

Analysis of Genetic Variability of Indigenous Chickens in the Cuvette-Ouest Department, Republic of Congo

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Abstract

Original Research Article

This study investigated the genetic variability of indigenous chickens from Congo using 14 microsatellite markers, of which 12 were found to be functional. The high amplification rates confirmed the quality of genetic typing and the reliability of the protocol used. Allelic and polymorphism analyses revealed strong heterogeneity among loci, with some highly informative markers showing substantial genetic richness, particularly in Northern populations. Principal Component Analysis (PCA) and phylogenetic tree analysis revealed a distinct genetic structure: the ALEME population represents a homogeneous genetic isolate, whereas EWO, OBILI, and OKA form an interconnected group characterized by significant gene flow. These results confirm the coexistence of genetic isolates and mixed genetic pools within village poultry systems and highlight the need to adopt differentiated conservation strategies to ensure the sustainable management of these local genetic resources.

Keywords: Genetics; indigenous chickens; department; Republic of Congo.

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INTRODUCTION

Local avian genetic resources occupy a crucial place in rural farming systems throughout sub-Saharan Africa. Indigenous chickens, in particular, play a major socio-economic role by ensuring household food security, generating additional income, and preserving a genetic heritage shaped by generations of natural selection. Their hardiness, adaptability to extensive production systems, and relative resistance to diseases make them a genetic reservoir of strategic importance for breeding and conservation programs (FAO, 2015; Msoffe *et al.*, 2005).

In the Republic of Congo, family poultry farming relies heavily on the rearing of local chickens. In the Cuvette-Ouest Department, this activity represents an important source of livelihood for rural populations while contributing to household economic resilience. However, despite their importance, Congolese indigenous chickens remain poorly documented in terms of their genetic diversity. This gap contrasts with studies conducted in other African regions, where a high level of genetic heterogeneity has been reported, associated with

farming practices, agroecological conditions, and intercommunity exchanges (Gueye, 2009; Lyimo *et al.*, 2014).

Knowledge of the genetic variability of indigenous chickens is essential for guiding sustainable management strategies, developing appropriate breeding programs, and preventing the erosion of local genetic resources. In a context marked by climate change and the intensification of production systems, the promotion and valorization of these populations appear to be major challenges for strengthening the sustainability of Congolese poultry production.

Genetic analyses based on molecular markers, particularly microsatellites, constitute a reliable and widely used tool for assessing both intra- and inter-population diversity. They make it possible to evaluate levels of polymorphism, detect genetic structuring, and identify potential signatures of differentiation among populations (FAO, 2011; Mpenda *et al.*, 2019).

In this context, the present study aims to analyze the genetic variability of indigenous chickens in the

Cuvette-Ouest Department of the Republic of Congo using microsatellite markers. It seeks to contribute to a better understanding of local poultry diversity and to provide a scientific basis for the conservation and sustainable valorization of national avian genetic resources.

MATERIALS AND METHODS

Study Area Description

The present study was conducted in the Cuvette-Ouest Department, located in the northwestern part of the Republic of Congo, specifically in the town of Ewo. The study was carried out between March 2023 and January 2024 and focused on the analysis of the genetic

variability of indigenous chickens in the Cuvette-Ouest Department of the Republic of Congo.

The Cuvette-Ouest Department is situated in the northwestern region of the Republic of Congo and straddles the Equator, with approximately two-tenths of its territory lying in the Northern Hemisphere. It is bordered by the Sangha Department to the north, Gabon to the west, the Cuvette Department to the east, and the Plateaux Department to the south (Figure 1).

The department covers an area of approximately 26,000 km², representing about 7% of the country's total land area, and has a population of 73,011 inhabitants.

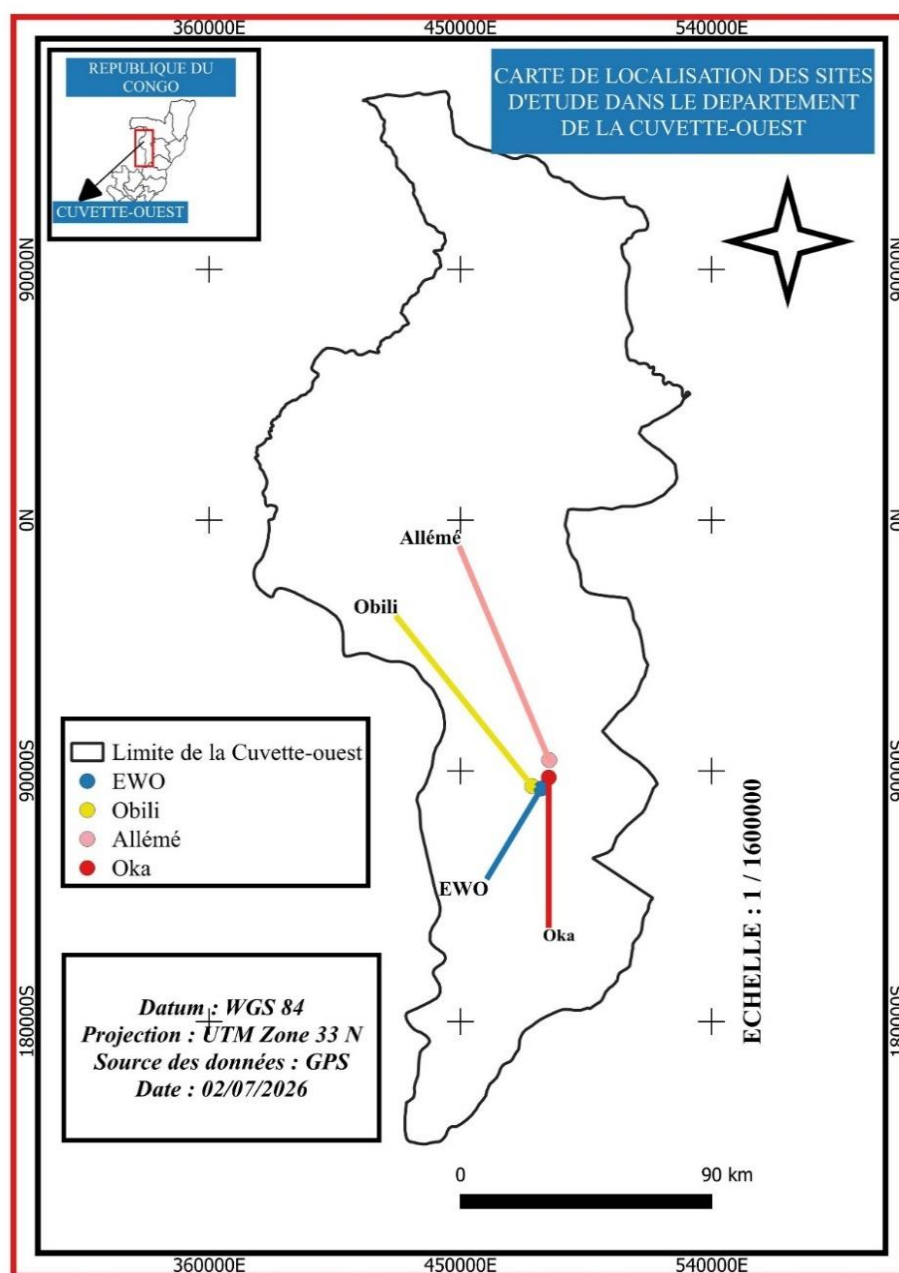


Figure 1. Presentation of the study area

Materials

Biological Material

The biological material consisted of local chickens (*Gallus gallus*), as well as blood and DNA samples collected from these birds. This species was selected because of its important contribution to poverty alleviation in rural areas. However, the different breeds or strains that may exist within these local chicken populations have not yet been inventoried or characterized. Therefore, this species represents an appropriate model for a molecular characterization study.

Methods

Blood Sampling and Sample Processing

Blood samples were collected from 25 indigenous chickens originating from the Cuvette-Ouest Department in the Republic of Congo.

A volume of 5 mL of blood was collected by puncture of the jugular vein into serum tubes containing EDTA as an anticoagulant (Figure 2).



Figure 2: Blood collection from the jugular vein

After gentle inversion of the contents (blood and anticoagulant) without vigorous shaking, the tubes were placed in tube racks and transported to the Laboratory of the Faculty of Health Sciences at Marien Ngouabi University.

At the laboratory, the blood samples were centrifuged at 3,000 rpm for 10 minutes using a ROTOFIX 32 A centrifuge (Figure 3). The supernatant

(plasma) was transferred into cryotubes using a 1000 μ L micropipette and stored initially at -4°C in a refrigerator and subsequently at -20°C in a freezer.

The samples were then shipped in a hermetically sealed cooler to the Laboratory of the Institute for Health Research, Epidemiological Surveillance and Training (IRSSEF) in Dakar, Senegal, for molecular analyses (Figure 4).



Figure 3: Centrifuge used for blood centrifugation



Figure 4: Transfer of plasma into the cryotube (left); Sample packaging for shipment (right)

Genomic DNA Extraction

Total genomic DNA was extracted from blood samples collected from Congolese indigenous chickens during the field survey using the QIAamp DNA Mini Kit (Qiagen®). The use of this kit enables efficient DNA extraction even from small volumes of blood.

Quality Control of Extracted DNA

The quality of the extracted DNA was assessed using a Qubit Flex fluorometer and TapeStation analysis, providing both quantitative and qualitative evaluation of DNA samples.

Selection of Markers and Primer Preparation

A panel of 14 microsatellite markers was selected for this study. All markers belonged to the set of

microsatellites recommended by the FAO for the assessment of genetic diversity and population structure in chicken breeds worldwide (FAO, 2011). The primers received were resuspended in ultrapure water to obtain a final concentration of 250 pmol/μL.

Microsatellite Genotyping Using Sequencing Technology

This step initially involved the amplification of microsatellite loci using the Polymerase Chain Reaction (PCR) technique, generating DNA fragments of variable lengths corresponding to different alleles.

Preparation of the PCR Reaction Mixture

A PCR master mix was prepared according to the composition presented in Table 1.

Table 1. Components used for the preparation of the PCR reaction mixture.

Reagents	Volume per reaction (μL)
10X Buffer	1.6
dNTPs (2.5 mM)	2.6
Forward primer (10 μM)	0.2
Reverse primer (10 μM)	0.3
H ₂ O	7.1
Taq polymerase enzyme (5 U)	0.1
MgCl ₂ (25 mM)	1.8
DNA template	2

Amplification

After optimization of the protocol, the analysis was performed using 96-well plates divided into four sections, with each section corresponding to a specific microsatellite marker. A total of four 96-well plates were used for the study. The layouts of the four plates were designed using Microsoft Excel. Each plate contained 23 DNA samples and one negative control consisting of nuclease-free water.

For each well, 2 μL of genomic DNA (corresponding to approximately 20 ng) and 13 μL of the PCR master mix were added. The labeled plates were subsequently loaded into a ProFlex thermal cycler programmed according to the PCR amplification conditions described in (Table 2).

Table 2: PCR cycling conditions used for microsatellite amplification

Cycles	Steps	Température (°C)	Time
1	Initial dénaturation	94	15 min
	Dénaturation	94	30 s
	Annealing	60	45 s
	Extension	72	1 min
1	Final extension	72	10 min

The post-PCR product was subsequently stored at 4°C, and quantification was performed prior to sequencing on Oxford Nanopore.

Oxford Nanopore sequencing

In our study, we performed targeted multiplex sequencing to specifically target regions of interest containing microsatellite markers. We adapted the approach from the Midnight Kit protocol, originally developed for whole-genome sequencing of SARS-CoV-2 using Oxford Nanopore technology (Figures 5 and 6).

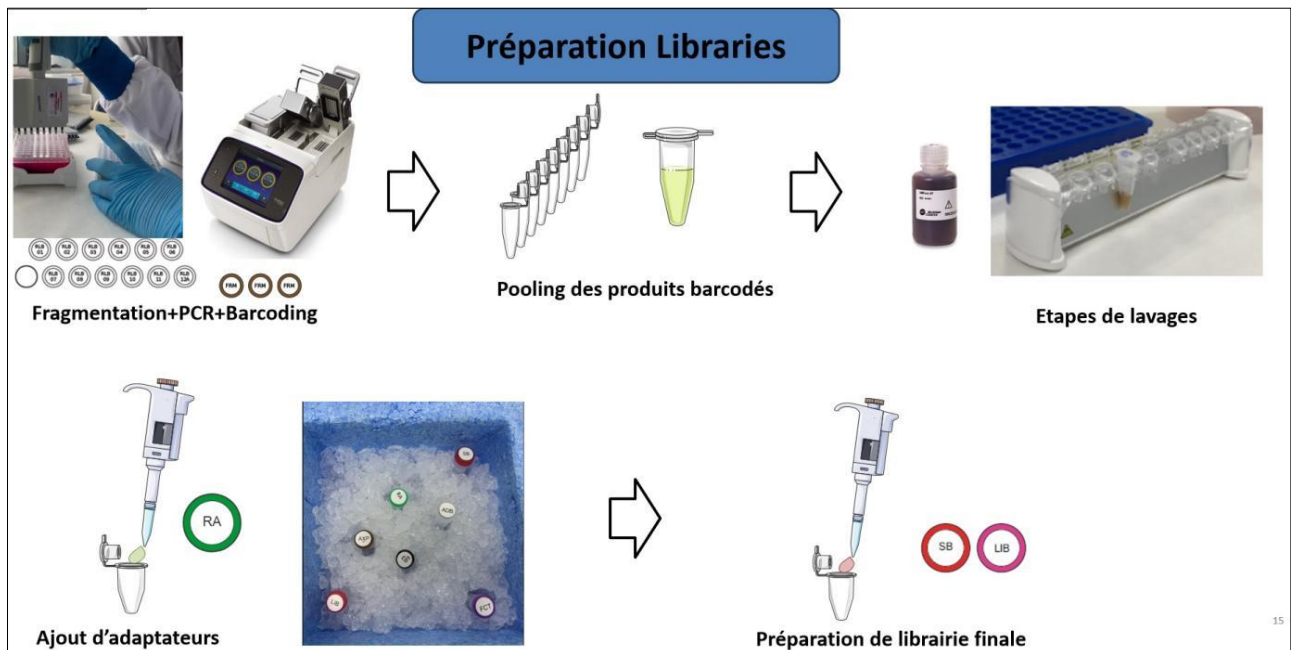

Figure 5: Library préparation

Figure 6: Oxford Nanopore sequencing procedure

Bioinformatic Analysis of Sequencing Data

1. Quality Control of Sequencing Reads

Before any downstream analysis, it is essential to assess the quality of sequencing data. The pycoQC tool was used to evaluate and visualize the quality of reads generated by Nanopore sequencing through an interactive report providing detailed information on read length distribution and quality scores.

2. Alignment of Sequencing Reads to the Reference Genome

Raw reads were aligned to the *Gallus gallus* reference genome using Minimap2, a fast and efficient tool specifically designed for long-read sequencing data. The alignment step enabled the accurate positioning of sequences on the reference genome and prepared the data for subsequent variant identification. Following alignment, the resulting files were converted into BAM format, sorted, and indexed to facilitate their processing during subsequent analyses.

3. Variant Calling and Filtering

The identification of single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) was performed using bcftools. After generating the VCF file containing the detected variants, filtering was applied to retain only variants meeting quality criteria. Only variants with a quality score above a predefined threshold (QUAL > 30 and DP > 10) were retained to improve the reliability and robustness of downstream analyses.

4. Analysis and Visualization

The obtained results were analyzed and visualized using the IGV (Integrative Genomics Viewer) tool for manual inspection of alignments and identified mutations. To investigate the genetic structure of the dataset, a Principal Component Analysis (PCA) was performed using R software version 4.4.0. This analysis allowed the visualization of individual distribution based on genetic similarities and the identification of potential clusters among samples.

The VCF files from all samples were combined to generate a common genotyping matrix. The merged data were subsequently converted into a format suitable for analysis in R, and dimensionality reduction was applied to extract the main axes of genetic variation. The results were represented as graphical plots to facilitate the interpretation of relationships among samples and to highlight potential subpopulations.

RESULTS AND DISCUSSION

Results

Identification of Microsatellites in Chickens from Northern Congo

This graph displays the relationship between individual samples (rows, e.g., EW_1 for the northern population) and the genetic markers (microsatellites) used (columns, e.g., ADL0268, MCW0034).

The color indicates a measurement value (usually the detection intensity or genotyping success rate).

1. Structure and Scale of the Graphs

Axes:

- Rows (Y-axis): The different sampled chickens.
- Columns (X-axis): The 13 microsatellite loci (genetic markers) used for genotyping. Color

Code: The scale on the right ranges from white/very light color (value close to 0.0) to dark blue (value close to 1.0). In this identification context, dark blue generally indicates successful genotyping (the allele was detected and correctly identified), whereas white indicates genotyping failure or missing data.

2. Observation and Interpretation

The heatmap shows a remarkable genotyping success pattern across the tested markers.

Overall Genotyping Success

- The vast majority of cells are dark blue (values close to 1.0) in the graph.
- This indicates a very high genotyping success rate for the entire study. The selected markers were robust, and the quality of DNA samples as well as the experimental protocols were satisfactory.

Specific Genotyping Failures (Missing Data)

Two specific columns appear clearly white (missing data or genotyping failure) for all samples: the MCW0222 locus and the MCW0295 locus.

3. Interpretation and Implications

Failure of the MCW0222 and MCW0295 Loci

The fact that all individuals failed genotyping for the MCW0222 and MCW0295 markers strongly suggests that:

- Primer design issues: The PCR primers specific to these two loci may not have performed efficiently with the DNA from the studied chickens, possibly due to mutations occurring in the primer-binding regions, resulting in amplification failure.
- Protocol standardization: Regardless of the technical cause, these two markers were excluded or could not be used in the genetic analyses (PCA and phylogenetic tree construction) presented in the previous figures because they did not provide any informative data for the studied population.

Impact on Genetic Analysis

- The genetic structure analysis was therefore performed using 12 microsatellite loci (14 loci initially selected minus the two failed markers), rather than the 14 markers originally planned.

Figure 7 confirms the excellent quality of the data obtained for 12 out of the 14 tested microsatellite

markers. However, it also clearly identifies two markers (MCW0222 and MCW0295) that systematically failed

across all samples from Northern Congo and therefore did not contribute to the genetic structure analysis.

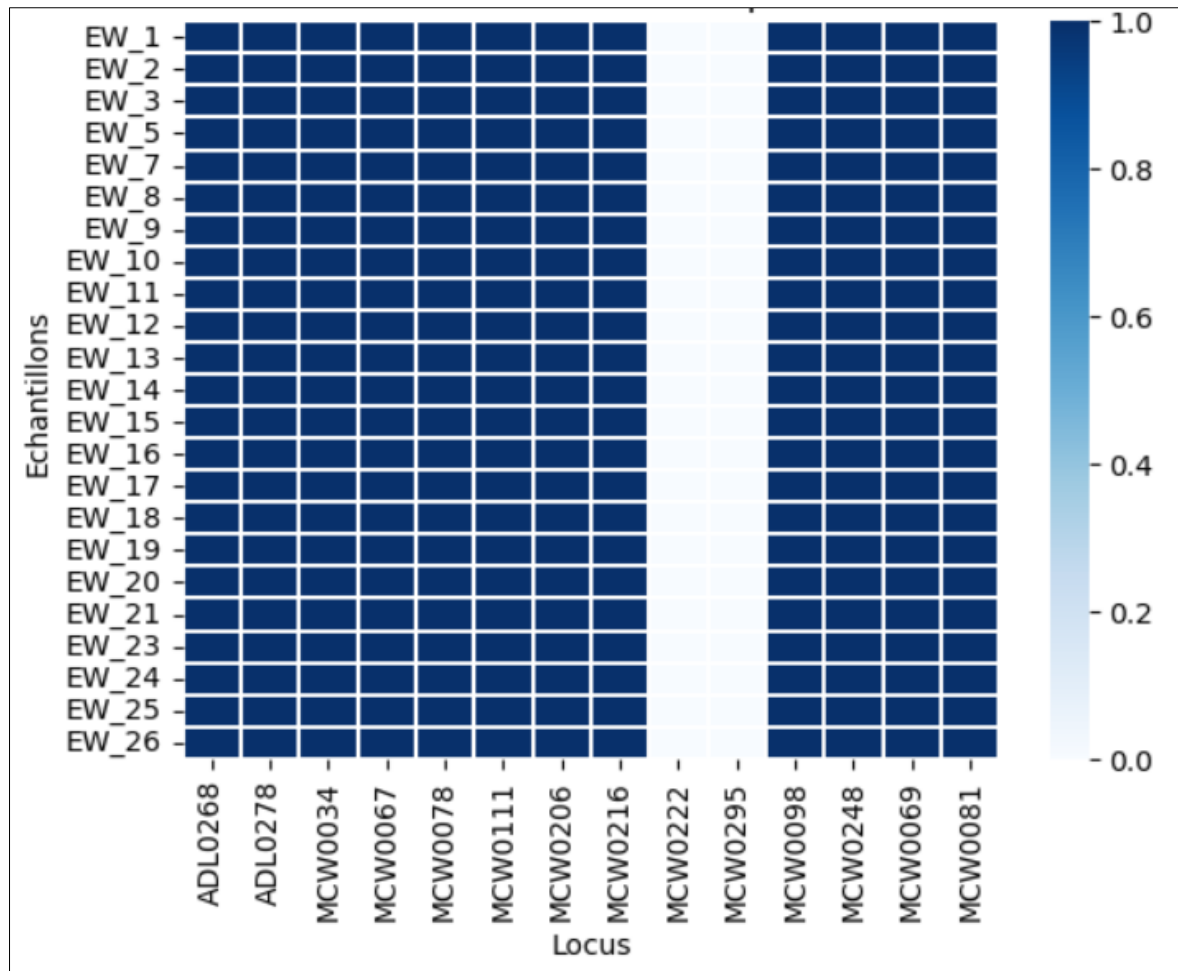


Figure 7: Identification of Microsatellites

Allelic Diversity of Microsatellites

Figure 8 presents the allelic richness of each genetic marker in chickens from Northern Congo (ALEME, EWO, OBILI, OKA).

1. Observation of Informative Loci

As shown in Figure 8, genetic diversity varies considerably among loci:

Highly Polymorphic Loci (Highly Diverse)

- The MCW0206 locus showed the highest diversity, with 11 different alleles.
- The MCW0034 and MCW0067 loci followed, each displaying 8 alleles.
- The MCW0098 locus presented 7 alleles.

- The MCW0248 and MCW0216 loci each exhibited 4 alleles.

Low Polymorphism Loci

- Several loci (ADL0268, MCW0111, MCW0081, MCW0078, and MCW0069) displayed only 1 or 2 alleles, making them less informative for assessing genetic diversity within this population.

Confirmation of Failed Loci: The MCW0222 and MCW0295 loci showed an allele number equal to zero, confirming that these markers were not successfully genotyped.

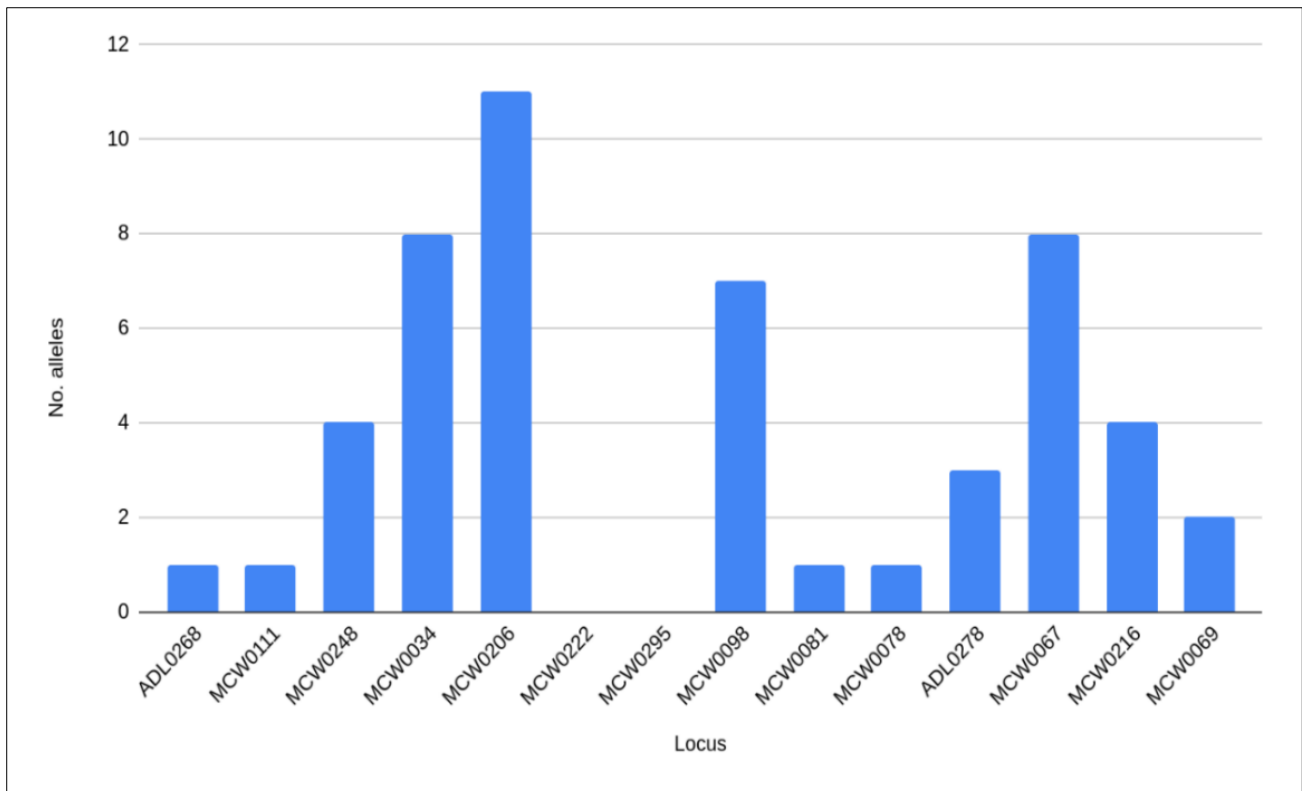


Figure 8: Identification of the number of alleles per locus

Genetic Structure

The two graphs (Figure 9) represent the results of a Principal Component Analysis (PCA), a statistical method used to visualize genetic diversity and relationships among individuals or populations.

Axes and Variance

- The horizontal axis (PC1) explains 34.49% of the total variance in the genetic data.
- The vertical axis (PC2) explains 28.2% of the total variance.
- Together, these two principal components account for more than 62% of the total genetic variation, which represents a highly significant proportion of the observed diversity.

Individual-Based Plot (Left)

- Each point represents an individual chicken.
- Individuals from the same region (represented by colors) tend to cluster together. For Example:
 - The red points (ALEME) are mainly distributed in the upper-left quadrant.
 - The light blue points (OKA) are more dispersed and overlap with the other groups.

Regional Plot with Ellipses (Center)

- This graph provides the most informative representation of population structure. The ellipses represent the confidence regions (or dispersion areas) of individuals from each region.

Clear Population Structure (Isolation)

- The ALEME region (red) shows a very narrow and distinct ellipse, indicating that chickens from this region are genetically highly similar to each other (low within-population diversity) and clearly separated from the other populations.

Overlap (Gene Flow)

- The ellipses representing EWO (dark blue), OBILI (blue), and OKA (green) populations show substantial overlap.
- This suggests higher genetic diversity within these populations and, importantly, greater gene flow (or interbreeding) among them, making genetic differentiation between these three regions difficult to distinguish.

2. Phylogenetic Tree (Right)

The phylogenetic tree illustrates the evolutionary relationships (genetic relatedness) among the different strains or individuals analyzed. The colors correspond to the regions identified in the PCA (red = ALEME, dark blue = EWO, etc.).

Evolutionary Distance

- The horizontal scale represents the genetic distance (branch length). A short distance (short branches) indicates a high degree of genetic similarity. Clades (Groups)
- The tree shows that the analyzed strains are grouped into eight clades (Clade 1 to Clade 8).

Western Region Clades (ALEME/EWO)

- Strains from the ALEME (red) and EWO (dark blue) regions dominate the upper clades (Clades 1 to 5).
- For example, Clade 1 is almost exclusively composed of ALEME individuals. This finding confirms the strong genetic isolation of the ALEME population observed in the PCA.

Mixed Clades

- Some clades show a mixture of colors (e.g., Clades 6, 7, and 8), which corresponds to the genetic overlap observed among the EWO, OBILI, and OKA regions in the PCA.

Overall, the combined analyses (PCA and phylogenetic tree) clearly demonstrate the existence of significant genetic structuring among chickens from Northern Congo:

1. The chicken population from the ALEME region is genetically the most distinct and isolated, showing low internal diversity and a high level of divergence compared with the other populations.
2. The populations from the EWO, OBILI, and OKA regions are less differentiated and share a significant proportion of their genetic diversity, suggesting gene exchange or a recent common evolutionary history.

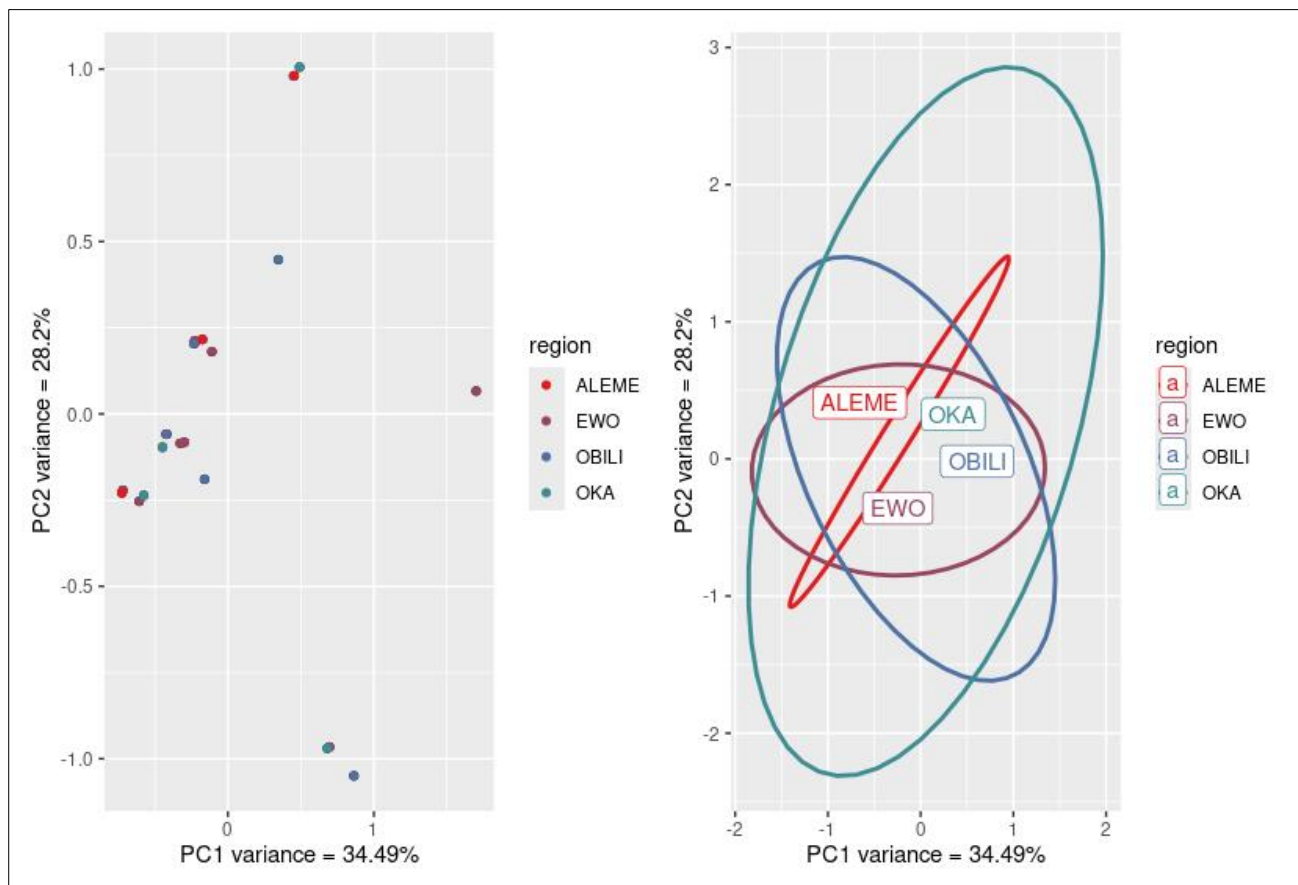


Figure 9: Principal Component Analysis (PCA) of chickens from Northern Congo

Phylogeny of Chickens from Northern Congo

This phylogenetic tree (Figure 10) confirms and further refines the observation of the complex genetic structure within the northern region, as previously suggested by the initial PCA analysis.

1. Tree Structure

Genetic Distance (X-axis)

- The genetic distance is relatively limited (maximum value of 0.1); however, the tree displays clear branching patterns, indicating genetic divergence within the studied group.

Clades and Subgroups

- The tree is divided into several distinct sub-clades, confirming that the northern chicken population is not genetically homogeneous.

2. Confirmation of ALEME Genetic Isolation

The tree highlights the presence of a particularly divergent lineage:

Upper Clade (Red/ALEME)

- The upper branch of the tree is largely dominated by red-colored individuals (EW_12, EW_11, EW_13, EW_14, EW_26).

Interpretation:

These individuals (likely belonging to the ALEME population, as suggested by the previous PCA) form a monophyletic clade (a group derived from a common exclusive ancestor) or are very close to forming one. This represents the clearest phylogenetic evidence that the ALEME population is genetically isolated: individuals from this population are more closely related to each other than to the other chickens from Northern Congo.

3. Genetic Mixing Among the Other Northern Regions

The Remaining Part of the Tree (Blue/Green)

- The rest of the tree (representing the majority of genetic diversity) consists of a mixture of the other colors (blue, turquoise, and green), corresponding to the EWO, OBILI, and OKA regions.

Interpretation:

Individuals from these three regions do not form region-specific clades; instead, they are intermixed throughout the tree. This confirms that gene flow is high and/or that the divergence history among EWO, OBILI, and OKA is very recent, as previously indicated by the overlapping ellipses observed in the PCA

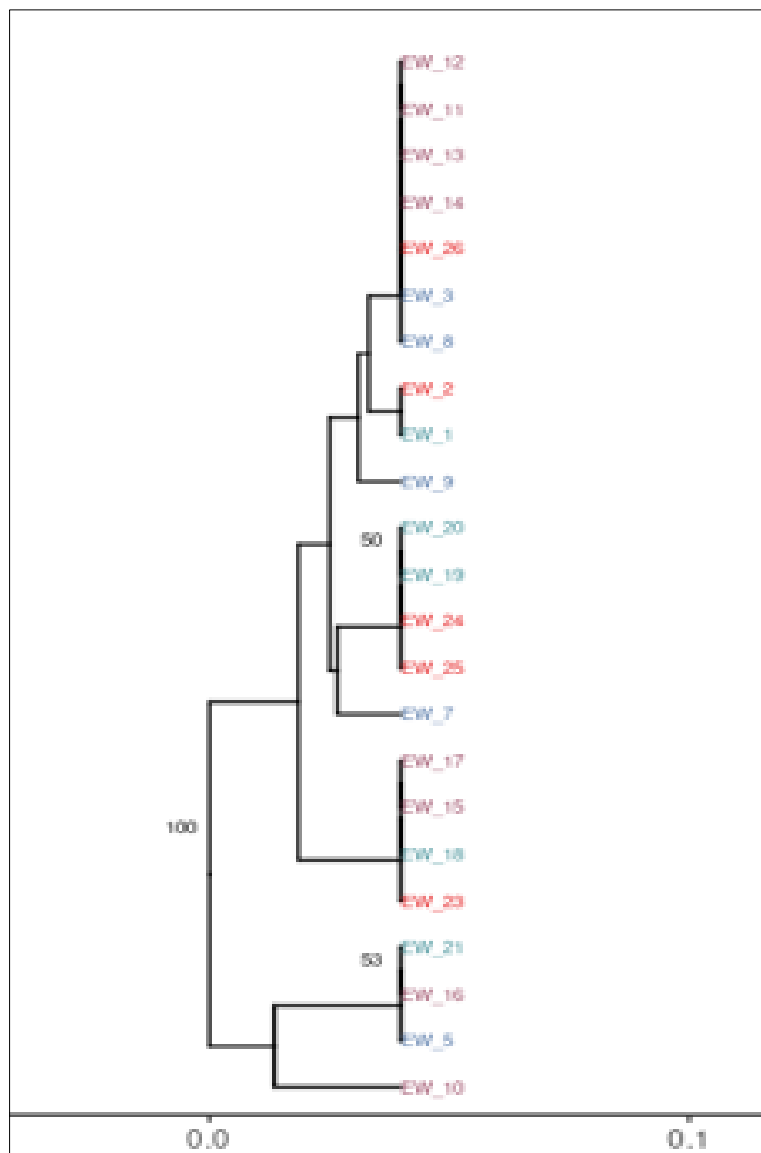


Figure 10: Phylogenetic tree of chicken populations from the Northern region

DISCUSSION

The microsatellite identification phase allowed the assessment of the quality of genetic typing and the robustness of the markers used in the study of chickens from Northern and Southern Congo. The obtained

heatmaps revealed a high similarity of genetic profiles between the two regions, reflecting methodological consistency and excellent quality of the collected genetic data. Indeed, the majority of loci showed a genotyping success rate close to 100%, as demonstrated by the high

values (close to 1.0) observed for most markers. This result confirms the good quality of the extracted DNA, the reliability of the primers used, and the standardization of the PCR amplification protocol (FAO, 2011; Weigend and Romanov, 2001).

However, two markers, MCW0222 and MCW0295, systematically failed to amplify in all samples, whether they originated from Northern or Southern Congo. This consistent observation across all populations suggests an inherent failure associated with these two loci. Several previous studies have reported similar failures for some microsatellite markers developed from commercial breeds or experimental lines when applied to African local chicken populations (Wimmers *et al.*, 2000; Granevitze *et al.*, 2007; Mtileni *et al.*, 2011).

These failures may be explained by several hypotheses:

- Mutations in PCR primer-binding sites, making amplification impossible or inefficient (Rosenberg *et al.*, 2002; Lyimo *et al.*, 2014).
- Structural genomic differences between Congolese local chicken breeds and the domestic lines used to design the primers for these microsatellites (Muchadeyi *et al.*, 2007).
- Specific experimental conditions, such as DNA concentration, annealing temperature, or reaction mixture composition, which may affect the amplification rate of certain loci (FAO, 2011).

Therefore, the lack of amplification of MCW0222 and MCW0295 does not compromise the overall validity of the results; however, it reduces the number of effectively usable markers from 14 to 12 functional loci. According to Weigend and Romanov (2001), a panel of 10 to 15 polymorphic microsatellite markers is sufficient for a reliable assessment of genetic diversity and population structure in chickens. Consequently, the use of the remaining 12 loci provides adequate statistical power for subsequent analyses (PCA, phylogenetic tree analysis, and diversity estimations).

From a methodological perspective, this observation highlights the importance of prior validation of markers in local populations before their large-scale application. As recommended by FAO (2011) and Leroy *et al.*, (2012), marker selection should be based on their ability to amplify reliably across different genetic backgrounds, particularly in non-commercial breeds exhibiting high genomic variability.

Finally, the overall genotyping success obtained with the 12 functional markers demonstrates the quality and rigor of the protocol employed. This technical performance represents an essential prerequisite for any genetic structure study, ensuring the reliability of subsequent analyses of chickens from Northern Congo.

The analysis of allelic diversity in chickens from Northern Congo (Alemé, Ewo, Obili, and Oka) revealed a high level of variability among the different loci studied. This heterogeneity among markers is a common finding in microsatellite-based studies, as the variability of each locus depends on its genomic position, local selection pressures, and the demographic history of the population (Weigend and Romanov, 2001; FAO, 2011).

The results indicate that some loci, particularly MCW0206, MCW0034, and MCW0067, exhibit high polymorphism (11, 8, and 8 alleles, respectively). These highly polymorphic loci represent reliable indicators of the genetic richness of the population. A high number of alleles per locus reflects substantial genetic variability, generally associated with a broad genetic base and low levels of inbreeding (Muchadeyi *et al.*, 2007; Mtileni *et al.*, 2011). The high polymorphism observed for the MCW0206 locus, also reported in other African local chicken populations (Mwacharo *et al.*, 2007; Khanyile *et al.*, 2015), suggests that this marker is highly informative and potentially associated with genomic regions involved in adaptive traits, such as pathogen resistance or climatic adaptation.

Conversely, other loci, such as ADL0268, MCW0111, MCW0081, MCW0078, and MCW0069, displayed low allelic diversity (1 to 2 alleles). These low-polymorphism loci may indicate more conserved genomic regions subjected to low mutation rates or stabilizing selection (Rosenberg *et al.*, 2002). The observed low diversity could also result from a founder effect or genetic drift, which is particularly plausible in isolated local populations or populations with small effective sizes (Leroy *et al.*, 2012; Fotsa *et al.*, 2010b).

Furthermore, the absence of detected alleles for the MCW0222 and MCW0295 loci suggests either genotyping failure or incompatibility of these markers with specific sequences present in chickens from this region. Similar cases have been reported by Wimmers *et al.*, (2000) and Granevitze *et al.*, (2007), who observed that some microsatellite markers developed for commercial breeds may show limited polymorphism or fail to amplify in African local breeds due to ancient genetic divergence.

The unequal distribution of allelic diversity among loci therefore reflects the complex genomic structure and evolutionary history of chickens from Northern Congo. As highlighted by FAO (2011) and Weigend and Romanov (2001), such allelic heterogeneity reflects both the adaptive potential of the population and the necessity of using a broad set of polymorphic markers to accurately assess true genetic diversity.

Thus, the high diversity observed at certain loci (particularly MCW0206, MCW0034, and MCW0067) represents an encouraging indicator of the maintenance of substantial genetic variability within this population. This diversity constitutes an essential resource for the conservation, management, and future improvement of local chicken populations, as it determines their ability to adapt to environmental pressures and socio-economic changes.

The Principal Component Analysis (PCA) and the phylogenetic tree obtained from microsatellite markers revealed a structured but heterogeneous genetic organization of chicken populations from Northern Congo. The first two principal components (PC1 = 34.49% and PC2 = 28.2%) together explained more than 62% of the total genetic variance, indicating a high discriminatory capacity of the markers used.

The PCA revealed a clear population structure according to geographical origin. Individuals from the ALEME region formed a homogeneous group clearly separated from the others, characterized by a very narrow ellipse. This pattern indicates low within-population diversity and marked genetic differentiation compared with the other regions (EWO, OBILI, and OKA). These observations are consistent with findings reported by Muchadeyi *et al.*, (2007) and Lyimo *et al.*, (2014), who emphasized that isolated village populations in sub-Saharan Africa, often characterized by limited animal exchanges and restricted breeding practices, exhibit well-defined genetic structures.

In contrast, populations from EWO, OBILI, and OKA showed considerable overlap of ellipses in the factorial space, indicating high within-population diversity and substantial gene flow among villages. This situation is likely explained by animal mobility between rural farms, local trade, and community exchanges, which promote interregional crossbreeding (Mtileni *et al.*, 2011; Leroy *et al.*, 2012). These gene flows contribute to reducing regional genetic differentiation, as observed in other African village chicken populations (FAO, 2011; Khanyile *et al.*, 2015).

The genetic homogeneity observed in the ALEME population suggests a founder effect or increased genetic drift. This phenomenon is typical of small geographically isolated populations, where a limited number of breeding individuals and uncontrolled mating patterns lead to progressive loss of genetic diversity over generations (Weigend and Romanov, 2001; Granevitze *et al.*, 2007).

Conversely, the high genetic diversity observed in the other populations reflects an important adaptive resilience, allowing the maintenance of genetic variability favorable to natural selection and environmental adaptation (Benabdeljelil *et al.*, 2003; Leroy *et al.*, 2012).

The phylogenetic tree reinforces the observations obtained from the PCA: individuals from ALEME are grouped into a distinct and homogeneous clade, whereas individuals from EWO, OBILI, and OKA are distributed across several mixed clades. The presence of shared clades among these three regions confirms continuous gene flow and a close evolutionary relationship between these populations. These results are consistent with studies by Ngeno *et al.*, (2015) and Mahrous *et al.*, (2019), who reported that the genetic structure of local chickens is strongly influenced by geographical factors, but also by community-based farming practices and informal animal exchange networks.

From the perspective of genetic resource management and conservation, the observed structure calls for a differentiated strategy. The ALEME population, being genetically distinct, represents a unique reservoir of specific diversity that should be conserved separately to prevent the erosion of its distinctive genetic characteristics. Conversely, the EWO, OBILI, and OKA populations, due to their genetic admixture, may be considered as a common genetic pool that could serve as a basis for participatory improvement programs. According to FAO (2011) and Weigend and Romanov (2001), the combination of genetically distinct but complementary populations represents an effective strategy for maintaining variability while improving productivity.

Overall, the PCA and phylogenetic tree demonstrate that the genetic diversity of chickens from Northern Congo relies on both the existence of a genetic isolate (ALEME) and a continuum of interconnected populations (EWO, OBILI, and OKA). This organization reflects the dual nature of local farming systems: village-based self-sufficiency on one hand, and community animal exchange networks on the other. These two dynamics coexist and shape the genetic structure of Congolese poultry populations.

The detailed phylogenetic tree of chickens from Northern Congo confirms and refines the observations obtained from the Principal Component Analysis (PCA). Although the maximum genetic distance is relatively low (approximately 0.1), the presence of distinct clades indicates internal genetic structuring within this geographical area.

The upper branch of the tree is strongly dominated by individuals from the ALEME population (EW_12, EW_11, EW_13, EW_14, EW_26), forming a nearly monophyletic clade. This observation supports the PCA results, where the ellipse corresponding to ALEME was small and isolated compared with the other northern populations.

This genetic homogeneity and isolation suggest that the ALEME population represents a genetic isolate, probably resulting from limited inbreeding practices and restricted gene flow with neighboring populations. Studies on African indigenous chickens have shown that isolated populations, such as those from Ethiopia and Zimbabwe, exhibit similar monophyletic clades associated with geographical or cultural isolation (Tadelle *et al.*, 2003; Muchadeyi *et al.*, 2007).

The existence of a distinct lineage within ALEME is particularly important for genetic conservation, as it represents a unique source of allelic diversity and specific genetic variants.

In contrast, individuals from the EWO, OBILI, and OKA regions (represented by blue, turquoise, and green colors) are interspersed throughout the tree, without forming distinct regional clades. This genetic mixture reflects substantial gene flow among these populations, likely resulting from animal mobility and frequent exchange of breeding individuals between villages (Wragg *et al.*, 2012; Gondwe *et al.*, 2007).

The genetic overlap and absence of distinct clades also indicate recent divergence among these populations, which limits regional differentiation detectable at the microsatellite level. These findings confirm the overlapping ellipses observed in the PCA and demonstrate that gene flow can maintain high genetic diversity while reducing spatial genetic structuring.

This contrasting genetic structure has important implications for genetic resource management:

- The ALEME population, as a genetic isolate, should be prioritized for both ex-situ and in situ conservation programs in order to preserve its unique variants and genetic integrity.
- The EWO, OBILI, and OKA populations could benefit from integrated breeding programs aimed at maintaining genetic diversity while improving production-related traits, given their high genetic connectivity and gene flow.

These results highlight the importance of considering local differences in genetic structuring when managing village chicken populations, even within the same agro-ecological zone

CONCLUSION

The microsatellite analysis conducted on indigenous chickens from Congo revealed substantial genetic diversity as well as differentiated genetic structuring among Northern and Southern populations. The quality of genetic typing was overall excellent, with an amplification success rate close to 100% for the majority of markers. Only two loci (MCW0222 and MCW0295) failed to amplify, a phenomenon frequently reported in studies involving African local breeds and

mainly reflecting genomic divergence or sequence incompatibilities. Nevertheless, the use of the 12 functional markers ensured sufficient analytical power to characterize the genetic variability of these populations.

In chickens from Northern Congo, the observed allelic diversity varied considerably among loci, with some highly polymorphic markers revealing significant genetic richness. This heterogeneity reflects the influence of local evolutionary history, genetic drift, and, in some cases, natural selection acting on adaptive traits. The results also showed that some populations, particularly ALEME, exhibit an isolated and homogeneous genetic structure, whereas others (EWO, OBILI, and OKA) are characterized by substantial gene flow, promoting high within-population diversity.

Multivariate analyses (PCA) and the phylogenetic tree confirmed this structural duality: ALEME represents a clearly distinct genetic isolate, while the other northern populations form a genetic continuum reflecting regular exchanges of breeding individuals among villages. These dynamics, already documented in other African contexts, highlight the importance of community-based farming practices in shaping the genetic structure of local poultry populations.

Overall, these results demonstrate significant genetic potential for the conservation and improvement of indigenous Congolese chickens. The data obtained provide a solid foundation for the implementation of sustainable genetic management programs, combining the preservation of distinct lineages such as ALEME with the rational utilization of mixed genetic pools such as EWO, OBILI, and OKA. The information generated will also contribute to strengthening national strategies for the valorization of poultry genetic resources in response to challenges related to climate resilience and food security

REFERENCES

- FAO. (2011). Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines No. 9. Rome, Italy: Food and Agriculture Organization of the United Nations.
- FAO. (2015). The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture. Edited by B.D. Scherf & D. Pilling. Rome: Food and Agriculture Organization of the United Nations.
- Fotsa, J. C., Rognon, X., Tixier-Boichard, M., Coquerelle, G., Poné, D. K., et Bordas, A. (2010b). Genetic diversity of local chickens in Cameroon using microsatellite markers. *Livestock Research for Rural Development*, 22(6), 99–110.
- Gondwe, T., Msoffe, P. L., Wotton, S. B., et Simianer, H. (2007). Genetic diversity of village chickens in Malawi using microsatellite markers.

- Tropical Animal Health and Production, 39(8), 553–563.
- Granevitze, Z., Hillel, J., Chen, G. H., Cuc, N. T. K., Feldman, M., Eding, H., et Weigend, S. (2007). Genetic diversity within chicken populations from different continents and management histories. *Animal Genetics*, 38(6), 576–583.
 - Gueye, E. F. (2009). The role of networks in information dissemination to family poultry farmers. *World's Poultry Science Journal*, 65(1), 115–126.
 - Khanyile, K. S., Dzomba, E. F., et Muchadeyi, F. C. (2015). Population genetic structure, linkage disequilibrium and effective population size of conserved and extensively raised village chickens of South Africa. *Frontiers in Genetics*, 6, 13.
 - Leroy, G., Baumung, R., Notter, D. R., Verrier, E., et Wurzi nger, M. (2012). Genetic diversity, inbreeding and breeding practices in local livestock populations. *Animal Genetics*, 43(2), 153–164.
 - Lyimo, C. M., Weigend, A., Janßen-Tapken, U., Msoffe, P. L. M., Simianer, H., & Weigend, S. (2014). Assessing the genetic diversity of five Tanzanian chicken ecotypes using molecular tools. *South African Journal of Animal Science*, 44(1), 80–86.
 - Mahrous, K. F., Hassan, H. M., et Shafey, H. I. (2019). Phylogenetic relationships among native chicken strains in Egypt using microsatellite markers. *Tropical Animal Health and Production*, 51(6), 1503–1511.
 - Mpenda, F. N., Schilling, M. A., Campbell, Z., Mngumi, E. B., & Buza, J. J. (2019). The genetic diversity of local African chickens: A potential for improving the breed. *Journal of Applied Poultry Research*, 28(1), 1–12.
 - Msoffe, P. L., Mtambo, M. M., Minga, U. M., Gwakisa, P. S., Mdegela, R. H., & Olsen, J. E. (2005). Genetic diversity and ecological structuring of *Salmonella Gallinarum* from chickens in Tanzania. *Poultry Science*, 84(6), 1051–1058.
 - Mtileni, B. J., Muchadeyi, F. C., Maiwashe, A., Groeneveld, E., et Weigend, S. (2011). Genetic diversity and conservation of South African indigenous chicken populations. *Journal of Animal Breeding and Genetics*, 128(3), 209–218.
 - Muchadeyi, F. C., Eding, H., Simianer, H., Wollny, C. B. A., Groeneveld, E., et Weigend, S. (2007). Mitochondrial DNA D-loop sequences suggest a Southeast Asian and Indian origin of Zimbabwean village chickens. *Animal Genetics*, 38(6), 615–623.
 - Mwacharo, J. M., Nomura, K., Hanada, H., Jianlin, H., Hanotte, O., & Amano, T. (2007). Genetic relationships among Kenyan and other East African indigenous chickens. *Animal Genetics*, 38(5), 485–490.
 - Ngeno, K., van der Waaij, E. H., Megens, H. J., van Arendonk, J. A. M., Crooijmans, R. P. M. A., et Kahi, A. K. (2015). Genetic diversity of different indigenous chicken ecotypes using microsatellite markers in Kenya. *Animal Genetic Resources*, 56, 87–95.
 - Rosenberg, N. A., Pritchard, J. K., Weber, J. L., Cann, H. M., Kidd, K. K., Zhivotovsky, L. A., & Feldman, M. W. (2002). Genetic structure of human populations. *Science*, 298(5602), 2381–2385.
 - Tadelle, D., Million, T., Alemu, Y., et Peters, K. J. (2003). Phenotypic and genetic characterization of indigenous chickens in Ethiopia. *Animal Genetic Resources Information*, 35, 21–33.
 - Weigend, S., et Romanov, M. N. (2001). Current strategies for the assessment and evaluation of genetic diversity in chicken resources. *World's Poultry Science Journal*, 57(3), 275–288.
 - Wimmers, K., Ponsuksili, S., Hardge, T., Valle-Zárate, A., Mathur, P. K., et Horst, P. (2000). Genetic distinctness of African, Asian and South American local chickens. *Animal Genetics*, 31(3), 159–165.
 - Wragg, D., Dawson, G., *et al.*, derson, L. (2012). Population structure of indigenous chickens in East Africa. *Poultry Science*, 91(6), 1364–1374.