High-resolution genomic analysis of four local Vietnamese chicken breeds

Moyse Evelyne1 | Duy Nguyen Van1,2 | Dor Amelyne1 | Thinh Nguyen Hoang2 | Luc Do Duc2 | Moula Nassim1 | Ton Vu Dinh2 | Farnir Frédéric1

1Biostatistics & Bioinformatics, FARAH-PAD, Faculty of Veterinary Medicine, University of Liege, Liège, Belgium
2Faculty of Animal Science, Vietnam National University of Agriculture, Hanoi, Vietnam

Correspondence
Farnir Frédéric, Biostatistics & Bioinformatics, FARAH-PAD, Faculty of Veterinary Medicine, University of Liege, Quartier Vallée 2 – Avenue de Cureghem, 6 (B43), 4000 Liège, Belgium.
Email: f.farnir@uliege.be

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Abstract
In Vietnam, local chicken breeds account for over 70% of the national poultry population. Although these breeds are abundant, their productivity is low and their use is threatened by the extensive importation of foreign productive breeds. In this context, conservation programmes targeting several emblematic breeds have been established. The goal of these programmes was to characterize endangered breeds and maintain a pool of characteristic birds for preserving their genetic heritage. To contribute to these programmes, we comprehensively characterized four Vietnamese local chicken breeds (Dong Tao, Ho, Mia and Mong) at the genomic level using high-density single-nucleotide polymorphism (SNP) genotyping. Despite originating in geographically close areas, Dong Tao and Ho were evidently different from each other as well as from Mong and Mia, which shared a more recent common ancestor. The genomic inbreeding coefficient revealed high homozygosity amongst the four breeds (10%–20%). The observation of clear differentiation at the genomic level supported the presence of distinct breeds; nonetheless, the occurrence of crossbred birds in a presumably purebred sample demonstrated the need to apply genomic tools to unambiguously assign the birds to the correct breed. Moreover, the occurrence of substantial inbreeding and the presence of subgroups in certain breeds warranted attention to create future nuclei for use in the conservation of these local breeds.

Keywords
genomics, local chickens, Vietnam

1 | INTRODUCTION

Vietnam is an agricultural country, with 70% of the human population living in rural areas (Desvaux, Vu Dinh, Phan Dang, & Pham Thi, 2008). In these areas, nearly 50%–90% of households own livestock (Burgos, Hong Hanh, Roland-Holst, & Burgos, 2007). Moreover, according to Ngoc (2020), approximately 7,864,700 households raised poultry in 2019. Local chickens in Vietnam account for over 70% of the national poultry population (Lan Phuong, Dong Xuan, & Szalay, 2015). There are 21 local chicken breeds, of which Ri, Tau Vang, Mia, Dong Tao and Ho are the most popular and historical breeds in specific regions (Pham Cong, 2016). Although these breeds are abundant, their productivity is low. Moreover, to meet consumer demand, livestock
farming in Vietnam is evolving towards more intensive breeding strategies. Accordingly, to improve productivity, many farmers have started rearing exotic chickens, with a small proportion of hybrid birds obtained from crosses between exotic and local breeds (Nguyen Van, Nguyen Thi, Tran Thi, Do Manh, & Dang, 2015). Such recent breeding approaches markedly affected the number of remaining native breeds and the size of their populations. Therefore, national programmes aimed at the conservation of Vietnamese animal genetic resources have been initiated since the 1990s, to prevent the extinction of local breeds.

Local chickens are well adapted to rural environments, with rudimentary housing conditions, coarse nutritional intake and continuous pathogen exposure. This adaptability indicates that specific genetic profiles have likely been selected naturally and artificially during the formation of these breeds. Consequently, the preservation of local chicken breeds is crucial for households, for which these breeds serve as a means of livelihood. Moreover, from the viewpoint of national policies, these breeds represent genetic resources characterized by robustness under local conditions, with potential application as materials for genetic improvement in future breeding programmes (Besbes, 2009; Rischkowsky & Pilling, 2007). Moreover, Vietnamese local chicken breeds are an essential part of cultural and social activities (FAO, 2008; Lan Phuong et al., 2015). In this context, establishing conservation programmes targeting several emblematic breeds is imperative. The goal of these programmes was to characterize endangered breeds and maintain a pool of characteristic birds for preserving their genetic heritage.

To this end, in the present study, four Vietnamese local chicken breeds (Dong Tao, Ho, Mia and Mong) included in the conservation programmes of the Ministry of Agriculture and Rural Development (MARD) of Vietnam, in collaboration with the Vietnam National University of Agriculture (VNUA) and the National Institute of Animal Sciences (NIAS), were examined. Of these, Dong Tao and Ho have small populations, being considered vulnerable according to the FAO criteria (FAO, 2008; Lan Phuong et al., 2015). These two breeds are two of the most important local chickens in Vietnam. Dong Tao chickens are famous for their massive body size, stout legs and good meat quality (Lan Phuong et al., 2015; Nguyen Van et al., 2015). Ho chickens are characterized by their large body size and diverse feather colours (Nguyen Van, Moula, et al., 2015). Historically, birds of these two breeds were offered as prestigious presents to the King (FAO, 2008; Lan Phuong et al., 2015). These four chicken breeds are typically raised in neighbouring provinces in northern Vietnam (Figure 1) and have originated from unique townships, namely the Ho Township for Ho, the Dong Tao Township for Dong Tao, the Duong Lam Township for Mia and the Tien Phong Township for Mong. Conservation programmes aimed at increasing the population sizes of these breeds have been initiated. In 2014, only 681 birds from 34 households were listed as Ho chickens (Vu Dinh, Bui Huu, Dao Thi, & Nguyen Van, 2014), whereas 200 birds each from 105 households were listed as Dong Tao chickens (Vu Dinh, Dao Thi, & Nguyen Van, 2013). Comparatively, more Mong and Mia chickens are present, with over 200 households rearing 65–2600 Mia chickens (Cuc et al., 2016) and over 200 households rearing 60–1800 Mong chickens (Cuc et al., 2016).

In villages, chickens live outside the households, and there is no mating control in most cases (Pham et al., 2013). In addition, for commercial and economic reasons, farmers who maintain local chicken breeds may cross these local chickens with other breeds. In the context of geographical proximity and desire to increase the productivity of local breeds without strict mating control, the risk of inbreeding or crossbreeding and the corresponding disadvantage are evident.

To date, the characterization of Vietnamese local chicken breeds was based primarily on phenotypic observations, although differences between breeds are often subtle. Therefore, the current phenotypic definitions of breeds may not correspond to the systematic differences at the genomic level. Recently, several studies have attempted to investigate the genetic structure of local Vietnamese chicken populations using microsatellite DNA markers (Berthouly et al., 2009; Cuc et al., 2010; Pham et al., 2013). Using a clustering approach, Cuc, Weigend, Tieu, and Simianer (2011) reported that Dong Tao chickens formed a single unique cluster, whereas Ho and Mia chickens formed an admixed cluster with three other breeds. Another study involving 17 Vietnamese local chicken breeds showed that Dong Tao and Mia could not be distinguished, whereas Ho and Mong formed a single cluster (Pham et al., 2013). Moreover, evaluations of the genetic diversity and conservation potential of these breeds have revealed contradictory results. Whilst Cuc et al. (2011) reported that Dong Tao chickens made the highest genetic contribution to the genetic diversity of Vietnamese chickens, Pham et al. (2013) identified its little genetic contribution. Nevertheless, these two studies observed a low genetic contribution of Mia chickens.

In this light, further studies are warranted to address these ambiguities. High-density single-nucleotide polymorphism (HD SNP) genotyping can provide more reliable estimates of genetic variation than reduced sets of microsatellites. Moreover, HD SNP genotyping allows...
for an accurate inference of the inbreeding and cross-breeding history of a particular population, as evidenced in recent studies on the genetic diversity of chicken breeds (Fleming et al., 2016; Johansson & Nelson, 2015; Mekchay et al., 2014; Muir et al., 2008; Zhao et al., 2018). The inbreeding coefficient is the expected proportion of loci that are homozygous by descent in an inbred individual (Falconer & Mackay, 1996). Controlling inbreeding within populations is a key step to maintain genetic diversity (Curik, Ferencaković, & Sölkner, 2014; Gandini, Stella, Del Corvo, & Jansen, 2014). Consequently, assessing inbreeding at both individual and population levels is a major concern for improving the efficiency of conservation and selection programmes.

To this end, the first aim of the present study was to evaluate whether the phenotypic definitions of the four Vietnamese local chicken breeds corresponded to their different genomic structures. If true, this would allow for a full recognition of these breeds. The next aim was to evaluate the genetic diversity of each potential breed as a step towards assessing the effectiveness of the current conservation programmes.

2 | MATERIALS AND METHODS

2.1 | Chicken populations

A total of 96 individuals were sampled from four populations of Vietnamese local chickens (28 Ho, 32 Dong Tao, 18 Mong and 18 Mia) distributed in four provinces (Bac Ninh, Hung Yen, Ha Nam and Hanoi, respectively). The sample distribution is presented in Table S1 and Figure 1. All individuals were from the same conservation programme in Vietnam, originating from either private farms or public institutions (VNUA and NIAS) involved in this programme. Neither pedigree nor phenotypic information was available.
We used stratified sampling for flocks, ensuring a distance of at least 500 m between the selected flocks and random sampling of one or two individuals in each selected flock.

### 2.2 DNA extraction and genotyping

Genomic DNA was extracted from the blood samples of all chickens (Table 1) using the phenol–chloroform method (Sambrook & Russell, 2001) with minor modifications. The DNA stocks were diluted to a working concentration of 50 ng µl⁻¹ for HD SNP genotyping using the Affymetrix GeneTitan platform. The HD array for chickens allows for the simultaneous genotyping of approximately 580,000 SNPs. This array comprises SNPs on 28 autosomal chromosomes, two sex chromosomes (Z and W for poultry) and two linkage groups (LGE64 and LGE 22C19W28). Only SNPs on the 28 autosomes were considered in the present study.

The assessment of genomic similarity between pairs of birds revealed two highly similar pairs of Ho chickens with over 90% identical genotypes. Accordingly, these birds were very likely to be related; thus, one bird from each pair was excluded from subsequent analyses. The remaining 94 individuals achieved call rates higher than 0.95. SNPs with more than 10% missing genotypes or minor allele frequencies lower than 1% were removed, yielding a consensus panel of 454,297 autosomal SNPs for the analysis of population genetic structure.

We applied a second quality control within each breed. Specifically, SNPs with within-breed minor allelic frequencies lower than 0.05 or SNPs with significant ($p < .0001$) deviation from the Hardy–Weinberg equilibrium (Wigginton, Cutler, & Abecasis, 2005) were filtered out. This second filter led to 368,652, 383,792, 405,535 and 432,631 SNPs for Ho, Dong Tao, Mong and Mia breeds, respectively. Of note, removing monomorphic SNPs within breeds may lead to the elimination of SNPs that are polymorphic across breeds. Due to the large number of available SNPs, we did not consider the potential effect of these filtered markers as important for subsequent analyses. Genetic diversity analyses were performed on the remaining 308,307 SNPs shared by all four breeds.

### 2.3 Population genetic structure

We used $F_{ST}$ (Weir & Cockerham, 1984) to assess the population genetic differentiation with PLINK 1.9 (Purcell et al., 2007). To investigate the genetic structure of breeds, we used a variational Bayesian framework implemented in fastSTRUCTURE (Raj, Stephens, & Pritchard, 2014). We conducted the analysis using the standard model with a simple prior and with the number of groups, $K$, ranging from 1 to 8. A fastSTRUCTURE script (chooseK.py) was used to select the $K$ value that maximized the marginal likelihood. When a subgroup was detected, we performed another round of fastSTRUCTURE analysis within this subgroup to differentiate the breeds in this subgroup. R software (version 3.3.2; R Core Team, 2016) was used to visualize individual admixture proportions.

We also performed principal component analysis (PCA) to classify the individuals based on a reduced number of significant orthogonal principal components. We used the ADE-4 package in R (Dray & Dufour, 2007) to perform PCA.

To observe the phylogenetic relationship, a distance matrix based on the identity by the state of the genotypes was generated using PLINK. A neighbour-joining tree was then created using the APE package in R (Paradis & Schliep, 2019).

### 2.4 Genetic variation within breeds

To assess the genetic variation within each breed, we obtained the number of SNPs as well as the observed and expected heterozygosity for each breed using PLINK.

#### 2.4.1 Linkage disequilibrium (LD)

The extent of LD was estimated using the $r^2$ statistics, calculated between marker pairs (Slatkin & Excoffier, 1996). To reduce computation, pairwise $r^2$ values were calculated for SNPs within a window of 5 Mb for each chromosome and breed. We also computed $r^2$ values for more distant SNPs: we used 100,000 random combinations of markers distant from 5 to 50 Mb as well as 20,000 random combinations for SNPs on different chromosomes in each breed. To test whether the obtained long-distance $r^2$ values were consistent with expected values, we performed 20,000 simulations to determine background LD. To this end, we generated $n$ genotypes at two loci using random allelic frequencies ($n = 18, 26$ and $32$ to mimic the sample sizes of our breeds) and obtained an empirical distribution of these random $r^2$ values. Finally, we plotted the real $r^2$ values as a function of intermarker distances using R.

**Table 1** Pairwise weighted $F_{ST}$

<table>
<thead>
<tr>
<th>Breed</th>
<th>Dong Tao</th>
<th>Ho</th>
<th>Mia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ho</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mia</td>
<td>0.05</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Mong</td>
<td>0.07</td>
<td>0.10</td>
<td>0.04</td>
</tr>
</tbody>
</table>
2.4.2 | Inbreeding

We estimated the extent of inbreeding using the RZooRoH package in R (Bertrand, Kadri, Flori, Gautier, & Druet, 2019). RZooRoH uses a hidden Markov model to analyse the runs of homozygosity (ROH). This method assumes that individual genomes are composed of homozygous-by-descent (HBD) and non-HBD stretches, corresponding to different states in the Markov model. The HBD stretches were further split into $K$ distinct states corresponding to different distances from the common ancestor. In the present study, we considered 13 classes: one non-HBD class and 12 HBD classes corresponding approximately to the distances of 1, 2, 4, 8, ..., and 2048 generations to the common ancestor (Druet & Gautier, 2017). For each HBD class, the genome-wide HBD probability was estimated as the probability of distances belonging to that class averaged over the whole genome. A global inbreeding coefficient ($F_{zooRoH}$) was obtained by integrating the HBD probabilities across all classes.

One-way ANOVA was used to compare the four breeds with respect to the global inbreeding coefficients. Pairwise comparisons were performed using planned contrasts, with correction for multiple comparisons following the Benjamini–Hochberg procedure (false discovery rate, FDR) (Benjamini & Hochberg, 1995). The Bartlett test was used to compare the variances of the four breeds. All statistical analyses were performed using R.

Moreover, the Viterbi algorithm (Rabiner, 1989) implemented in the RZooRoH package was used to identify the HBD segments. Because the package assigns each marker to one class, HBD segments can be defined as the stretches of consecutive markers assigned to the same class. Thus, the HBD segments also represent ROH.

2.4.3 | Past effective population size (Ne)

LD-based Ne was calculated for each breed. Under the assumption of linear population growth, the expected $r^2$ between neutral markers is given as follows:

$$E[r^2] = \frac{1}{\alpha + 4Ne \ast c} + \frac{1}{n},$$

(Qanbari, Hansen, Weigend, Preisinger, & Simianer, 2010; Tenesa et al., 2007), where $\alpha = 1$ (or 2) if the mutation is (not) taken into account, $n$ is the number of SNPs in the tested chromosome and $c$ is the genetic distance in Morgan units.

As shown by Hayes, Visscher, McPartlan, and Goddard (2003), LD between distant SNPs estimates more recent Ne than LD between close SNPs. Therefore, the evolution of Ne can be estimated since LD between loci with a genetic distance of $c$ reflects the ancestral effective population size $1/(2c)$ generations ago (Hayes et al., 2003). Given the high variability of the recombination rates across chromosomes in chicken, the genetic distance was calculated for a marker interval of physical length $x_i$ (in Kb) on chromosome $i$ as $c_i = \rho_i \ast x_i$, where $\rho_i$ is the recombination rate of chromosome $i$ (in M/Kb), as reported by Groenen et al. (2009).

The marker pairs were sorted according to the genetic distances between markers, and the mean LD was then obtained at every 0.05, 0.5 and 1 cM for distance ranges of 0.1–1, 1–10 and 10–20 cM, respectively (Sargolzaei, Schenkel, Jansen, & Schaeffer, 2008). Finally, we plotted the Ne values as a function of the number of generations using R. A linear model was used to fit Ne as a function of the number of generations according to the breed.

3 | RESULTS

3.1 | Population genetic structure

The average genetic differentiation ($F_{ST}$) between the four breeds was 0.08. Ho was the most differentiated from the remaining three breeds. In contrast, Mia and Mong showed the least genetic differentiation (Table 1).

Analysis using fastSTRUCTURE revealed that the assumption of the presence of four groups maximized the marginal likelihood of the entire data set (Figure S1). The results indicated that Mia and Mong originated from the same ancestral population (Figure 2a, orange group). Ho chickens formed a single cluster (blue group), with a mean blue membership posterior probability of 0.93. Incidentally, one supposedly Ho individual showed an admixed pattern, with the orange, green and blue memberships posterior probabilities of 0.60, 0.27 and 0.13, respectively, revealing its crossbred origin. Dong Tao was divided into two subgroups (green and red groups), with variable levels of admixture with the other two breeds.

To further resolve the putative difference between Mia and Mong, a separate analysis involving only these two breeds was performed using $K$ values ranging from 1 to 4. The value of $K$ maximizing the marginal likelihood was 1, confirming that these two breeds have originated from the same ancestral population.

Figure 2b, top presents a two-dimensional graph of the first two principal components (PC1 and PC2) of the PCA of all samples. PC1 and PC2 explained 3.99% and 2.79% of the variation, respectively, and they separated the four breeds into three groups. Except for the single Ho bird
showing an admixed pattern, all Ho and Dong Tao chickens were clearly distinct, whereas Mia and Mong chickens remained admixed. These results were consistent with the findings of $F_{ST}$ and fastSTRUCTURE analyses. Interestingly, for Dong Tao chickens, individuals with PC2 values exceeding 300 corresponded to the red group in the fastSTRUCTURE graph. Moreover, the Ho breed was divided into two subgroups by PC2.

A second PCA was performed only on Mia and Mong (Figure 2b, down). With the exception of one bird that was very close to Mia, all Mong chickens formed a separate and moderately homogeneous group. In contrast, Mia chickens exhibited a scattered distribution. Specifically, five birds with PC1 value below −100 were distant from the other birds and formed the pure orange group in the fastSTRUCTURE graph (orange membership > 0.90).

In the neighbour-joining tree (Figure 2c), three branches were formed from a common ancestor. One of these branches was further divided into two groups: Mia (including one Mong bird) and Mong. Another branch corresponded to the Ho breed, which was further divided into subgroups. Finally, the last branch corresponded to the Dong Tao breed, which was further divided into subgroups. Once again, the “ambiguous” single Ho bird identified in the previous analyses was detected to be closer to the Dong Tao breed in this analysis. Of note, the substructuring of the breeds into subgroups cannot be associated with the other identified variables in the present study; in particular, these differences do not match the possible origins of the samples (i.e., private flocks, NIAH or VNUA).

### 3.2 Genetic diversity

The proportion of polymorphic SNPs (Table 2) showed that the genomes of Dong Tao and Ho harboured significantly less informative SNPs than those of the remaining two breeds. The observed heterozygosity was the lowest for Mong chickens but the highest for Ho chickens, with a small but significant difference ($p < 0.0001$).

#### 3.2.1 LD

As expected, LD decreased with increasing distances between SNPs in all breeds (Figure 3a). The highest $r^2$ was recorded in Ho chickens, whereas the fastest LD decay was detected in Mia chickens. Similar patterns were observed for the three chicken chromosome types (Figure 3a). The mean $r^2$ between SNP pairs from different chromosomes was 0.049, 0.057, 0.061 and 0.065 for Dong Tao, Mong, Mia and Ho chickens, respectively (Figure 3b). In macrochromosomes, distances of 1.35, 2.25, 20 and 35 Mb led to the mean $r^2$ of unlinked markers in Mia, Mong, Dong Tao and Ho chickens, respectively.

The simulated average $r^2$ values using random genotypes were 0.031, 0.039 and 0.055 for Dong Tao, Ho and Mia (or Mong) chickens, respectively. The observed $r^2$ values were significantly ($p < 0.0001$) higher than the simulated ones, thereby revealing a non-random association amongst the loci in our samples.

#### 3.2.2 Inbreeding coefficient

**Global inbreeding**

One-way ANOVA revealed significant differences in global inbreeding coefficients amongst the four breeds ($R^2_{adj} = 0.30, p < 0.001$; Table 2). Pairwise comparisons revealed that Mia chickens achieved significantly lower values than chickens of the remaining three breeds ($p < 0.001$). Mong chickens achieved significantly higher values than Dong Tao ($p = 0.036$) and Ho chickens ($p = 0.036$). Moreover, differences in the variances were obvious. Specifically, Ho and Mong chickens showed significantly higher variances than Mia and Dong Tao chickens.

In total, 88,400 HBD segments (or ROH) were detected (Table 2). The variations amongst samples in terms of the number and length of ROH are presented in Figure 4a. Birds from the same breed were clustered on the plot. A gradient of the number of ROH could be visualized on this graph. Mia chickens tended to have the highest number of ROH, followed by Mong, Dong Tao and Ho chickens. In addition, the total ROH length was similar amongst Mong, Dong Tao and Ho chickens but lower in Mia chickens. In other words, Mia chickens showed relatively more but smaller ROHs. Of note, chickens on the right side of the plot showed longer ROH, representing the most inbred individuals. Furthermore, majority of the inbred individuals originated from breeds with a reduced effective population size (i.e., Mong, Ho and Dong Tao).
The ZooRoH method provides information on the history of consanguinity. In the present study, a high contribution of ancient inbreeding was evident. Indeed, the highest percentage of HBD was observed in the most ancient class of $F_{\text{zooRoH}}$ in all breeds (Figure 4b). Overall, 25%–35% of the total inbreeding corresponded to the oldest class (i.e., approximately 2048 generations ago). In the Mia

**TABLE 2** Results from the diversity and inbreeding analyses within the four breeds

<table>
<thead>
<tr>
<th>Breed</th>
<th>n</th>
<th>Ho</th>
<th>He</th>
<th>$F_{\text{zooROH}}$</th>
<th>Mean #ROH</th>
<th>Min #ROH</th>
<th>Max #ROH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dong Tao</td>
<td>32</td>
<td>0.38</td>
<td>0.36</td>
<td>0.17 ± 0.004</td>
<td>893.5 ± 63.13</td>
<td>787</td>
<td>1037</td>
</tr>
<tr>
<td>Ho</td>
<td>26</td>
<td>0.39</td>
<td>0.36</td>
<td>0.17 ± 0.007</td>
<td>769.6 ± 111.33</td>
<td>505</td>
<td>1089</td>
</tr>
<tr>
<td>Mia</td>
<td>18</td>
<td>0.37</td>
<td>0.36</td>
<td>0.13 ± 0.004</td>
<td>1181 ± 78.60</td>
<td>960</td>
<td>1260</td>
</tr>
<tr>
<td>Mong</td>
<td>18</td>
<td>0.35</td>
<td>0.35</td>
<td>0.19 ± 0.010</td>
<td>1030.1 ± 58.85</td>
<td>944</td>
<td>1125</td>
</tr>
</tbody>
</table>

Note: $n =$ number of birds; Ho = observed heterozygosity; He = expected heterozygosity; $F_{\text{zooROH}} =$ average (±SEM) inbreeding coefficient computed using zooROH; Mean (Min, Max) # of ROH = average (minimum, maximum) number of ROH per individual.

**FIGURE 3** Results of the linkage disequilibrium analyses. (a, top) decay of the average pairwise linkage disequilibrium ($r^2$) as a function of the distances between SNPs, the breeds and the chromosome types. (b, down) distribution of the $r^2$ values between random SNPs located on distinct chromosomes for the four breeds (DT = Dong Tao, HO = HO, MA = Mia, MN = Mong). White dot indicates the median. White cross indicates the mean. The thick black bar in the centre indicates the interquartile range, and the thin black line extended from it represents the 95% confidence intervals. The shape of data distribution is shown with the kernel density estimation placed on each side.

**Age-based partitioning of inbreeding**

The ZooRoH method provides information on the history of consanguinity. In the present study, a high contribution of ancient inbreeding was evident. Indeed, the highest percentage of HBD was observed in the most ancient class of $F_{\text{zooRoH}}$ in all breeds (Figure 4b). Overall, 25%–35% of the total inbreeding corresponded to the oldest class (i.e., approximately 2048 generations ago). In the Mia
FIGURE 4 Results of the inbreeding analyses. (a, top) number of ROH segments as a function of the total ROH size for each individual. (b, bottom left) contributions of the distances to the common ancestors to the individual inbreeding (up) and relative contributions of these distances to the average inbreeding in each breed (down). (c, bottom right) estimation of the evolution of the effective population sizes over the last 500 generations (up) and zoom on the last 50 generations (down)
breed, this proportion reached 68% of the total inbreed-
ing (Figure 3b), indicating that, as shown previously, Mia chickens carried numerous smaller ROH, which explained its higher number of ROH but lower global inbreeding coefficient.

Very recent inbreeding, corresponding to the first two classes, was close to zero in all breeds. Ho chickens showed the highest contributions from recent classes, with 6% of the total inbreeding originating from the eight generations’ class and 14% from the 16 generations’ class. In contrast, these two classes represented only 4% of the total inbreeding in the other breeds. In Dong Tao and Mong, an intermediate pattern was observed, with high contributions from the 32 and 64 generations classes.

Furthermore, inbreeding originating from the 256 generations class contributed importantly to the global inbreeding in all breeds, whereas no inbreeding originated from the 1024 generations class in Mia and Mong.

3.2.3 Effective population size

The estimates of Ne over past generations were obtained from the LD data (Figure 4c). In the remote past, Mia chickens had a higher Ne than chickens of the remaining three breeds. However, a decreasing trend was apparent over the last generations in all breeds. Modelling the Ne decline over the last 500 generations using linear regression analysis revealed the highest slope of the regression line for Mia (β = 2.79) but the lowest slope for Ho (β = 1.426). In other words, although Mia showed the highest Ne over the last 500 generations, the decline in Ne was more obvious in this breed.

For all breeds, higher slopes of regression lines were noted when modelling the decline over the last 50 generations than over the last 500 generations (β = 5.43, 3.98, 3.61 and 2.25 for Mia, Mong, Dong Tao and Ho chickens, respectively), suggesting an accelerated decline in Ne over the last 50 years.

4 DISCUSSION

The first aim of the present study was to investigate the utility of HD SNP genotyping to explore the potential genetic differentiation of four Vietnamese local chicken breeds originating from geographically close areas. All analyses revealed three clear clusters, with one unique cluster each for Ho and Dong Tao chickens and an admixed cluster comprising Mia and Mong chickens; therefore, Mia and Mong shared a more recent common ancestor than the remaining two breeds.

The distinction between Ho and Dong Tao chickens was in concordance with previous reports (Cuc et al., 2010; Pham et al., 2013). Moreover, clustering between Mia and other Vietnamese breeds has been reported in previous studies, although the breeds forming these clusters are not consistent across studies. The clear genetic differentiation between Ho and Dong Tao could be explained by the selection and breeding for very specific morphological traits as well as the long period of reproductive isolation, with the corresponding genetic drifts to conserve these unique traits. These processes likely drove the co-existence of these breeds as small conservation flocks (Zanetti, De Marchi, Dalvit, & Cassandro, 2010).

Despite the clear distinction between Ho and Dong Tao, all analyses of population structure showed at least two subgroups in both breeds, consistent with previous reports (Pham et al., 2013). As mentioned earlier, although the sampled flocks were reared either on private farms in a district or on the NIAH/VNUA farms, the subgroups did not match these distinct origins. These results demonstrated the importance of genomic information for creating the nuclei of “purebred” animals and controlling mating to prevent a possible drift within the populations, potentially leading to genetic separation within these breeds. Moreover, at the individual level, all results supported the identification of a crossbred bird in the Ho sample. This finding also showed how genomic information can assist in controlling crossbreeding.

Regarding genetic diversity, the lowest allelic diversity was observed in Ho chickens but the highest in Mia chickens. For each breed, the extent of observed heterozygosity was less than that reported previously (Berthouly et al., 2009; Pham et al., 2013). This observation reflects the possible loss of genetic diversity or, more likely, the differences in measures based on microsatellites (in previous studies) and SNPs (in the present study), since microsatellites are generally much more polymorphic than SNPs. Therefore, for a more comprehensive comparison with the findings of microsatellite-based studies, SNP haplotypes, rather than individual SNP genotypes, must be established. However, our results are comparable to the reports on chickens in other countries. For instance, the observed heterozygosity was approximately 0.22 in conserved Chinese chicken populations (Zhang et al., 2018) and 0.21–0.34 across six Italian chicken populations (Strillacci et al., 2017).

The effects of breeding strategies (purebred or cross-bred lines and conservation nuclei) on the rate of LD are well-documented (Fu, Dekkers, Lee, & Abasht, 2015; Khanyile, Dzomba, & Muchadeyi, 2015; Seo et al., 2014). In the present study, the four breeds were part of a conservation programme. For all breeds, the long-distance and interchromosomal $r^2$ values tended to exceed the values expected by chance, indicating a non-random association
of alleles resulting from population genetic forces, such as drift. Moreover, populations with smaller effective sizes exhibited greater LD. In particular, the magnitude of LD was greater and extended over longer distances in Ho chickens than in the remaining three breeds, indicating a different breeding history of the Ho breed and/or its smaller initial nucleus size. In terms of breed conservation, this observation and the associated rapid decline of Ne underline the need for efficient genetic management in the conservation programme.

Our inbreeding analysis offers a different perspective. At the global level, Mia chickens achieved the lowest inbreeding coefficient but the highest number of ROH. As previously reported in cattle (Solè et al., 2017), global inbreeding was mostly associated with very ancient inbreeding in all breeds, particularly Mia, in which 68% of the total inbreeding was attributed to the most ancient classes. In contrast, Ho chickens showed the highest contributions from recent classes (i.e., inbreeding due to crosses from the last eight to 16 generations); this recent inbreeding explained the specific LD pattern observed in this breed.

The global inbreeding coefficient of Dong Tao chickens was similar to that of Ho chickens, although there was less between-individual variability. Moreover, the total length of ROH was similar between Ho and Mong chickens, albeit with a moderate number of ROH. These characteristics are typical of small populations (Ceballos, Joshi, Clark, Ramsay, & Wilson, 2018).

Finally, the utility of ZooRoH has previously been tested in humans (Tenesa et al., 2007), dogs, sheep and cattle (Peripolli et al., 2017). The present study confirmed that this method is also useful for studying inbreeding in chickens.

5 CONCLUSIONS

Despite being geographically close, the Dong Tao and Ho breeds are clearly different at the genomic scale. Moreover, the two breeds differ from Mong and Mia, which share a more recent common ancestor. This similarity between Mong and Mia could lead to prioritize Mong individuals in the conservation programme rather than Mia, if necessary. This would allow the conservation of an endangered breed whilst at the same time conserving most of the genomic content of the other breed. The extent of global genomic inbreeding measured using ZooRoH revealed high homozygosity amongst the four breeds, with inbreeding coefficients of 10%–20%. The occurrence of frequent inbreeding and the presence of subgroups within some breeds underline the need for the creation of future nuclei for use in the conservation of these breeds. Considering the specific phenotypic and cultural characteristics of the tested local breeds, our genomic information underscores the need for efforts to maintain genetic variability and continuously monitor mating strategies for avoiding the loss of biodiversity and preventing further genetic separation within these breeds.

AUTHORS’ CONTRIBUTIONS

FF and VDT conceived the experiments. NVD, NHT, DDL and MN selected the genotyped samples and performed genotyping experiments. DA and FF helped in data analysis. FF contributed greatly to the writing of the manuscript. ME analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study have been deposited in the Dryad repository (https://doi.org/10.5061/dryad.d208kps07). The codes for statistical analyses are available from the corresponding author upon request.

ORCID

Farnir Frédéric https://orcid.org/0000-0003-3430-9930

REFERENCES


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