Genetic Relationship Between Vietnamese Chicken Populations and Chicken Populations from Different Continents

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Abstract: The project aims at assessing genetic diversity between Vietnamese local chicken breeds and other chicken breeds from various continents and analyzing genetic relationship between them. A total of 43 chicken breeds was genotyping using 29 Microsatellite markers. The result showed that the gene pool of Vietnamese local chickens had 26 unique alleles. Within genetic variability of Vietnamese local chicken was high compared to other chicken group except for Vietnamese exotic breeds of Chinese origin. The Vietnamese local populations closely clustered with the Red Jungle fowl population and one Chinese native breed but separated with clusters of other native Chinese and European breeds, with the African cluster in the middle. Clustering of 43 populations also revealed correspond to known breed history and geographical distribution in which Vietnamese chickens are more closely related to Red Jungle fowl populations than the Chinese breeds. This study suggested that gene flow between Vietnamese local chickens and wild ancestors could occurred.

Keyword: Genetic Differentiation, Vietnamese Local Chicken, Microsatellite, Unique Alleles

1. Introduction

Poultry production plays an important role in providing food and cash income for small scale farmers. Despite their importance, their population sizes have been assumed decreasing with some breeds becoming endangered and under extinctive threat. This is due to low performance of local chickens, farmer's preference of exotic breeds that have a high performance, mixed rearing with other breeds and lack of suitable conservation strategies to protect local chickens. The erosion of local chickens may be linked with the loss of valuable genetic variability and unique characteristics. The convention on biological diversity has put the need to conserve farm animal genetic diversity on the agenda. In farm animal diversity conservation, a unified approach accounting for two main roads to conservation has been established. This includes prevention of breed extinction and management of within breed genetic variability with the main objective of controlling genetic drift [1].

Some previous study [2-5] showed that Vietnamese chicken revealed high genetic diversity. However, understanding of the genetic diversity of Vietnamese local chicken in comparison with other chicken breeds in the world and the relationship between them have still limited. There is a need to investigate genetic diversity using source of information as molecular markers as a prerequisite for the development of effective conservation programs for Vietnamese chicken breeds in the global context. In the current study, we used 43 chicken breeds from the previous reports [2, 4, 6] which include Vietnamese local chickens, exotic breeds of Chinese origin, commercial populations, local breeds from China, Europe, Africa with a wide range of origins and histories. The genotyping results obtained for Vietnamese and other populations, which were carried out in the same laboratory using the 29 consistent microsatellite markers, are comparable. The project aims at assessing genetic diversity between Vietnamese local chicken breeds and other chicken breeds from various continents and analyzing genetic relationship between them.
2. Material and Method

2.1. Study Populations and Blood Sampling

Three data sets, which include a set of 9 Vietnamese local chickens and a set of 2 Vietnamese exotic breeds of Chinese origin that were already genotyped as reported by the study [4], were used for analysing. All Vietnamese local chickens were randomly collected. Sampling design is showed in table 1.

| Agro ecological zones | Study areas (province) | Breeds | Abbreviation | Blood sampling |
|-----------------------|------------------------|--------|--------------|----------------|
| Northwest             | Son La                 | H'mong | HM2_VN       | 31             |
|                       | Hung Yen               | Dong Tao | DT_VN    | 32             |
|                       | Bac Ninh               | Ho      | Ho_VN       | 32             |
|                       | Duong Lam, Ha Tay      | Mia     | Mia_VN      | 32             |
|                       | Hoa Duc, Ha Tay        | Ri      | Ri_VN       | 32             |
|                       | Ba Vi, Ha Tay          | Te      | Te_VN       | 8              |
|                       | NIAH                   |         |             | 24             |
| Red river             | Khanh Hoa              | Choi    | Choi_VN     | 33             |
| South central coast   | Long An                | Tau Vang | TV_VN      | 33             |
|                       |                        | Ac      | Ac_VN       | 32             |
|                       |                        | Tam Hoang | TH_VN    | 32             |
|                       |                        | NIAH    |              | 32             |
|                       |                        | Luong Phuong | LP_VN | 32             |
| Total                 |                        |         |              | 353            |

The third set to use for analysing in this study is 32 chicken populations (n = 1121) originating from various chicken production systems and continents, including Asia, Africa, European, purebred and Jungle fowl. These reference populations were already genotyped and reported by these studies [2, 6]. The set of these chicken populations included one population from Vietnam, 10 native Chinese breeds, 4 local African breeds, 8 breeds of different phylogenetic origins collected in Europe, 6 purebred lines and 3 Red Junglefowl populations.

Samples of Chinese native breeds were randomly taken from conservation flocks kept at the Poultry Institute, Academy of Chinese Agricultural Sciences, Yangzhou, China. African populations from Zimbabwe, Sudan and Malawi are all non-descriptive populations not highly selected for any production trait [6]. Six purebred lines including broiler dam line, broiler sire line, 2 brown egg layers and 2 white egg layer lines as well as 8 European breeds were selected from the AVIANDIV project, a European collaborative project on chicken biodiversity. These European populations from France, Italy, Norway, Island and Germany (4 populations) are the fancy breeds with intensive selection for desirable phenotype of particularly traits. While the purebred lines were managed as closed flocks with no migration from outside populations with known breed history and pedigree as well as under well controlled breeding, the Red Junglefowl populations, including 2 populations from China and one from Thai Land, are populations with no any management regime [2]. Information on these breeds is summarized in table 2.

2.2. Microsatellite Genotyping

A drop of venous blood was collected from the ulnar vein onto FTA@Micro Card (Whatman Bio Science, UK), which enables storage of blood samples under local conditions without additional cooling. DNA was isolated by using the phenol-chloroform method.

The DNA polymorphism was assessed using a set of 29 microsatellite markers recommended by the study [7] for assessing chicken genetic diversity. Polymerase chain reaction (PCR) was done according to the study [2]. The RFLPscan software (Scanalytics Division of SSP, Billerica, USA) was applied for Electrophoregram processing and allele scoring. Allele scoring were adjusted using standard alleles. The genotyping results obtained for Vietnamese and other populations, which were carried out in the same laboratory using the consistent microsatellite markers, are comparable (Table 2).

| Continent | Country | Breed        | Abbreviation | N  | Alleles/locus | H_e | H_s | Fis |
|-----------|---------|--------------|--------------|----|---------------|-----|-----|-----|
| Asia      | Vietnam | H'mong 2     | HM1_VN       | 36 | 6.7           | 0.670 | 0.620 | 0.075 |
|           |         | Chahua 2     | CHA_Chi      | 38 | 4.6           | 0.550 | 0.503 | 0.093 |
|           |         | Gushi 2      | GUS_Chi      | 40 | 3.4           | 0.440 | 0.434 | 0.014 |
|           |         | Bai 2        | BAI_Chi      | 34 | 4.2           | 0.537 | 0.498 | 0.074 |
|           |         | Dagu 2       | DAG_Chi      | 25 | 5.2           | 0.634 | 0.640 | -0.011 |
|           |         | Dou 2        | DOU_Chi      | 33 | 3.8           | 0.531 | 0.528 | 0.004 |
|           |         | Langshan 2   | LAN_Chi      | 40 | 4.2           | 0.542 | 0.513 | -0.133 |
|           |         | Wugu 2       | WUG_Chi      | 40 | 4.6           | 0.577 | 0.564 | 0.022 |
|           |         | Xiaoshan 2   | XIS_Chi      | 40 | 4.5           | 0.608 | 0.608 | 0.000 |
|           |         | Beijing Fatty | YOU_Chi   | 38  | 4.4           | 0.553 | 0.512 | -0.36 |
|           |         | Wannan three yellow 2 | WTY_Chi | 32 | 6.3           | 0.644 | 0.605 | 0.061 |
### 2.3. Statistical Analyses

#### 2.3.1. Allelic Diversity and Genetic Diversity

Total number of alleles, allele frequency, the numbers of alleles per locus, expected and observed heterozygosity per populations were calculated using Microsatellite toolkit software package [8].

Observed heterozygosity at a locus is given by direct counting of the number of heterozygotes in the sample divided by the number of individuals typed at the locus. An unbiased estimate of expected heterozygosity is given [9].

\[
H = \frac{2n}{2n-1} \left[ 1 - \sum_{i=1}^{k} p_i^2 \right]
\]

Where: \( n \) is the number of individuals \( x \) is allele frequency at locus 1, \( k \) is number of alleles at locus 1.

Estimates of Inbreeding coefficients (\( F_{st} \)) and their significant level within each of Vietnamese populations were done by FSTAT [10] and GENEPOP computer packages [11], respectively. \( F_{st} \) values were calculated as follows:

\[
F_{st} = \frac{H_{exp} - H_{obs}}{H_{exp}}
\]

Where: \( F_{st} \) was the inbreeding coefficient of an individual relative to the sub-population, \( H_{exp} \) was the mean of expected heterozygosity in populations, \( H_{obs} \) was the mean of observed heterozygosity in populations. Multiple estimates over loci are averaged to obtain a mean estimate.

### 2.3.2. Breed Differentiation

Two methods based on the similarity in allele frequencies between populations, which provided different insights into genetic variation and differentiation were used in this study. They are Wright’s F statistics [12] and Marker Estimated Kinship methods [13]. Wright’s F statistics was the standard instrument to investigate population sub-division and partition genetic variation into between and within population components and might be used as indicators for the relative importance of the breeds within a species. Whereas F-statistics had an attention to differences between populations, Marker Estimated Kinship was interested in resemblance between them.

The degree of population subdivision will be assessed using the Wright’s fixation indices [14] and are described as:

\[
1 - F_{st} = (1 - F_{is})(1 - F_{ss})
\]

Where:
- \( F_{st} \) is the inbreeding coefficient of an individual relative to the whole set of populations,
- \( F_{is} \) is inbreeding coefficient of an individual relative to entire population,
- \( F_{ss} \) is inbreeding coefficient of an individual relative to the sub-population it belongs to.

Taking all 43 populations into consideration, Wright’s fixation indices (\( F_{is}, F_{st} \) and \( F_{ss} \)), according to the study [12] were calculated in order to estimate the partitioning of the variance between and within populations using FSTAT software package.

The similarity indices between and within populations were calculated from allele frequencies using Malecot’s definition of similarity [15]. The Malecot similarity is advantageous over other similarity measures because it can be calculated directly from allele frequency. The mean

| Continent | Country | Breed | Abbreviation | N | Alleles/locus | \( H_c \) | \( H_s \) | \( F_s \) |
|-----------|---------|-------|--------------|---|--------------|--------|--------|--------|
| African   | Malawi 1 | MAL   | 60           | 5.9 | 0.607        | 0.554  | 0.088  |
|           | Sudan 1 | SUD   | 48           | 5.6 | 0.561        | 0.517  | 0.081  |
|           | Zimbabwe 1 | ZIM   | 50           | 6.7 | 0.642        | 0.590  | 0.082  |
|           | Zimbabwe 3 | ZZIM 1 | 51           | 6.1 | 0.650        | 0.605  | 0.070  |
|           | France 2 | MAR_Fr| 30           | 3.8 | 0.560        | 0.498  | 0.111  |
|           | Island 1 | ICL_Is| 30           | 4.2 | 0.540        | 0.510  | 0.056  |
|           | Norway 3 | JAE_Nor| 30           | 2.6 | 0.314        | 0.325  | -0.035 |
| Europe    | Italy 3  | Padova| 30           | 2.8 | 0.403        | 0.349  | 0.136  |
|           | Germany 2 | LAK_Ger| 30           | 2.9 | 0.428        | 0.320  | 0.255  |
|           | China 3  | WFT_Ger| 30           | 2.8 | 0.396        | 0.324  | 0.185  |
|           | China 1  | CRJF_SC | 30          | 3.8 | 0.540        | 0.536  | 0.004  |
| Germany   | Germany 1 | OFM_Ger| 30           | 2.6 | 0.407        | 0.355  | 0.129  |
|           | China 3  | Westf_Totleger | 30     | 2.8 | 0.596        | 0.324  | 0.185  |
|           | China 1  | GALLUS galls galus | 30   | 3.8 | 0.540        | 0.536  | 0.004  |
| Germany   | Germany 1 | Ost_Moenen | 30       | 2.9 | 0.428        | 0.320  | 0.255  |
|           | China 3  | Lakenfelder | 30     | 2.8 | 0.396        | 0.324  | 0.185  |
|           | China 1  | GALLUS galls galus | 30   | 3.8 | 0.540        | 0.536  | 0.004  |
| Jungle fowl | China 1  | GALLUS galls spadiceus | 26  | 4.7 | 0.638        | 0.578  | 0.096  |
|           | China 1  | Thai Land | 30         | 5.7 | 0.655        | 0.630  | 0.039  |
|           | Purebred | White egg layer | 30      | 2.7 | 0.338        | 0.310  | 0.086  |
|           | Purebred | lines | 30           | 1.8 | 0.212        | 0.218  | -0.032 |
|           | Purebred | Brown egg layer | 30     | 2.9 | 0.418        | 0.391  | 0.065  |
|           | Purebred | lines | 30           | 2.9 | 0.393        | 0.399  | -0.015 |
|           | Purebred | Broiler dam and | 30   | 3.8 | 0.584        | 0.585  | -0.002 |
|           | Purebred | sire | 30           | 3.8 | 0.547        | 0.526  | 0.039  |

Key: \( H_c \): Expected heterozygosity; \( H_s \): Observed heterozygosity; \( F_s \): Inbreeding coefficient within population; \(^1\) No management or selection; \(^2\) Conservation flocks; \(^3\) Fancy breeds which were selected for a given standard; \(^4\) Selected for quantitative traits.
Malecot (calculated over multiple loci) was expected to be equal to the coefficient of kinship [15]. MEK between and within populations were calculated using MEK [15]. Calculation of MEK between and within populations was based on these similarity indices using the Weighted Drift Similarity model. This procedure was recommended as a suitable method for analysis of individuals within a population [15]. The model was represented as:

$$\log(1 - S_{ij}) = \log(1 - f_{ij}) + \log(1 - S_L) + \text{error}_{ij}$$

Where: $S_{ij}$ is the similarity between population i and j for locus L, $f_{ij}$ is the kinship between i and j, $S_L$ is the probability of alleles alike in state for locus L.

### 2.3.3. Phylogenetic Network Trees

Phylogenetic network trees were constructed by the Splitstree software [16]. The kinship matrix was transformed into a distance matrix, which was used as an input file for to build phylogenetic network tree. Advantages of the phylogenetic network tree is that it not only represents evolutionary relationships between populations but also shows any network in which populations are represented, i.e. besides constructing phylogenetic tree, split and reticulate networks are also constituted.

The split network is obtained as a combinatorial generalization of phylogenetic trees and is designed to represent incompatibilities within and between data sets. The reticulate network represents evolutionary histories in the presence of reticulate events such as hybridization, horizontal gene transfer, or recombination.

### 3. Results and Discussion

#### 3.1. Allelic Diversity

There was rich allelic variation for microsatellite genotyped. The allelic diversity of microsatellite in the 9 Vietnamese local and 2 Vietnamese exotic populations as well as for a total of 43 populations is shown in Table 3. The group of Vietnamese local chickens showed an approximately 2-fold higher mean number of alleles per locus (10.24 ± 5.83) compared with the Vietnamese breeds of Chinese origin (5.84± 2.67). Overall, 364 alleles were detected, resulting in a mean number of alleles per locus (± SD) of 12.55 ± 7.00. Extreme loci are ranging from MCW165 (3 alleles) and Lei 234 (33 alleles).

The gene pool of Vietnamese local chickens had 26 alleles, which were absent in the other gene pools. Twenty one of these 26 private alleles appeared at a frequency of less than one percent, and the remainder varied from 1.21 to 2.60 percent (Table 3). In turn, the 2 Vietnamese exotic breeds had only one private alleles (a frequency of 3.13), which were missing in the other populations.

### Table 3. Observed Allele Size Ranges and Number of Alleles in the All Populations and the Size and Frequency of Alleles Unique to the Vietnamese Populations.

| Locus   | All 43 Populations | 11 Vietnamese Populations | Unique Alleles |
|---------|--------------------|----------------------------|----------------|
|         | Allele range (bp)  | No. Alleles N= 1534        | No. Alleles N= 289 | No. Alleles N= 64 | |
| MCW 330 | 250 – 300          | 9                          | 9              | 5              | 250 (0.17)* |
| MCW 295 | 88 – 110           | 12                         | 10             | 6              | 225 (0.35); 227 (0.87) |
| MCW 248 | 209 – 227          | 10                         | 7              | 3              | 136 (0.35); 142 (0.17); 146 (1.21) |
| MCW 222 | 220 – 226          | 4                          | 4              | 4              | 249 (0.17) |
| MCW 216 | 136 – 149          | 11                         | 10             | 6              | 292 (1.38); 305 (0.52) |
| MCW 206 | 221 – 249          | 14                         | 12             | 7              | |
| MCW 183 | 292 – 326          | 21                         | 17             | 8              | |
| MCW 165 | 114 – 118          | 3                          | 3              | 3              | |
| MCW 123 | 76 – 100           | 12                         | 12             | 4              | |
| MCW 111 | 96 – 120           | 13                         | 8              | 8              | |
| MCW 104 | 190 – 236          | 24                         | 19             | 12             | 236 (1.21) |
| MCW 103 | 262 – 274          | 4                          | 2              | 3              | 262 (3.13)* |
| MCW 098 | 261 – 267          | 4                          | 3              | 2              | |
| MCW 081 | 112 – 145          | 14                         | 10             | 4              | 115 (0.52); 131 (0.35); 137 (0.17) |
| MCW 080 | 264 – 283          | 20                         | 15             | 7              | 265 (0.52); 283 (0.35) |
| MCW 078 | 135 – 147          | 6                          | 6              | 4              | |
| MCW 069 | 156 – 176          | 11                         | 11             | 8              | 156 (0.52) |
| MCW 067 | 176 – 199          | 9                          | 6              | 4              | 183 (0.52) |
| MCW 037 | 154 – 160          | 7                          | 6              | 6              | |
| MCW 034 | 212 – 246          | 18                         | 17             | 9              | |
| MCW 020 | 175 – 187          | 6                          | 6              | 4              | 175 (0.69) |
| MCW 016 | 162 – 206          | 16                         | 12             | 8              | 184 (0.52); 188 (0.87) |
| MCW 014 | 158 – 188          | 16                         | 11             | 4              | 260 (0.18); 348 (1.26); 372 (0.36) |
| LEI 234 | 216 – 380          | 33                         | 28             | 13             | 357 (2.60); 361 (0.17) |
| LEI 166 | 350 – 376          | 13                         | 10             | 4              | 241 (0.52) |
| LEI 094 | 241 – 289          | 24                         | 20             | 9              | |
| ADL 278 | 114 – 129          | 14                         | 11             | 7              | |
| ADL 268 | 104 – 118          | 8                          | 7              | 5              | |
| ADL 112 | 120 – 130          | 8                          | 5              | 4              | |
Compared to the previous microsatellite-based analyses for Vietnamese local chickens, a mean number of alleles per locus in this study is higher than that observed in the reports of [3, 5, 17]. The higher obtained value could be attributed to the higher number of breeds and number of loci used. This study analyzed national chicken genetic resources distributed in both the Northern and Southern part of Vietnam using 29 microsatellite markers and 11 admixture chicken populations presenting four different phenotypes from a North-eastern province were analysed using only 25 microsatellite markers in the report of these studies [3, 18] showed that the mean number of alleles per locus of five Swedish chicken using 24 microsatellites was 4.7 alleles. Fathi [19] reported that 5 Saudi chicken breeds represented 4.4 alleles using 25 microsatellites. Okumu [20] studied on 8 Kenyan chicken breeds indicated 15.7 alleles in 18 microsatellites.

Overall, a mean value of number of 12.55 alleles per locus observed in 43 populations with the high number of Vietnamese local breeds in this study is higher than that estimated for 23 local populations from Africa, Asia and South America [21] using 22 microsatellite markers or for 64 populations from different continents [2] using the same markers as this study. These probably suggest that Vietnamese gene pool may contribute considerable genetic diversity. The further analyses on genetic diversity with and between breeds might be complementary to this inference. According to suggestion [1], Vietnamese chicken genetic resources, which are proposed to add much to diversity, are probable to prioritise to be conserved.

Private alleles are defined as allelic variants restricted to single populations [22]. An estimation of gene flow can be practiced through the abundance of private alleles [23]. Compared to Vietnamese exotic chickens of China descent, Vietnamese local populations revealed much higher unique alleles (26 alleles vs. 1 allele). In the study [2], the Vietnamese H’mong chickens also displayed the highest number of private alleles compared with the other 63 populations. A population displayed highest the number of unique alleles are probably the first to split to the other populations. These findings support the researches of Fumihito and West [24-25] who proposed that chickens were first domesticated in Southeast Asia and were taken north to become established in China.

### 3.2. Genetic Diversity

Compared to other populations, the average expected and observed heterozygosity of the Vietnamese local populations is higher than that of the Chinese, African, European, purebred and Red Jungle fowl populations. The results are illustrated in Table 4.

**Table 4.** Average observed and expected heterozygosity frequencies of Vietnamese local, Vietnamese exotic, Chinese, African, European, purebred and Jungle fowl populations.

| Population            | n  | $H_E \pm SD$ | $H_O \pm SD$ |
|-----------------------|----|--------------|--------------|
| Vietnamese local      | 9  | 0.634 ± 0.034| 0.597 ± 0.033|
| Vietnamese exotic     | 2  | 0.654 ± 0.037| 0.632 ± 0.036|
| Chinese               | 10 | 0.562 ± 0.059| 0.541 ± 0.063|
| African               | 4  | 0.615 ± 0.041| 0.567 ± 0.039|
| European              | 8  | 0.428 ± 0.083| 0.372 ± 0.084|
| Pure breed            | 6  | 0.415 ± 0.137| 0.405 ± 0.135|
| Jungle fowl           | 3  | 0.611 ± 0.062| 0.581 ± 0.047|

Key: $H_E$: Expected heterozygosity; $H_O$: Observed heterozygosity

Compared to other population, Vietnamese local group showed higher expected heterozygosity. This is in agreement with the previous reports. For instance, the expected heterozygosity varied from 0.505 to 0.678 in the study on 78 Chinese chickens [26]. The lower heterozygosity reported in Japanese native and Red Junglefowl populations ranged from 0.349 to 0.501 [27]. In the study on local Asian and European chickens, [3] found the range of heterozygosity between 0.429 and 0.625. Moreover, Vietnamese Hmong breed was reported as a population with highest genetic diversity compared to the other 63 populations from different continents and management histories [2]. High values of expected heterozygosity found in Vietnamese local chickens is probably complementary to the inference based on their high mean number of alleles per locus and their great number of private alleles that Vietnamese gene pool harbours abundant reservoir of genetic diversity.

[28] researched on chicken breeds of different continents showing that the highest expected heterozygosity was found in the African group and the lowest expected heterozygosity was found in the European group. [18] and Okumu [20] indicated that the expected heterozygosity of Swelsh and Kenya chicken breeds was from 0.231 dën 0.515 and from 0.351 to 0.434, respectively.

### 3.3. Breed Differentiation

In comparison of the partitioning of the variance between and within populations of the Vietnamese breeds to that of other populations, the mean $F_{st}$, $F_{is}$ and $F_{sd}$ values of the Vietnamese local, Vietnamese exotic, China, African, European, Purebred and Jungle fowl populations were estimated (Table 5). In contract to purebred which reached...
the maximum between population variation ($F_{ST} \pm SD = 0.387 \pm 0.024$) and the highest heterozygote deficiency ($F_{IT} \pm SD = 0.403 \pm 0.026$), Vietnamese group performed the minimum between population variation ($F_{ST} \pm SD = 0.052 \pm 0.018$) and the second lowest heterozygote deficiency ($F_{IT} \pm SD = 0.109 \pm 0.111$). Surprisingly, $F_{ST}$ value of Vietnamese local group approximately equals that of populations from various countries of African continent.

### Table 5. Overall population ($F_{IT}$), between populations ($F_{ST}$) and within population ($F_{IT}$) inbreeding coefficients of the Vietnamese, Asian, African, European, Purebred and Jungle fowl populations.

| Population                | $F_{IT} \pm SE$ | $F_{ST} \pm SE$ | $F_{IS} \pm SE$ |
|---------------------------|-----------------|-----------------|-----------------|
| Vietnamese local          | 0.109 ± 0.111   | 0.052 ± 0.006   | 0.061 ± 0.010   |
| Vietnamese exotic         | 0.094 ± 0.018   | 0.062 ± 0.008   | 0.034 ± 0.017   |
| Chinese                   | 0.176 ± 0.012   | 0.171 ± 0.010   | 0.006 ± 0.011   |
| African                   | 0.138 ± 0.018   | 0.060 ± 0.009   | 0.083 ± 0.013   |
| European                  | 0.404 ± 0.140   | 0.313 ± 0.016   | 0.133 ± 0.015   |
| Pure breed                | 0.403 ± 0.026   | 0.387 ± 0.024   | 0.026 ± 0.016   |
| Jungle fowl               | 0.213 ± 0.026   | 0.175 ± 0.014   | 0.046 ± 0.024   |

Overall deficit of heterozygotes was reported for Vietnamese local chickens (Binh et al., 2007). A similar phenomenon has been presumed for almost all of Vietnamese local populations used in this study due to small population sizes or uncontrolled mating.

An association between the level of inbreeding and the type of management and origin was concluded by Granevitze [2]. Considering all 43 populations, inbreeding coefficients ($F_{IT}$) of Vietnamese local chickens were higher that of other populations except for the African and European groups because the European populations used to compare in the current study were fancy breeds, which were selected for a given standard [2], and culling chickens based on ranking criteria was practiced in Zimbabwe [6]. The result of this study was consistent with the other reports on native chickens. An excess of heterozygosity was found in the current study was consistent with the other reports on native chickens [29]. Tadano [27] reported that the Japanese native chickens were more inbreeding than the Red Junglefowl populations. Muchadeyi [6] displaced a high value of inbreeding in the scavenging African populations than in the pure breeds. Recently, Berthouly [3] revealed that a low level of inbreeding in the Asian breeds compared to the European breeds.

In comparison with previous researches on local chickens from different countries, the higher value was found by the study [26] in 78 Chinese chicken populations and no genetic differentiation (0.008) was estimated in the Zimbabwe population kept in the five agro-ecological zones [6]. The value of 0.052 is below 0.15 and therefore describes a moderate differentiation between populations (Wright, 1968). This indicated a presence of sub-structure of the Vietnamese local chickens.

The closer association of the African populations reported by Muchadeyi [6] can be one the reason to explain why their genetic distinction was similar to the country context of Vietnam. As expected, $F_{IT}$ estimates for commercial breeds was high because they were based on different founder breeds, kept as closed flocks and selected for different productive traits. In a similar way, a possible explanation for the high $F_{IT}$ value of the European group is due to intensive selection practiced in these populations [2].

### 3.4. Phylogenetic Network Trees

The matrix of marker estimated kinships within and between populations of Vietnamese and other populations is estimated to built the tree. The overall structure of the tree, which exhibited the estimated relationship between 43 populations, is showed in figure 1.

![Figure 1. Phylogenetic network from marker estimated kinships of 43 populations.](image)

The Vietnamese local populations closely clustered with the Red Jungle fowl population and one Chinese native breed but separated with clusters of other native Chinese and European breeds, with the African cluster in the middle.

Clustering of 43 populations revealed also correspond to known breed history and geographical distribution. One Chinese breed, Chahua chicken, was clustered to Vietnamese groups because the chicken was kept in Junnan province, an adjacent area of Vietnam. Therefore, it provides evidence of gene flow between this chicken and the Vietnamese chicken gene pool. Moreover, a closer relationship between the Chinese Jungle fowl population and the Vietnamese local chickens than between them and the Chinese chickens in addition to closely clustering of the Vietnamese local populations with the Red Jungle fowl populations and the Chahua chicken could support the previous studies on domestication of chickens which reported domestication origin in Southeast Asia [24-25] or assumed Yunnan and bordering area as domestication centre [30].
4. Conclusion

The gene pool of Vietnamese local chickens had 26 unique alleles. Within genetic variability of Vietnamese local chicken was high compared to other chicken group except for Vietnamese exotic breeds of Chinese origin. The Vietnamese local populations closely clustered with the Red Jungle fowl population and one Chinese native breed but separated with clusters of other native Chinese and European breeds, with the African cluster in the middle.

Clustering of 43 populations revealed also correspond to known breed history and geographical distribution in which Vietnamese chickens are more closely related to Red Jungle fowl populations than the Chinese breeds. This study suggested that gene flow between Vietnamese local chickens and wild ancestors could occurred.

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