loci *MCW0098* and *MCW0216* were identified as having an infinite probability of being under directional selection (Figure S5). Two alleles of the locus *MCW0098* (MCW0098-257 and MCW0098-259) were specifically identified in the DT-HY population, whereas one allele (MCW0098-253) was specific to the Thai population (DT-U and DT-L). Six alleles (MCW0216-142, MCW0216-155, MCW0216-157, MCW0216-161, MCW0216-162 and MCW0216-163) at locus *MCW0216* were unique to the DT-HY population.

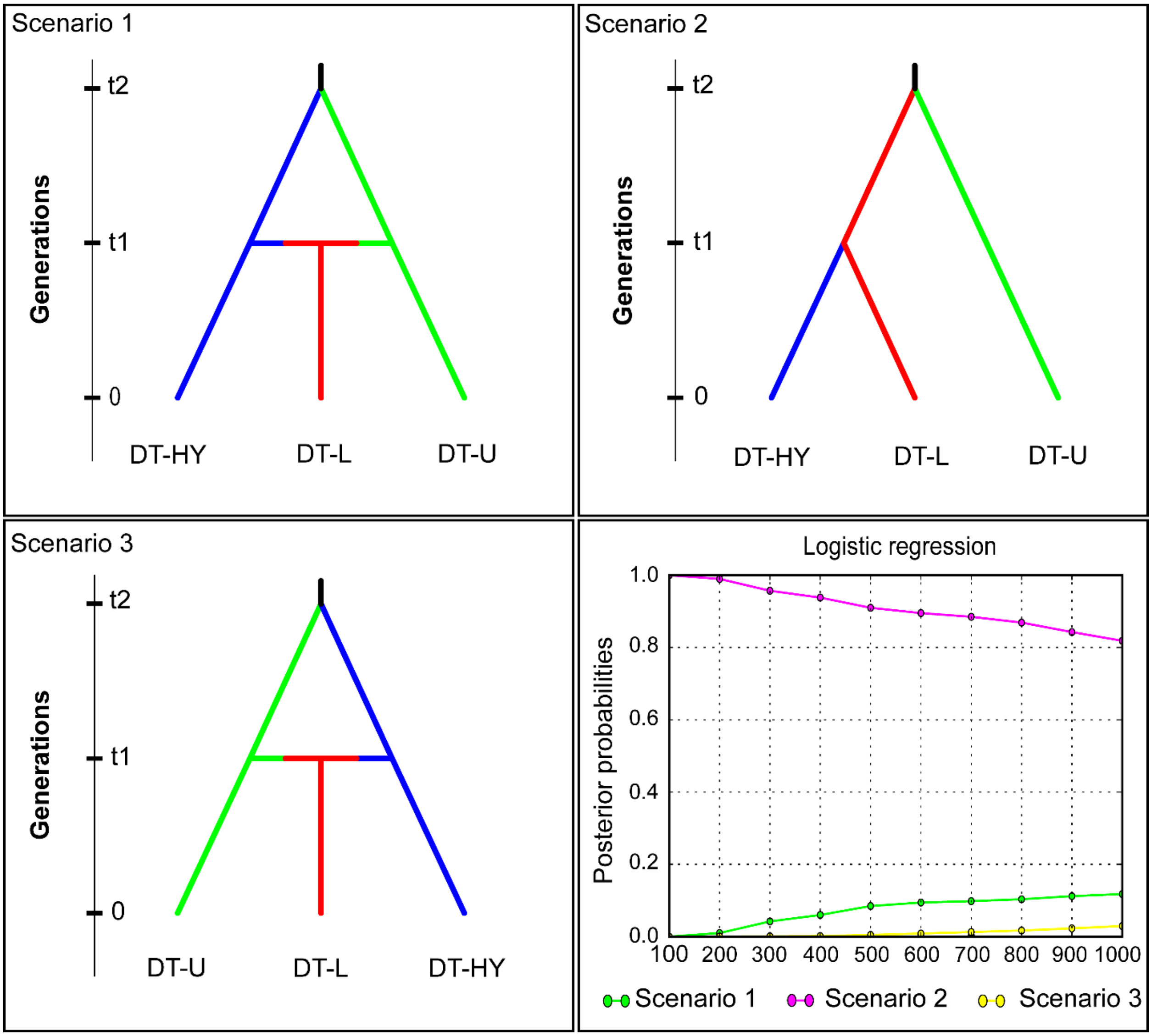

Figure 5. Evaluation of population history models for the Dong Tao (DT) chicken populations utilizing approximate Bayesian computation (ABC) inference.

The *M* ratio values were lower than the 0.68 threshold established by Garza and Williamson (2001), suggesting a rapid population decline in these groups over time (Tables 1 and S3). Wilcoxon signed-rank tests using both single-mutation model (SMM) and Two-Phase Mutation (TPM) models were conducted to assess recent population bottlenecks. The values ranged from 0.778 to 0.922 for SMM and from 0.760 to 0.801 for TPM across all populations. Mode-shift tests revealed a 'normal L-shaped distribution' for the DT-U and DT-HY population, indicating stability without recent bottlenecks. By contrast, the DT-L population exhibited a 'shifted mode,' suggesting recent bottlenecks (Table S11).

3.2. Genetic differences among DT populations, red junglefowl, and other indigenous and local chicken breeds in Thailand

The results of the PCoA, DAPC, FCA, and MDS analyses showed that DT-U and DT-HY chickens were grouped into clusters similar to those of other indigenous and local chicken breeds, whereas DT-L was clustered with red junglefowl (Figure S6–S9), as confirmed by the STRUCTURE analysis. Genotyping data of the DT breed were compared with baseline reference data from our previous studies, including indigenous and local Thai chicken breeds and red junglefowl. Population structure patterns were observed using STRUCTURE, with K-values ranging from 1 to 25 (Figure S10).

The STRUCTURE analysis demonstrated the highest posterior probability based on Evanno’s ΔK at $K = 2$, whereas a single peak was observed at $K = 20$ based on the mean ln P(K). At $K = 2$, DT-U and DT-HY chickens shared similar gene pool patterns with most Indigenous and local Thai chicken breeds such as Leung Hang Khao (Phitsanulok farm, Phitsanulok Panyanukun school, Nakhon Pathom, Nonthaburi), Chee (Nonthaburi), Pradu Hang Dam (Phitsanulok 2, Chiang Mai, Nakhon Pathom, Nonthaburi), Mae Hong Son, Chee Fah, Fah Luang, Wein Chang, Prama, Trad, Lao Pa Koi, Samae Dam, Rose, Shiang Hai, and Phuphan, and red junglefowl from Khon Kaen (*Gallus gallus gallus*). DT-L had similar gene pool patterns to Leung Hang Khao (Phitsanulok), Chee (Nakhon Pathom), Pradu Hang Dam (Phitsanulok 1), Kheaw Paree, Betong, Decoy, mixing-fighting-cock, Nin Kaset, and most red junglefowl from Thailand, except for *G. gallus spadiceus* from Khon Kaen. With a higher K value ($K = 20$), DT-L shared gene pool patterns with Betong, and Nin Kaset chickens. DT-U shares gene pool patterns with Pradu Hang Dam (Nakhon Pathom), Chee Fah (Chiang Rai), Fah Luang (Chiang Rai), Wein Chang, and Lao Pa Koi. DT-HY shares a gene pool pattern with Leung Hang Khao (Phitsanulok Panyanukun school), and red junglefowl from Huai Yang Pan (*G. gallus spadiceus*).

Additionally, as revealed by the higher *K* value (*K* = 25), DT-L shared a gene pool pattern with Betong and showed an admixture gene pool from mixing-fighting-cock and Nin Kaset, and red junglefowl from Songkhla (*G. gallus spadiceus*). DT-U chickens shared a gene pool similar to that of Leung Hang Khao (Phitsanulok), Chee (Nakhon Pathom), Pradu Hang Dam (Phitsanulok 1), Kheaw Paree, Decoy, Wein Chang, Prama (Lamphun) and Lao Pa Koi chickens. DT-HY shared a gene pool pattern similar to that of Leung Hang Khao (Phitsanulok Panyanukun School), and a partial gene pool with DT chickens from Udon Thani.

4. DISCUSSION

Domestic chickens are generally classified into commercial, indigenous, indigenous village, and local groups, each playing a pivotal role in the food supply chain and adapting to local conditions (Martin et al., 2015; DAD-IS, 2021; Wattanadilokcahtkun et al., 2023; Wongloet et al., 2023). Commercial chickens (e.g., White Leghorns, brown egg layers, and commercial broilers) suffer high mortality from heat stress and temperature fluctuations, whereas indigenous, indigenous village and local breeds maintain relatively stable production under high temperatures and humidity. The adaptability of these breeds suggests that specific genetic traits are selected by natural and artificial means. Their genetic makeup, which contains genes suited for tropical regions, enables their survival in harsh environments. This genetic potential could be utilized in future breeding programs for genetic improvement (Kutnyuk et al., 2001; Rischkowsky & Pilling, 2007; Besbes, 2009). Despite their abundance, indigenous and local chicken breeds have relatively low productivity compared with commercial chickens. Many farmers have turned to commercial breeds to boost productivity, often engaging in crossbreeding with both indigenous and local breeds (Tadelle et al., 2003; Nguyen et al., 2015; ABD, 2018). This new breeding strategy has significantly reduced the population size of indigenous breeds, leading to genetic admixture, which diminishes their adaptability in several indigenous and local chicken populations.

Once raised specifically to be offered to the royal family and mandarins, rare DT chickens are prized as a delicacy in Vietnam (Patowary, 2015). Indigenous to the Red River Delta in Northern Vietnam, they are valued for their exquisite meat and distinctively large body weight with unusually enlarged legs. This breed is endemic to Dong Tao Commune, Khoái Châu District, Hưng Yên Province, Vietnam. Rearing in Thailand for over six generations has allowed DT birds to adapt to local conditions, raising interesting questions about its diversity, origin, and relationship to the original Vietnamese breeds. As shown in this study, although a historical bottleneck was indicated in the three DT chicken populations, likely due to a small founder population, high variability was retained even in the two populations in Thailand, as observed from the high H_o and H_e values and the negative F value, similar to other local chickens (Zanetti et al., 2009; Restoux et al., 2022; Budi et al., 2023). The high genetic diversity and low inbreeding levels observed in DT chicken populations suggest that efficient management practices have been implemented.

Significant genetic differentiation among the three DT chicken populations was indicated based on F_{ST}, which was consistent with the AMOVA and clustering analyses. Population structure and ABC analyses suggested that the gene pool patterns of the DT-HY and DT-L populations were more similar than those of the DT-U population. F_{ST} and R_{ST} values were calculated to assess genetic differentiation. Observations of higher F_{ST} than R_{ST} values in most population pairs suggest that gene flow contributes to the high genetic differentiation observed (Calafell et al., 1998). This finding aligns with those of the examined populations, in which the chances of genetic exchange were very limited. Alternatively, this might be explained by the separation of the DT-L and DT-U populations from the DT-HY population, leading them to domesticate or adapt to distinct environmental conditions, such as those in Thailand (Meek et al., 2023).

MCW0098 and *MCW0216* were identified by BAYESCAN analysis as loci under directional selection in DT chickens and were located on chromosomes 4 and 13, possibly corresponding to regions with genes related to environmental adaptation (Park et al., 2006; Nassar et al., 2012). Studies have found that genes responsible for environmental adaptation in chickens, such as those for PPARG coactivator 1 alpha (*PPARGC1A*), 5-hydroxytryptamine receptor 2C (*HTR2C*), N-deacetylase and N-sulfotransferase 4 (*NDST4*), and non-SMC condensin I complex subunit G (*NCAPG*), are located on chromosome 4, whereas the golgi associated, gamma adaptin ear containing, ARF binding protein 3 (*GGA3*) gene is located on chromosome 13 (Gheyas et al., 2021; Shi et al., 2023). The *PPARGC1A* gene is recognized for regulating adaptive thermogenesis and energy metabolism, thereby influencing glucose uptake, gluconeogenesis, and mitochondrial biogenesis. The *HTR2C* gene affects stress responses and behavioral regulation, whereas *NDST4*, *NCAPG*, and *GGA3* are associated with abdominal fat, carcass traits, and intestinal damage caused by heat stress (Williams et al., 2005; Finck & Kelly, 2006; Česen et al., 2012; Brummett et al., 2014; Ma et al., 2019; Larkina et al., 2021a). This suggests that hitchhiking selection might have affected microsatellite loci, with functional gene selection influencing allelic frequency at nearby linked loci despite the neutrality of these microsatellites (Kim & Stephan, 2002). Although specific adaptive genes or mutations have not been directly assessed, this study highlights the potential for future research on the genomic regions responsible for environmental adaptation in DT chickens using high-throughput genomic methods and robust data analysis (Gheyas et al., 2022; Xu et al., 2023; Kebede et al., 2024; Doublet et al., 2024; Dementieva et al., 2024). Moreover, the small sample sizes may have introduced bias in the analysis. However, the accuracy of genetic assignment using over 20 loci with F_{ST} values above 0.10 approaches 100% (Carlsson, 2008). Therefore, genetic clustering data for DT chickens were considered reliable, even with populations of fewer than 10 individuals.

To investigate the genetic origin and introgression of the three DT chicken populations deeper, data from the DT chickens and the SCBP library were used. The results showed that DT-U and DT-HY clustered with indigenous and local breeds, whereas DT-L clustered with the majority of red junglefowl. This suggests that the gene pool patterns of DT-U and DT-HY are more closely related to indigenous and local chicken breeds in Thailand than to the red junglefowl, in contrast to the DT-L population. Future studies should incorporate larger sample sizes and a wider range of populations to gain a deeper understanding of the origins and adaptive processes of the DT chicken breed in Thailand and Vietnam. The inclusion of additional red junglefowl samples from Vietnam may clarify the gene pool dynamics of the DT-HY population. In this study, the DT chicken, a culturally significant breed in Vietnam, possessed unique characteristics that have garnered global interest. Traditional production may have led to unique genetic variants resulting from local climate adaptation. These chickens are considered valuable genetic resources for poultry breeding, and offer the potential to develop breeds adapted to specific environments in Vietnam and Thailand. Further research is required to evaluate their characteristics, aid genetic improvement programs, and ensure the maintenance of genetic diversity.

5. CONCLUSION

In summary, this study provided significant insights into the genetic diversity and population dynamics of DT chickens (known as Dragon Chicken) in Thailand and Vietnam. The findings revealed a high level of genetic variability and low inbreeding, suggesting effective management practices despite observed historical bottlenecks. Genetic analyses showed that populations DT-U and DT-HY shared genetic similarities with indigenous and local chicken breeds, whereas DT-L aligned more closely with red junglefowl. The identified genetic markers, *MCW0098* and *MCW0216*, underscore the potential influence of directional selection, which may be driven by environmental adaptation pressures. These findings underscore the value of DT chickens as a genetic resource for breeding programs that target climate resilience and productivity enhancement. Limitations of this study include a small sample size, which may limit the broader applicability of the findings. Future research should incorporate larger sample sizes and wider population ranges, including additional red junglefowl samples from Vietnam. High-throughput genomic methods such as SNP analysis can further elucidate adaptive genes and support comprehensive breeding strategies. Continued efforts are vital for preserving genetic diversity and ensuring the sustainable utilization of this unique poultry resource.

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CONFLICTS OF INTEREST

No potential conflict of interest was reported by the author(s)

DATA AVAILABILITY STATEMENT

All genotyping data are available from the Dryad Digital Repository Dataset (https://www.sci.ku.ac.th/scbp/; https://datadryad.org/stash/share/x2qlPmboMgCROXO8, updated on June 12, 2024). The detailed results of all calculations are also publicly available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.hhmgqnkm0, updated on June 13, 2024).

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