OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2024.109.121

# Research Article
# Assessment of the Genetic Diversity and Population Structure of Local Chickens of Five Gabonese Ecotypes Using 28 of the 30 Microsatellite Markers Recommended by the FAO

[1,2]Sylvère Mboumba, [2]Landry Erik Mombo, [3]Richard Habimana, [4]Christian Keambou, [5]Anique Gbotto, [6]Sarah Osama, [6]Pauline Asami and [6]Nasser Yao

[1]Département de Zootechnologie, Institut National Supérieur d'Agronomie et de Biotechnologies (INSAB), Université des Sciences et Techniques de Masuku (USTM), Franceville, Gabon
[2]Laboratoire de Biologie Moléculaire et Cellulaire (LABMC), Université des Sciences et Techniques de Masuku (USTM), Franceville, Gabon
[3]College of Agriculture and Veterinary Science, University of Rwanda, P.O. Box 57, Nyagatare, Rwanda
[4]Faculty of Science, University of Buea, Buea, Cameroon
[5]Laboratoire de Génomique Fonctionnelle et Amélioration Génétique (LaGeFAGe), UFR-SN, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire
[6]Biosciences Eastern and Central Africa - International Livestock Research Institute (BecA-ILRI) Hub, P.O. Box 30709, Nairobi, Kenya

## Abstract

**Background and Objective:** The management of livestock biodiversity has become an important issue for the international scientific community. For this purpose, we assessed genetic variation in local chicken (*Gallus gallus*) populations from five regions of Gabon. **Materials and Methods:** A total of 28 microsatellite markers were used to genotype 194 individuals, including one commercial line (Isa Brown) that was assessed for possible introgression into local gene pools. A total of 292 alleles were revealed in the whole population with an average of 10.429 alleles per locus. **Results:** The observed heterozygosity rate was 0.484, 0.472, 0.495, 0.483 and 0.495 for Franceville, Libreville, Makokou, Mouila and Oyem, respectively. These values are below the expected heterozygosity for each locality ($p<0.05$). This resulted in a positive inbreeding coefficient in the local chicken populations and a negative coefficient in the commercial chickens. Wright's F-statistics (Fit = 0.216; Fis = 0.110; Fst = 0.123) suggesting moderate differentiation of individuals. Analysis of molecular variance revealed that 83% of the total genetic diversity was attributed to within-population variation and the remaining 5 and 12% were attributed to differentiation between regions and individuals, respectively. The pairwise genetic distances of the populations were very small ($0.008 \leq GD \leq 0.017$) between local populations and very large ($0.833 \leq GD \leq 0.884$) when comparing the local populations to the commercial chicken population. The analysis of the structure of the whole population revealed three genetic entities. These results showed that the study population has a satisfactory genetic diversity and a low level of introgression of exotic genes into the identified local gene pool. **Conclusion:** This genetic diversity constitutes an important basis for the implementation of conservation and genetic improvement programmes for local chickens in Gabon.

Key words: Gallus gallus domesticus, Gabon, genetic diversity, Indigenous chicken, introgression, population structure

Citation: Mboumba, S., L.E. Mombo, R. Habimana, C. Keambou and A. Gbotto *et al*., 2024. Assessment of the genetic diversity and population structure of local chickens of five gabonese ecotypes using 28 of the 30 microsatellite markers recommended by the FAO. Int. J. Poult. Sci., 23: 109-121.

Corresponding Author: Sylvere Mboumba, Département de Zootechnologie, Institut National Supérieur d'Agronomie et de Biotechnologies (INSAB), Université des Sciences et Techniques de Masuku (USTM), Franceville, Gabon Tel: +241 66616063



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Currently, the importance of biodiversity is recognized and there is no doubt that it contributes to the preservation of ecosystems and species[1]. Genetic diversity promotes the adaptation of livestock to various environmental conditions and stress, including diseases, temporary food and/or water shortage, heat stress, humidity and many other factors[2]. In recent years, it has received much attention for its importance for continuous genetic improvements in livestock and poultry[3].

However, the genetic diversity of livestock is threatened. Genetic resources are being lost before their potential is characterized and evaluated[4]. The Food and Agriculture Organization (FAO) statistics reveal that 9% of domestic bird's breeds have already disappeared, 20% are threatened, while 36% of them are under unknown situation due to lack of information[5]. A large number of chicken breeds are at risk among these birds (33%), forty breeds of chickens are already declared missing[6]. The management of livestock biodiversity has become an important issue for the international scientific community. Due to major changes in production systems, several breeds have disappeared and others are diluted by crossing with commercial strains[7]. Therefore, there is a need for enhancement and conservation of local genetic resources. The poultry sector in Gabon is based on the intensive rearing of imported strains. Local breeds are very poorly known from scientific perspectives and are only exploited by local populations in traditional extensive systems. However, in Gabon, studies on the genetic diversity of local chicken populations are often limited to the morpho-biometric characteristics[8]. To date, there is no relevant information on the diversity and genetic structure of local chicken populations in Gabon.

Several studies have been conducted to assess the genetic diversity of chicken using microsatellite markers and the results reported have clearly demonstrated the usefulness of these panels for biodiversity and breeding studies[9,10].

The evaluation of genetic variation and relationships on the one hand and the structure of local chicken populations in Gabon on the other, can provide a better understanding of the differences and similarities between the populations studied and can serve as a basis for future genetic improvement programs and the establishment of effective conservation systems for this animal genetic resource. Therefore, this study aimed to assess the genetic variation and genetic relationships (within and between populations) and population structure of local chicken from different agroecological regions of Gabon using 28 microsatellite markers.

## MATERIALS AND METHODS

**Bird sampling and DNA extraction:** Five regions of Gabon, at least 500 km apart, were chosen for sampling on the basis of their agro-ecological profiles (Fig 1). A total of 196 unrelated birds were studied for genetic variability from north-west (n = 37), south-east (n = 35) south (n = 38), north (n = 38) and north-east (n = 36). In addition, a commercial line ISA-brown (ISA) (n = 12) which was widely used in the country, was studied. DNA was extracted from a drop of blood collected from the cubital vein of each bird onto Whatman FTA™ filter cards (Whatman International Ltd). Blood drops were allowed to dry under shade for about one hour and kept in separate envelop at room temperature until processing. Genomic DNA was isolated using a boiling method as described by Smith and Burgoyne[11], DNA concentration of each sample was measured via a spectrophotometer (NanoDrop 2000c-ThermoScientific) and stored at -20°C for further amplification.

**Amplification reactions:** Individuals were genotyped using 28 pair of primers fluorescently-labelled for the amplification of microsatellite loci, selected on the base of the huge polymorphism expressed by a high polymorphism information content and genome coverage[12]. The PCR was carried out in reaction volumes of 10 µL containing: 2.5 µL of DNA (10-20 ng/µL); 5.0 µL of Master Mix (OneTaq and DreamTag) 0.2 µL of the forward and reverse primer (10 µM each) and 2.1 µL of double distilled water. The ampli cation conditions were: Pre-denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 sec, the annealing temperature of primers ranging from 58-64°C based on the primer components for 1 min (Table 1), extension at 72°C for 2 min and a nal elongation step at 72°C for 10 min. PCR amplification was performed in an Applied Biosystems Thermocycler (PCR-Gene Amp PCR System 9700). The pooled PCR amplicons were denatured with Hi-Di formamide at 95°C for 3 min. Samples were analysed on an ABI PRISM 377 DNA. Sequencer GeneScan™-500 LIZ® (Applied Biosystems) was used as internal size standard. The fragment data from ABI PRISM 377 system were analysed and allele sizes scored with GeneMapper version 4.1 software (Applied Biosystems).

**Genetic relationship and population structure:** The genetic diversity of the whole population of chicken was analysed on two levels: the within and among population variability. The Hardy and Weinberg law was used to test the population equilibrium, based on the chi-square test, genotypic frequencies of non-conformity with the Hardy-Weinberg equilibrium (HWE) were calculated at the significance levels of $p<0.05$, $p<0.01$ and $p<0.001$.

Table 1: Information on the sequences of the 30 microsatellites recommended by the FAO for molecular characterisation studies in chickens

| Name | Chromosome | Primer sequence (5'->3') forward reverse | Annealing temperature (°C) | Genebank accession number | Allele range (bp) | Multiplex[1] group |
|---|---|---|---|---|---|---|
| ADL0268 | 1 | CTCCACCCCTCTCAGAACTA<br>CAACTTCCCATCTACCTACT | 60 | G01688 | 102-116 | 1 |
| MCW0206 | 2 | CTTGACAGTGATGCATTAAATG<br>ACATCTAGAATTGACTGTTCAC | 60 | AF030579 | 221-249 | 7 |
| LEI0166 | 3 | CTCCTGCCCTTAGCTACGCA<br>TATCCCCTGGCTGGGAGTTT | 60 | X85531 | 354-370 | 3 |
| MCW0295 | 4 | ATCACTACAGAACACCCTCTC<br>TATGTATGCACGCAGATATCC | 60 | G32052 | 88-106 | 2 |
| MCW0081 | 5 | GTTGCTGAGAGCCTGGTGCAG<br>CCTGTATGTGGAATTACTTCTC | 60 | ... | 112-135 | 2 |
| MCW0014 | 6 | TATTGGCTCTAGGAACTGTC<br>GAAATGAAGGTAAGACTAGC | 58 | ... | 164-182 | 4 |
| MCW0183 | 7 | ATCCCAGTGTCGAGTATCCGA<br>TGAGATTTACTGGAGCCTGCC | 58 | G31974 | 296-326 | 4 |
| ADL0278 | 8 | CCAGCAGTCTACCTTCCTAT<br>TGTCATCCAAGAACAGTGTG | 60 | G01698 | 114-126 | 1 |
| MCW0067 | 10 | GCACTACTGTGTGCTGCAGTTT<br>GAGATGTAGTTGCCACATTCCGAC | 60 | G31945 | 176-186 | 6 |
| MCW0104 | 13 | TAGCACAACTCAAGCTGTGTAG<br>AGACTTGCACAGCTGTGTACC | 60 | ... | 190-234 | 5 |
| MCW0123 | 14 | CCACTAGAAAAGAACATCCTC<br>GGCTGATGTAAGAAGGGATGA | 60 | ... | 76-100 | 5 |
| MCW0330 | 17 | TGGACCTCATCAGTCTGACAG<br>AATGTTCTCATAGAGTTCCTGC | 60 | G32085 | 256-300 | 6 |
| MCW0165 | 23 | CAGACATGCATGCCCAGATGA<br>GATCCAGTCCTGCAGGCTGC | 60 | ... | 114-118 | 5 |
| MCW0069 | E60C04W23 | GCACTCGAGAAAACTTCCTGCG<br>ATTGCTTCAGCAAGCATGGGAGGA | 60 | ... | 158-176 | 2 |
| MCW0248 | 1 | GTTGTTCAAAAGAAGATGCATG<br>TTGCATTAACTGGGCACTTTC | 60 | G32016 | 205-225 | 1 |
| MCW0111 | 1 | GCTCCATGTGAAGTGGTTTA<br>ATGTCCACTTGTCAATGATG | 60 | L48909 | 96-120 | 3 |
| MCW0020 | 1 | TCTTCTTTGACATGAATTGGCA<br>GCAAGGAAGATTTTGTACAAAATC | 60 | ... | 179-185 | 5 |
| MCW0034 | 2 | TGCCGCACTTACATACTTAGAGA<br>TGTCCTTCCAATTACATTCATGGG | 60 | ... | 212-364 | 2 |
| LEI0234 | 2 | ATGCATCAGATTGGTATTCAA<br>CGTGGCTGTGAACAAATATG | 60 | Z94837 | 216-364 | 3 |
| MCW0103 | 3 | AACTGCGTTGAGAGTGAATGC<br>TTTCCTAACTGGATGCTTCTG | 64 | G31956 | 266-270 | 7 |
| MCW0222 | 3 | GCAGTTACATTGAAATGATTCC<br>TTCTCAAAACACCTAGAAGAC | 60 | G31996 | 220-226 | 2 |
| MCW0016 | 3 | ATGGCGCAGAAGGCAAAGCGATAT<br>TGGCTTCTAAGCAGTTGCTATGG | 60 | ... | 162-206 | 3 |
| MCW0037 | 3 | ACCGGTGCCATCAATTACCTATTA<br>GAAAGCTCACATGACACTGCGAAA | 64 | ... | 154-160 | 3 |
| MCW0098 | 4 | GGCGCTTTGTGCTCTTCTCG<br>CGATGGTCGTAATTCTCACGT | 60 | ... | 261-265 | 6 |
| LEI0094 | 4 | GATCTCACCAGTATGAGCTGC<br>TCTCACACTGTAACACAGTGC | 60 | X83246 | 247-287 | 1 |
| MCW0284 | 4 | GCCTTAGGAAAAACTCCTAAGG<br>CAGAGCTGGATTGGTGTCAAG | 60 | G32043 | 235-243 | ... |
| MCW0078 | 5 | CCACACGAGAGGAGAAGGTCT<br>TAGCATATGAGTGTACTGAGCTTC | 60 | ... | 135-147 | 6 |
| LEI0192 | 6 | TGCCAGAGCTTCAGTCTGT<br>GTCATTACTGTTATGTTTATTGC | 60 | Z83797 | 244-370 | ... |
| ADL0112 | 10 | GGCTTAAGCTGACCCATTAT<br>ATCTCAAATGTAATGCGTGC | 58 | G01725 | 120-134 | 4 |
| MCW0216 | 13 | GGGTTTTACAGGATGGGACG<br>AGTTTCACTCCAGGGCTCG | 60 | AF030586 | 139-149 | 1 |

Genetic diversity within and between populations: To evaluate the informativeness of each marker, the polymorphic information content (PIC) of markers was calculated based on the allele frequencies[13] using Power Marker software[14].

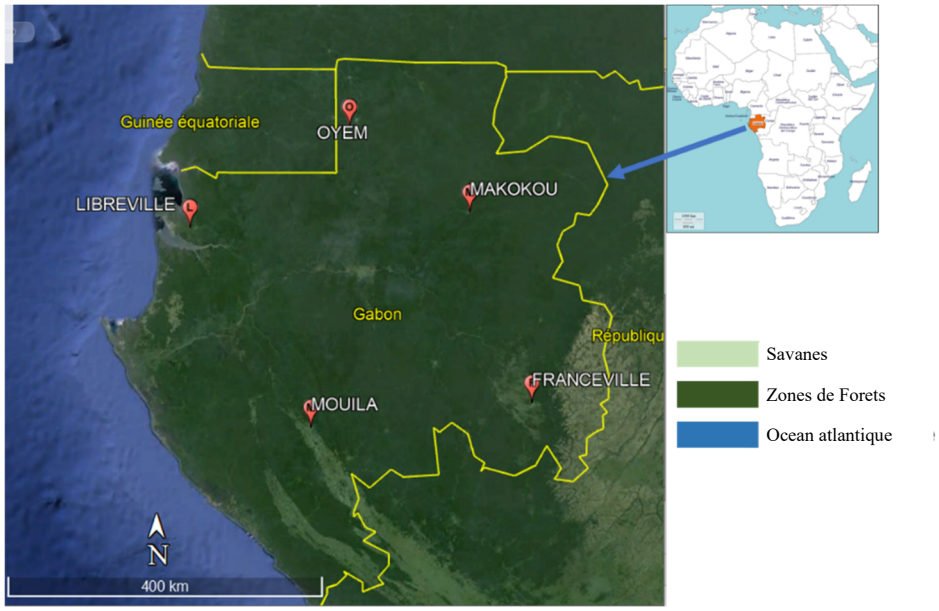



Fig. 1 : *Data collection sites

Chi-square and Fisher's exact (probability) test on a two-by-two contingency table was used to assess the deviations from Hardy-Weinberg Equilibrium (HWE) for each locus and each population. The genetic diversity was described using different parameters such as: number of Genotypes (NG) total number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), inbreeding coefficient over all populations (Fis), among populations (Fit) and within populations (Fst), Shannon index (I). Genetic variation within and among populations was estimated using the AMOVA (analysis of molecular variance) computed in GenAlEx software version 6.5[15]. The F statistics of Wright, Genetic distances and gene flow parameters were used to describe the genetic diversity between populations. The Nei's standard genetic distances were estimated among pairs of population using the standard approach based on allele frequencies in each population[16]. The POPGENE software version 1.31[17] was used to calculate these indices, with the level of significance set at $p<0.05$.

Darwin Version 6.0.10 software was used to establish a phylogenetic tree and unrooted cladogram according to pairwise kinship distance matrix between populations[18]. A consensus tree assessed by 2000 bootstraps all through the group of loci was created.

**Population structure:** We used two methods to study the population structure. First, to establish the phylogenetic tree of the local chicken in Gabon, we used the Neighbour-Joining method. The Darwin software v.6.0 according to pairwise kinship distance matrix between populations was performed to get a consensus tree assessed by 1000 bootstraps[18]. Second, the population genetic structure of the studied chicken ecotypes was inferred from the multi-loci genotypic data using a Bayesian approach employed in STRUCTURE software version 2.3.4[19]. The analysis involved an admixture model with correlated allele frequencies. The model was tested using 100000 iterations of Markov Chain Monte Carlo (MCMC) and a burn-in period of 50000 for each K value ranging from $1 \leq K \leq 20$. Each assessment of K was repeated ten times to check the repeatability of the results. The most probable number of clusters (ΔK) was calculated following the equations proposed by Evanno *et al.*[20] via STRUCTURE Harvester (University of California, USA) and CLUMPAK (Naama M Kopelman, Jonathan Mayzel; Tel Aviv University, Israel) was used to single out clustering types and bundle population structure deductions across K[21].

## RESULTS

**Genetic diversity within population:** Genetic diversity parameters were assessed with 28 SSR markers across all populations and the results are presented in Table 2 and 3. All (100%) SSR markers were polymorphic for the six populations: the PIC ranged from 0.324 to 0.899 respectively for markers MCW0103 and LEI0234 (Table 1). The number of genotypes (NGT) ranged from 8 (locus MCW103) to 63 (locus LEI0234) with an average of 19.393 for all loci. The 28 polymorphic

Table 2: Marker polymorphism and diversity parameters across studied populations in Gabon

| Marker | PIC | NGT | Na | Ne | Ho | uHe | He | F | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | I | Nm | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ADL0268 | 0.568 | 23 | 11 | 2.608 | 0.515 | 0.617 | 0.604 | 0.156 | 0.147 | 0.203 | 0.066 | 1.182 | 3.557 | 0.003 |
| MCW0014 | 0.458 | 14 | 10 | 2.039 | 0.468 | 0.511 | 0.500 | 0.077 | 0.066 | 0.210 | 0.155 | 0.831 | 1.368 | 0.001 |
| MCW0206 | 0.578 | 12 | 9 | 2.632 | 0.588 | 0.628 | 0.615 | 0.043 | 0.045 | 0.110 | 0.068 | 1.117 | 3.425 | 0.449 |
| MCW0183 | 0.697 | 26 | 10 | 3.492 | 0.673 | 0.726 | 0.712 | 0.052 | 0.055 | 0.112 | 0.060 | 1.440 | 3.885 | 0.003 |
| MCW0016 | 0.351 | 14 | 11 | 1.614 | 0.182 | 0.299 | 0.290 | 0.389 | 0.373 | 0.595 | 0.354 | 0.580 | 0.456 | 0.000 |
| MCW0034 | 0.789 | 41 | 14 | 4.662 | 0.728 | 0.794 | 0.778 | 0.065 | 0.065 | 0.119 | 0.058 | 1.800 | 4.061 | 0.003 |
| MCW0330 | 0.501 | 10 | 6 | 2.232 | 0.448 | 0.555 | 0.543 | 0.155 | 0.176 | 0.243 | 0.081 | 0.937 | 2.821 | 0.003 |
| MCW0295 | 0.413 | 17 | 10 | 1.782 | 0.419 | 0.419 | 0.409 | -0.020 | -0.025 | 0.221 | 0.240 | 0.819 | 0.792 | 0.043 |
| MCW0069 | 0.589 | 18 | 8 | 2.522 | 0.510 | 0.602 | 0.591 | 0.137 | 0.136 | 0.218 | 0.095 | 1.196 | 2.384 | 0.000 |
| MCW0248 | 0.483 | 9 | 8 | 1.789 | 0.463 | 0.440 | 0.430 | -0.029 | -0.076 | 0.154 | 0.214 | 0.720 | 0.920 | 0.000 |
| LEI0234 | 0.899 | 63 | 22 | 8.084 | 0.838 | 0.892 | 0.874 | 0.041 | 0.041 | 0.081 | 0.042 | 2.215 | 5.685 | 0.262 |
| MCW0123 | 0.540 | 19 | 11 | 2.193 | 0.443 | 0.539 | 0.527 | 0.163 | 0.158 | 0.191 | 0.039 | 1.093 | 6.172 | 0.001 |
| MCW0165 | 0.604 | 15 | 8 | 2.422 | 0.474 | 0.590 | 0.577 | 0.180 | 0.179 | 0.292 | 0.138 | 1.060 | 1.566 | 0.000 |
| MCW0037 | 0.505 | 10 | 7 | 1.809 | 0.415 | 0.454 | 0.445 | 0.096 | 0.066 | 0.263 | 0.210 | 0.745 | 0.939 | 0.000 |
| MCW0104 | 0.612 | 26 | 17 | 2.422 | 0.460 | 0.511 | 0.497 | 0.087 | 0.075 | 0.180 | 0.113 | 1.135 | 1.954 | 0.959 |
| LEI0094 | 0.774 | 30 | 16 | 4.049 | 0.772 | 0.767 | 0.751 | -0.031 | -0.028 | -0.005 | 0.022 | 1.674 | 11.201 | 0.330 |
| ADL0112 | 0.644 | 17 | 10 | 3.311 | 0.616 | 0.693 | 0.679 | 0.079 | 0.093 | 0.164 | 0.078 | 1.312 | 2.939 | 0.000 |
| MCW0067 | 0.441 | 8 | 6 | 1.931 | 0.379 | 0.478 | 0.468 | 0.183 | 0.190 | 0.291 | 0.125 | 0.789 | 1.755 | 0.000 |
| LEI0192 | 0.803 | 41 | 18 | 4.760 | 0.705 | 0.789 | 0.773 | 0.089 | 0.089 | 0.153 | 0.071 | 1.785 | 3.279 | 0.486 |
| MCW0103 | 0.324 | 8 | 7 | 1.546 | 0.253 | 0.344 | 0.336 | 0.238 | 0.248 | 0.476 | 0.303 | 0.563 | 0.574 | 0.000 |
| ADL0278 | 0.670 | 20 | 10 | 3.270 | 0.678 | 0.706 | 0.692 | 0.024 | 0.020 | 0.078 | 0.059 | 1.314 | 4.016 | 0.015 |
| MCW0078 | 0.695 | 18 | 11 | 3.439 | 0.515 | 0.707 | 0.694 | 0.233 | 0.258 | 0.302 | 0.059 | 1.383 | 4.016 | 0.000 |
| MCW0222 | 0.522 | 14 | 8 | 2.291 | 0.472 | 0.572 | 0.560 | 0.160 | 0.158 | 0.289 | 0.155 | 0.936 | 1.362 | 0.000 |
| MCW0081 | 0.522 | 17 | 11 | 2.150 | 0.507 | 0.541 | 0.530 | 0.035 | 0.043 | 0.111 | 0.071 | 0.989 | 3.259 | 0.348 |
| MCW0020 | 0.696 | 15 | 9 | 3.612 | 0.680 | 0.737 | 0.723 | 0.059 | 0.058 | 0.070 | 0.012 | 1.376 | 21.044 | 0.174 |
| MCW0098 | 0.474 | 9 | 7 | 1.908 | 0.479 | 0.477 | 0.469 | -0.034 | -0.023 | 0.245 | 0.262 | 0.708 | 0.703 | 0.000 |
| MCW0111 | 0.702 | 20 | 10 | 2.650 | 0.531 | 0.626 | 0.612 | 0.113 | 0.132 | 0.167 | 0.039 | 1.225 | 6.082 | 0.000 |
| MCW0284 | 0.440 | 9 | 7 | 1.932 | 0.306 | 0.504 | 0.475 | 0.373 | 0.355 | 0.522 | 0.259 | 0.710 | 0.717 | 0.000 |
| Total | | 543 | 292 | | | | | | | | | | | |
| Mean | 0.582 | 19.393 | 10.429 | 2.827 | 0.518 | 0.590 | 0.577 | 0.111 | 0.110 | 0.216 | 0.123 | 1.130 | 3.605 | |

PIC: Polymorphism Information content, NGT: Number of genotypes, Na: Number of alleles, Ne: Number of effective alleles, Ho: Observe Heterozygosity, uHe: unbiased expected Heterozygosity, He: expected Heterozygosity F: Fixation index; $F_{IS}$: inbreeding coefficient over all populations, $F_{IT}$: inbreeding coefficient among populations, $F_{ST}$: inbreeding coefficient within populations, I: Shannon's information index, Nm: Number of migrants, p-value: Hardy-Weinberg equilibrium p-value based on chi square test (There is a deviation from HWE at $p < 0.05$)

microsatellite markers had a total of 292 alleles (Table 2). The number of alleles across loci ranged from 6 (Loci MCW0330 to MCW0067) and 22 (LEI0234) with an average number of alleles of 10.429 (Table 2). The Effective number of alleles (Ne) ranged from 1.546-8.084 for Loci MCW0103 and LEI0234, respectively with an average number of 2.827. The observed and unbiased heterozygosity were calculated for each locus and population under the assumption of Hardy-Weinberg equilibrium. The expected and unbiased heterozygosity ranged from 0.290-0.874 and 0.299-0.892, respectively with a means of 0.577 and 0.590, respectively, while the observed heterozygosity ranged from 0.182-0.838 with an average number of 0.518. Within population, global inbreeding coefficients for over all populations and among population ($F_{IS}$, $F_{IT}$ and $F_{ST}$) were 0.110; 0.216 and 0.123, respectively, this resulted in a fixation index of 0.111 and meant that the mean number of migrants per generation in the overall population and across all the loci was 3.605.

**Genetic diversity between populations:** The genetic diversity indices of the different local hen populations in Gabon are summarised in Table 4. The average sum of alleles varied from 6.571±0.528 to 4.321±0.317 for Libreville and commercial chicken respectively. As for the number of private alleles, the highest value was found in the commercial chickens (54), followed by the Libreville chickens (40), while the four other localities had a very low number of private alleles (3 for Franceville, Makokou and Oyem and 4 for Mouila). On the other hand, for 4 other parameters (Ne, I, He, Ho), the exotic chickens had higher values than the local chickens. Furthermore, the lowest observed heterozygosity was in the Libreville (0.472±0.034) while the highest was recorded in exotic chicken population (0.682 ± 0.039).

The p-values of HWE for each population are reported in Table 2 and shows the results of the equilibrium test verified by the Popgene software. We can see that some loci deviated significantly from Hardy-Weinberg equilibrium while in the

Table 3: Tests for the Hardy-Weinberg equilibrium probability of loci in the six populations in Gabon

| | Commerciale | | Franceville | | Libreville | | Makokou | | Mouila | | Oyem | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | Prob. | Sig. | Prob. | Sig. | Prob. | Sig. | Prob. | Sig. | Prob. | Sig. | Prob. | Sig. |
| ADL0268 | 0.836 | ns | 0.000 | *** | 0.000 | *** | 0.002 | ** | 0.351 | ns | 0.000 | *** |
| MCW0014 | 0.662 | ns | 0.732 | ns | 0.000 | *** | 0.000 | *** | 0.772 | ns | 0.186 | ns |
| MCW0206 | 0.390 | ns | 0.412 | ns | 0.000 | *** | 0.918 | ns | 0.696 | ns | 0.253 | ns |
| MCW0183 | 0.026 | 0 | 0.000 | *** | 0.000 | *** | 0.001 | ** | 0.040 | 0 | 0.218 | ns |
| MCW0016 | 0.332 | ns | 0.000 | *** | 0.000 | *** | 0.931 | ns | 0.046 | 0 | 0.002 | ** |
| MCW0034 | 0.112 | ns | 0.409 | ns | 0.000 | *** | 0.009 | ** | 0.005 | ** | 0.000 | *** |
| MCW0330 | 0.189 | ns | 0.955 | ns | 0.000 | *** | 0.645 | ns | 0.385 | ns | 0.993 | ns |
| MCW0295 | 0.518 | ns | 0.935 | ns | 0.000 | *** | 0.051 | ns | 0.003 | ** | 0.733 | ns |
| MCW0069 | 0.635 | ns | 0.097 | ns | 0.000 | *** | 1.000 | ns | 0.000 | *** | 0.028 | 0 |
| MCW0248 | 0.019 | 0 | 0.852 | ns | 0.000 | *** | 0.390 | ns | 0.781 | ns | 0.962 | ns |
| LEI0234 | 0.702 | ns | 0.752 | ns | 0.000 | *** | 0.021 | 0 | 0.322 | ns | 0.000 | *** |
| MCW0123 | 0.014 | 0 | 0.000 | *** | 0.000 | *** | 0.644 | ns | 0.000 | *** | 0.110 | ns |
| MCW0165 | 0.319 | ns | 0.007 | ** | 0.000 | *** | 0.104 | ns | 0.074 | ns | 0.000 | *** |
| MCW0037 | 0.002 | ** | 0.087 | ns | 0.000 | *** | 0.350 | ns | 0.002 | ** | 0.264 | ns |
| MCW0104 | 0.720 | ns | 0.812 | ns | 0.000 | *** | 1.000 | ns | 0.662 | ns | 0.352 | ns |
| LEI0094 | 0.631 | ns | 0.000 | *** | 0.000 | *** | 0.988 | ns | 0.072 | ns | 0.493 | ns |
| ADL0112 | 0.038 | 0 | 0.166 | ns | 0.000 | *** | 0.428 | ns | 0.663 | ns | 0.666 | ns |
| MCW0067 | 0.501 | ns | 0.359 | ns | 0.000 | *** | 0.568 | ns | 0.011 | 0 | 0.000 | *** |
| LEI0192 | 0.819 | ns | 0.444 | ns | 0.000 | *** | 0.466 | ns | 0.469 | ns | 0.103 | ns |
| MCW0103 | 0.506 | ns | 0.147 | ns | 0.000 | *** | 0.658 | ns | 0.199 | ns | 0.000 | *** |
| ADL0278 | 0.185 | ns | 0.040 | 0 | 0.000 | *** | 0.911 | ns | 0.984 | ns | 0.303 | ns |
| MCW0078 | 0.821 | ns | 0.000 | *** | 0.000 | *** | 0.000 | *** | 0.000 | *** | 0.001 | *** |
| MCW0222 | 0.296 | ns | 0.753 | ns | 0.000 | *** | 0.012 | 0 | 0.002 | ** | 0.767 | ns |
| MCW0081 | 0.619 | ns | 0.143 | ns | 0.000 | *** | 0.013 | 0 | 0.996 | ns | 0.671 | ns |
| MCW0020 | 0.968 | ns | 0.195 | ns | 0.000 | *** | 0.023 | 0 | 0.004 | ** | 0.897 | ns |
| MCW0098 | 0.429 | ns | 0.794 | ns | 0.000 | *** | 0.667 | ns | 0.351 | ns | 0.309 | ns |
| MCW0111 | 0.190 | ns | 0.000 | *** | 0.004 | ** | 0.229 | ns | 0.045 | 0 | 0.120 | ns |
| MCW0284 | 0.343 | ns | 0.414 | ns | 0.372 | ns | 0.068 | ns | 0.128 | ns | 0.797 | ns |

p <0.05 show the genotype frequencies for nonconformity with Hardy-Weinberg Equilibrium (HWE) based on Chi square test

Table 4: Private alleles, Number of alleles, Effective number of alleles (Ne), Shannon index (I), observed (Ho), expected (He), Fixation index (F) and inbreeding coefficient (Fis) by population

| Population | Pa | Na | Ne | I | Ho | He | F | Fis |
|---|---|---|---|---|---|---|---|---|
| Commerciale | 54 | 4.321±0.317 | 3.137±0.242 | 1.193±0.070 | 0.682±0.039 | 0.637±0.023 | -0.084±0.060 | -0.071 |
| Franceville | 3 | 4.857±0.445 | 2.693±0.272 | 1.054±0.085 | 0.484±0.035 | 0.550±0.033 | 0.129±0.032 | 0.120 |
| Libreville | 40 | 6.571±0.528 | 2.812±0.252 | 1.193±0.081 | 0.472±0.034 | 0.580±0.029 | 0.198±0.038 | 0.186 |
| Makokou | 3 | 5.286±0.482 | 2.675±0.283 | 1.083±0.085 | 0.495±0.034 | 0.553±0.032 | 0.101±0.034 | 0.105 |
| Mouila | 4 | 5.571±0.521 | 2.811±0.302 | 1.123±0.089 | 0.483±0.034 | 0.565±0.033 | 0.161±0.026 | 0.145 |
| Oyem | 3 | 5.321±0.483 | 2.833±0.303 | 1.132±0.085 | 0.495±0.036 | 0.576±0.030 | 0.162±0.034 | 0.141 |

exotic population, 82% of the loci were in Hardy-Weinberg equilibrium. In the local chicken populations of Gabon, 68% were in equilibrium (Franceville, Oyem and Makokou), while in Mouila only 57% of the loci were in equilibrium. Almost all the loci in the Libreville population were in Hardy-Weinberg disequilibrium, with the exception of the MCW0284 locus.

Furthermore, under the Hardy Weinberg hypothesis, the selected ecotypes showed a significant ($p<0.05$) deficit of heterozygotes which is observable with the Fis values for each local population: 0.120, 0.186, 0.105, 0.145, 0.141 for Franceville, Libreville, Makokou, Mouila and Oyem, respectively. On the contrary, the exotic population showed a significant excess of heterozygotes (FIS = -0.071) (Table 4).

**Genetic relationship and population structure:** Analysis of molecular variance revealed that 83% of the total variation originated from variation within individuals. Twelve percent (12%) of the variation was due to differentiation among individuals. The lowest variability (5%) was due to among-populations variation that was represented by diversity among the population with an overall Fst value of 0.047 ($p<0.001$) (Table 5, Fig. 2).

The results of the principal co-ordinate analysis (PCoA) are presented in Fig. 3 and show groups of trees in which there is a group made up entirely of local chickens, another group made up entirely of exotic chickens and finally a third group

with a profile intermediate to the two previous groups. This intermediate group is made up of 7 individuals all from the local populations, no individuals from Makokou were part of this group.

The pairwise genetic distance matrix of the Gabonese chicken populations (Table 6) showed low genetic distances, e.g., the genetic distance between Franceville-Makokou and Mouila-Oyem, which was 0.017 and 0.008, respectively.

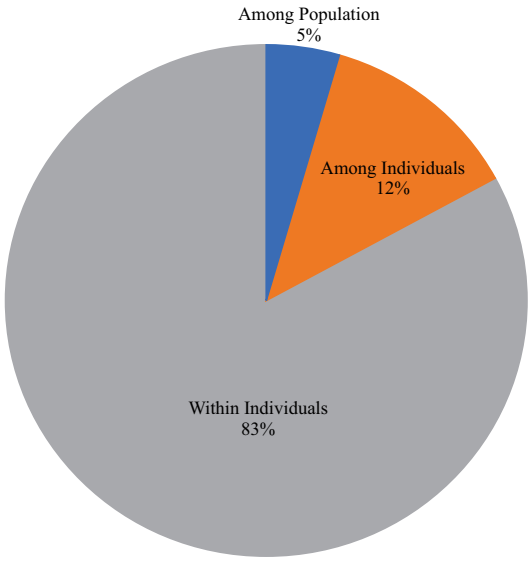



Fig. 2: Percentage distribution of molecular variance

However, when comparing each of these local populations with the exotic chicken population, the genetic distances were very large (about 0.9).

The phylogenetic relationship by the Neighbour-Joining tree showed three clusters with no grouping according to ecotypes (Fig. 4). The first mixed cluster consisted of both local chickens from all ecotypes without distinction and commercial chickens. The second cluster (consisting of about 30 individuals) and cluster 3, were all made up solely of local chickens. By examining the result of the principal co-ordinate analysis (Fig. 3) and the dendrogram of the total population (Fig. 4), three main groups can be distinguished, which in turn have subgroups. The tree analysis showed that the grouping of individuals was independent of geographical origin or ecotype.

To study the structuring of genetic diversity within individual populations, the Nei distance[16] was estimated between pairs of populations (Table 6). The highest genetic distances (0.833; 0.879; 0.884; 0.838; 0.859) were recorded between the commercial chicken population and the five local populations (Franceville, Libreville, Makokou, Mouila and Oyem, respectively).

The Bayesian analysis using the software STRUCTURE indicated the presence of three, maybe four main clusters in the entire set of accessions. The highest value for ΔK, the rate of change in the log probability of the data between successive potential numbers of clusters, was obtained for K = 3 just followed by K = 4 (Fig. 5). The estimated log

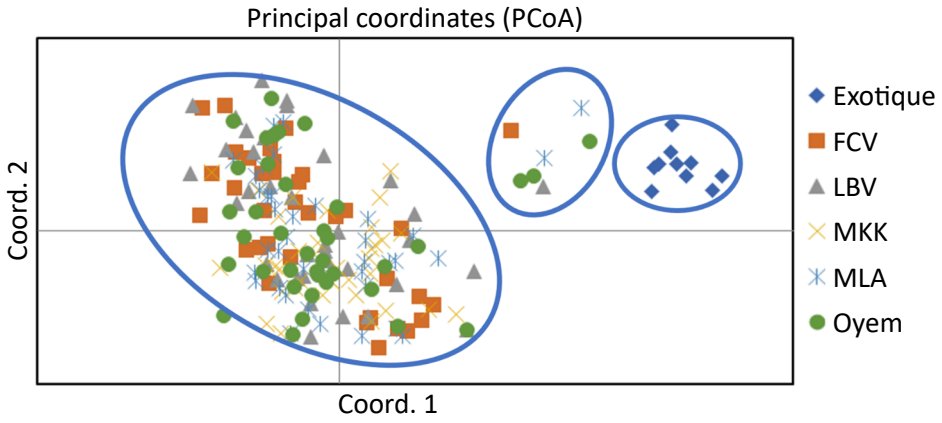



Fig. 3: Principal Coordinates Analysis (PCoA)

Table 5: Analysis of molecular variance calculated on the basis of the allelic distance matrix of Wright's F-statistics among Gabon local chickens

| Source | Degree of freedom | Sum of square | Mean square | Estimated variances | Estimated variances (%) | F-statistics |
|---|---|---|---|---|---|---|
| Among populations | 5 | 171.591 | 34.318 | 0.396 | 5 | Fst = 0.047 |
| Among individuals | 188 | 1725.814 | 9.180 | 1.058 | 12 | Fis = 0.130 |
| Within individuals | 194 | 1370.203 | 7.063 | 7.063 | 83 | Fit = 0.171 |
| Total | 387 | 3267.607 | | 8.518 | 100 | |

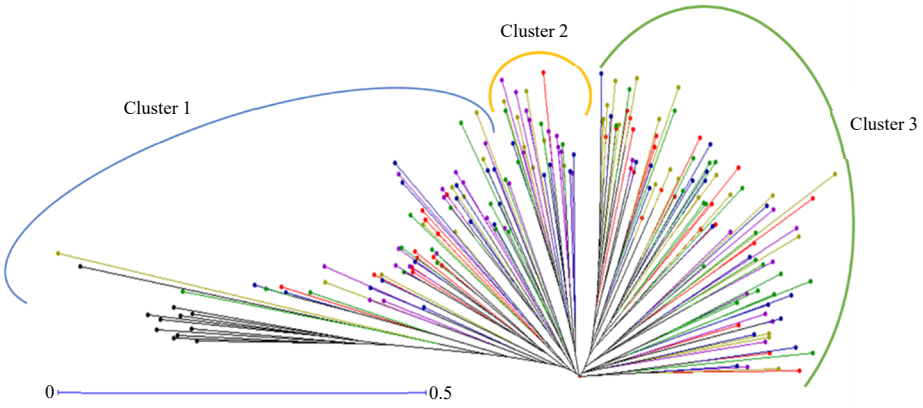



Fig. 4: Phylogenetic tree based on the neighbour-joining describing the relationships between local chickens. The colours correspond to the different agro-ecological zones

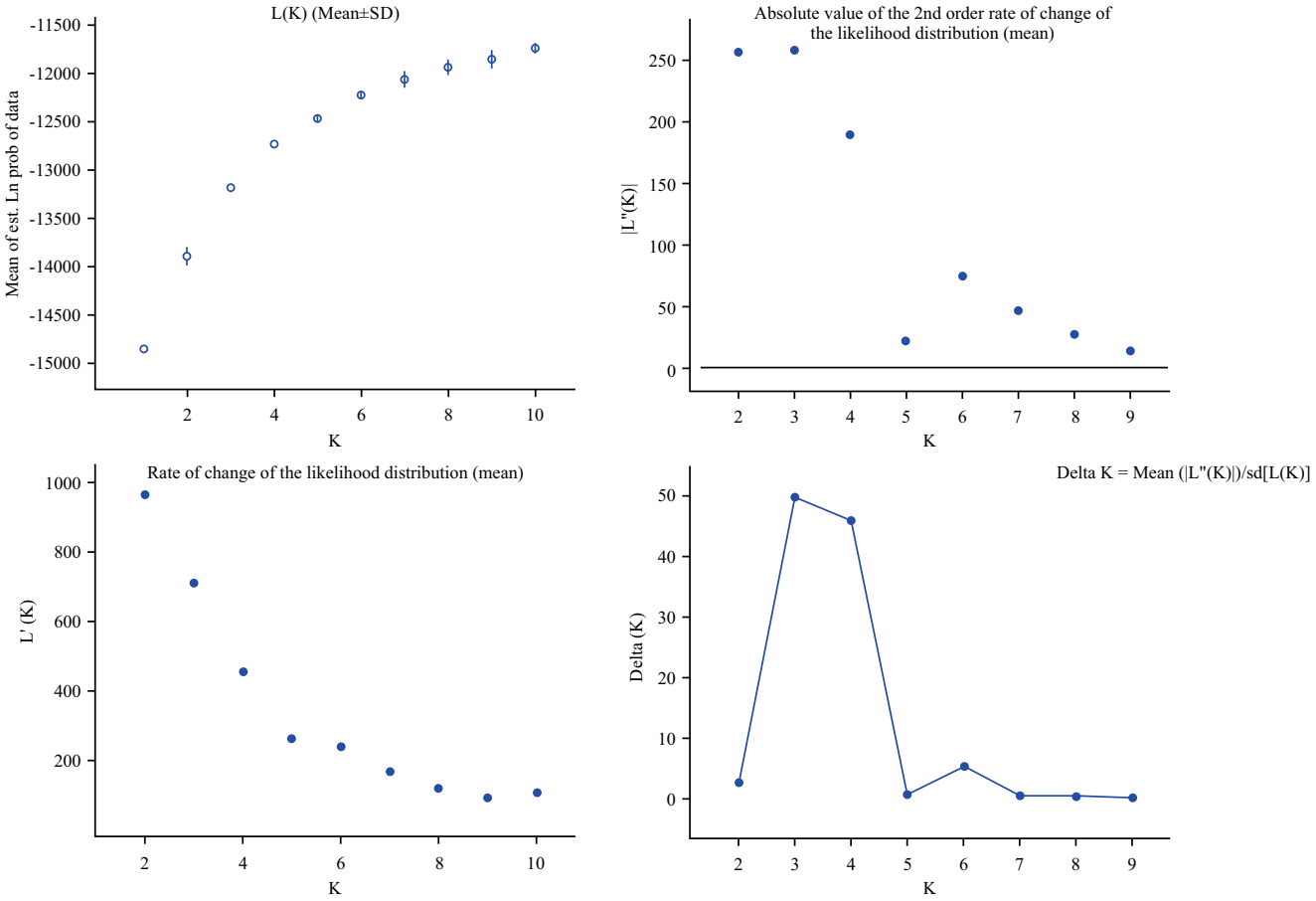



Fig. 5: Evolution of the mean estimate of ln probability of data and Delta K (ΔK) approximating the more possible number of clusters in Gabon local chickens

probability of the data (Table 7) was higher under K = 3 (-13179.6) than under K = 4 (-12728.6). The results were plotted to evaluate the geographical relationships of the population and individuals in different genetic clusters (Fig. 6 and 7) and confirmed the population structure revealed by the Neighbour-Joining tree (Fig. 4).

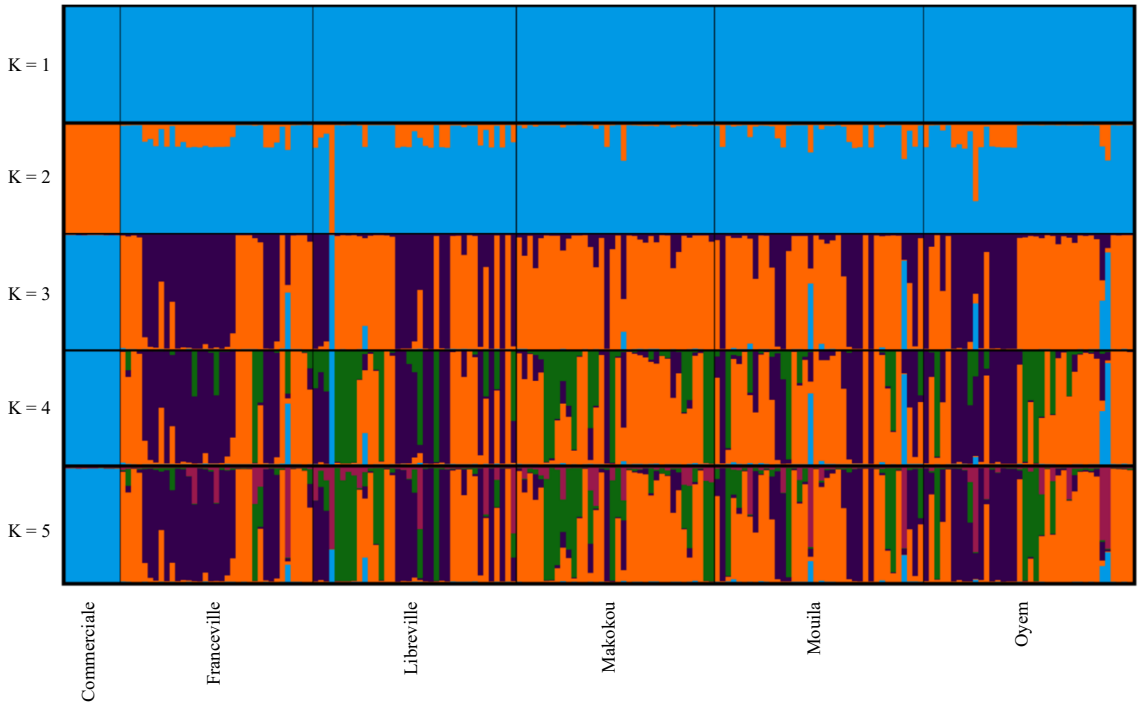
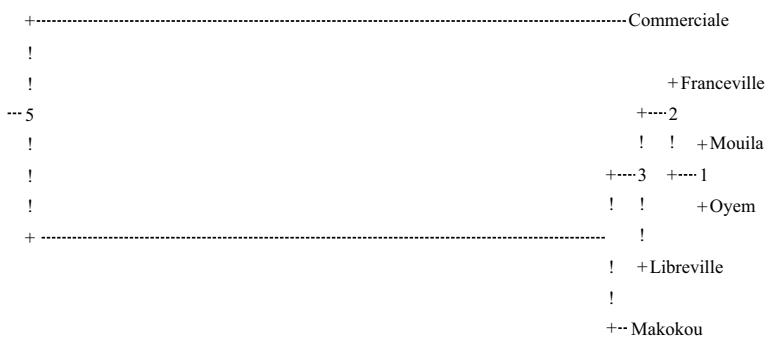



Fig. 6: Genetic structure of six chicken populations (194 individuals in total) based on amplification profiles of 28 microsatellite loci. The bar plot shows clustering of individuals according to genetic populations, using the software Structure, with $1 \leq K \leq 10$ (PRITCHARD *et al.*[19]). Each colour represents one Population and a vertical bar, broken into K coloured segments, with lengths proportional to each of the K inferred clusters, represents an individual of the population




| Between | And | Length |
|---|---|---|
| 5 | Commerciale | 45.44709 |
| 5 | 4 | 44.05981 |
| 4 | 3 | 0.45572 |
| 3 | 2 | 0.16611 |
| 2 | Franceville | 0.76545 |
| 2 | 1 | 0.16998 |
| 1 | Mouila | 0.59547 |
| 1 | Oyem | 0.59547 |
| 3 | Libreville | 0.93156 |
| 4 | Makokou | 1.38728 |

Fig. 7: Phylogenetic relationships between the six chicken populations

Table 6: Pairwise Population Matrix of Nei Unbiased Genetic Distance and Fst Values, Genetic Distance below the diagonal

| | Commercial | Franceville | Libreville | Makokou | Mouila | Oyem |
|---|---|---|---|---|---|---|
| Commercial | 0.000 | 0.171 | 0.166 | 0.175 | 0.166 | 0.165 |
| Franceville | 0.833 | 0.000 | 0.001 | 0.016 | 0.005 | 0.000 |
| Libreville | 0.879 | 0.010 | 0.000 | 0.009 | 0.001 | 0.000 |
| Makokou | 0.884 | 0.017 | 0.014 | 0.000 | 0.013 | 0.005 |
| Mouila | 0.838 | 0.013 | 0.010 | 0.016 | 0.000 | 0.000 |
| Oyem | 0.859 | 0.008 | 0.008 | 0.012 | 0.008 | 0.000 |

Table 7: Number of clusters (K) based on the progression of the average estimate of Ln likelihood of data in Gabon local chicken.

| K | Reps | Mean LnP(K) | SD LnP(K) | Ln'(K) | \|Ln''(K)\| | Delta K |
|---|---|---|---|---|---|---|
| 1 | 10 | -14852.8 | 0.274064 | — | — | — |
| 2 | 10 | -13888.3 | 92.665530 | 964.48 | 255.82 | 2.760681 |
| 3 | 10 | -13179.6 | 5.175197 | 708.66 | 257.63 | 49.781680 |
| 4 | 10 | -12728.6 | 4.115945 | 451.03 | 188.84 | 45.880110 |
| 5 | 10 | -12466.4 | 25.855700 | 262.19 | 21.49 | 0.831151 |
| 6 | 10 | -12225.7 | 13.818570 | 240.70 | 74.24 | 5.372480 |
| 7 | 10 | -12059.3 | 77.484300 | 166.46 | 46.40 | 0.598831 |
| 8 | 10 | -11939.2 | 62.449380 | 120.06 | 27.47 | 0.439876 |
| 9 | 10 | -11846.6 | 87.939880 | 92.59 | 13.43 | 0.152718 |
| 10 | 10 | -11740.6 | 49.833090 | 106.02 | — | — |

## DISCUSSION

In this study, we assessed the genetic diversity and relationships between and within five population of local chicken from Gabon and one exotic line based on genotyping individuals at 28 microsatellite loci.

**The rate of polymorphic loci:** All microsatellite loci were found polymorphic at the 95% threshold in all populations. This result indicates that microsatellites are effective molecular markers for the study of genetic diversity of these chicken populations. The average PIC is a value that provides information about the polymorphism of the alleles[22]. In our study, 71.43% of all loci were highly informative, only 28.57% were moderately informative (PIC between 0.25 and 0.50). The average PIC value was 0.582, the highest value was recorded at locus LEI0234 with a PIC = 0.899. The lowest value was recorded at locus MCW0103 (PIC = 0.324). These results confirm that the loci used were appropriate for estimating the genetic diversity of the local chicken populations in Gabon. The average PIC found in this study (0.582) was similar to that obtained in Cameroon (0.570)[10], lower than the PIC obtained in Rwanda (0.645)[9] and higher than the PIC obtained in Burkina Faso (0.541)[23].

**Number of alleles:** The average number of alleles per marker in this study (10.429), was higher than those reported in previous studies, which were on average 6.35[23], 9.04[10]; 7.09[24] and 4.2[25]. This, despite a number of individuals genotyped here two times smaller than those in the study by Keambou *et al.*[10] in Cameroon, for example. The values obtained in this study were in the same range as observed by Habimana *et al.*[9] in Rwanda (10.893) and Ethiopian chicken ecotypes (10.6) found by Dana[26]. But this value was lower than those obtained by Clementino *et al.*[27] in Brazilian (13.3), by Li *et al.*[28] in Bhutanese (14.17)[28] and by Putman and Carbone[29] in Chinese (16.8) chicken populations.

Heterozygosity is a factor generally taken into account in studies of genetic diversity within populations. The average heterozygosity of a population is an element for identifying the level of stability of the said population. In fact, a low heterozygosity of the population indicates a high genetic stability of the population[30] . In this study, Ho of the different populations of local chicken in Gabon varies from 0.182 to 0.838 with an overall average value of 0.518, while He varies from 0.290 to 0.874 with an overall average of 0.577.

**Genetic diversity:** The average expected heterozygosity (0.577) is statistically significant ($p<0.05$) and higher than the observed heterozygosity (0.518) indicating a positive difference and suggesting a deficiency of heterozygotes in the studied population. This difference between expected and observed heterozygosity may be the result of several factors such as the location, the sample size, the population structure and the source of markers[22]. The gene diversity (heterozygosity) over loci observed (0.518) was similar to that observed in Burkina Faso[23] (0.521) and Cote d'Ivoire[31] (0.528) respectively and lower than those reported by Habimana *et al.*[9] in Rwanda (0.616) and Keambou *et al.*[10] in Cameroun (0.60) on local chicken.

Under the assumption of Hardy Weinberg, selected ecotypes showed a significant ($p<0.05$) deficit of heterozygotes that is observable with the Fis values of each local population, while the exotic population showed a significant excess of heterozygotes. The mean Fis values for the populations of Franceville (Fis = 0.120), Libreville (Fis = 0.186), Makokou (Fis = 0.105), Mouila (Fis = 0.145) and Oyem (Fis = 0.141) were all positive and suggested a heterozygote deficit in all the local chicken populations studied. Three main factors could explain the observed imbalance: inbreeding through modification of frequencies and genotype, leading to a progressive loss of genetic variability over generations. The existence of null alleles, as a mutation in the flanking sequences of the microsatellite could

lead to the presence of null alleles and thus no PCR amplification. Finally, the Wahlund effect[32,33] resulting in the presence of subpopulations within each ecotype.

The study of Private Alleles (PA) in local populations showed that there was a great genetic diversity between the populations. Indeed, the number of private alleles was highest in Libreville (40), followed, far behind, by Mouila (4). Franceville, Makokou and Oyem recorded 3 private alleles. The total number of private alleles in this study (107) was higher than that found in Rwanda (60) by Habimana *et al.*[9] and Cameroon (24) by Keambou *et al.*[10]. This parameter is a good indicator of the relationship and structure of a population. However, the control of the economic trait linked to these alleles needs to be done in further studies.

Analysis of molecular variability (AMOVA) revealed that the overall diversity among local chickens in the five regions of Gabon was very low and mainly due to diversity among individuals within the population (83%). Similar results were found in Burkina Faso[23]; in Rwanda[9], in Cameroon[10] and Côte d'Ivoire[31].

Wright's F-statistics (Fis, Fit, Fst) provide important information about the evolutionary processes that influence the structure of genetic variation within and between populations[34]. In our study, the Fis values of the local chicken in Gabon (12%, 18.6%, 10.5%, 14.5% and 14.1%) are higher than those reported by Yacouba *et al.*[23], Habimana *et al.*[9], Keambou *et al.*[10] and Loukou *et al.*[31], in Burkina Faso, Rwanda, Cameroon and Côte d'Ivoire, respectively. However, they were lower than those reported by Özdemir and Cassandro[35] who worked on local subpopulations of Turkish chickens from Denizli using 19 microsatellites. These values were also found to be lower than those obtained by Kaya and Yıldız[22] for indigenous Turkish chicken (30.1%) with 10 SSR loci.

Of the fixation indices, Fst is directly related to the variance in allele frequency between populations and, conversely, to the degree of similarity between individuals within populations[34]. Fst was calculated between populations; the genetic differentiation parameter was almost zero between local populations. This reflects the absence of geographical and genetic structuring between these populations, which would therefore be homogeneous. This clear rapprochement presupposes exchanges of animals between these regions. In fact, the local chicken in Gabon is an animal that is widely used as a present during various traditional ceremonies, particularly customary marriages that are often interregional. This could be the origin of a strong sharing of genetic material between these populations of chickens. On the other hand, there is a distinction between the exotic chicken population and the local populations ($0.165 \leq Fst \leq 175$) which can be explained by the isolation of the exotic chickens' farms. Indeed, exotic chickens are generally used in Gabon in industrial, intensive or semi-intensive breeding. While local chickens are raised in a free-range system.

The genetic distance (GD) matrix between the populations indicates very low GD values within the local populations ($0.008 \leq GD \leq 0.017$) confirming similarity and thus membership of the same genetic group. This is not the case when comparing the five local populations to the exotic population: the genetic distance is very high in the different cases ($0.833 \leq GD \leq 0.884$), which means that the populations of Libreville, Franceville, Makokou, Mouila and Oyem have no genetic similarity with the exotic chickens and do not belong to the same genetic group, confirming the above result on genetic differentiation (Fst). Comparison of the genetic structures of the 5 populations of Gabonese local chicken did not reveal any distinct structuring. The geographical distances between these ecotypes may not be sufficient to promote isolation but rather gene flow between ecotypes. Indeed, gene flow (Nm) between pairs of local population can be separated in two major groups. The first group of population showed a relative high value ($14.140 \leq Nm \leq 20.409$) and the second one with high value ($25.517 \leq Nm \leq 33.050$) whereas it was lower between local and exotic populations ($1.181 \leq Nm \leq 1.268$). Overall, the values reflect a significant exchange of genes between local populations only.

Using grouping method[19], individuals with similar genotypes were grouped together to form a single population (Fig. 4). For a high value of K (1 to 10), the local population showed a very low internal heterogeneity. Separation between the local populations was not possible because of the low values of genetic distances between them. Similar results were observed by Muchadeyi *et al.*[36] during the structuring of the Zimbabwe traditional chickens but the studies in South Africa and Cameroon[10,37] showed a structure of traditional chickens in subpopulations following geographical origin and phenotype. It appeared that the three gene pools resulting from the structuring (Fig. 4) of the total population, the local population forms two pools with almost similar profiles and the exotic population forms the third gene pool. These results also show that there is very little introgression of commercial (exotic) chickens into the local chicken populations in Gabon due to the low interbreeding rate between the commercial strains and the local population. Thus, the local pools in Gabon currently retain this uniqueness and these initial genetic traits.

## CONCLUSION

This is the first study in Gabon on molecular characterization of indigenous chicken. This study allowed us to understand the structure of the population of local chicken from different agroecological zones of Gabon. It showed that there is no apparent structuring and thus, no differentiation between the 5 ecotypes (Libreville, Mouila, Oyem, Makokou and Franceville). Thus, the variances obtained in the intra- and inter-population genetic diversity of local chicken in Gabon can shed light on conservation strategies and enable priorities to be better established. This study highlights the uniqueness of the local hen in Gabon and provides a decision-making tool for developing conservation and improvement programmes for our local breeds, without upsetting the unique genetic structure of hen populations.

## REFERENCES


1. Fadlaoui, A., 2006. Modélisation bioéconomique de la conservation des ressources génétiques animales. Ph.D. Thesis, Université Catholique de Louvain.
2. FAO, 2011. Draft guidelines on molecular genetic characterization of animal genetic resources. Rome Comm. Genet. Food Agric. Sixth Sess. 61-63. https://openknowledge.fao.org/items/b7a00762-aa91-4991-9221-2d7ee5029dd9.
3. Tadano, R., M. Sekino, M. Nishibori and M. Tsudzuki, 2007. Microsatellite marker analysis for the genetic relationships among Japanese long-tailed chicken breeds. Poult. Sci., 86: 460-469.
4. FAO, 2005. Interactions du genre, de la biodiversité agricole et des savoirs locaux au service de la sécurité alimentaire. Food and Agriculture Organization, Rome, Italy, Pages: 190.
5. Zanetti, E., 2009. Genetic, phenotypic and proteomic characterisation of local chicken breeds. Ph.D. Thesis, University of Padua.
6. FAO, 2007. The state of the world's animal genetic resources for food and agriculture. Food and Agriculture Organization, Rome, Italy, Pages: 511.
7. FAO., 2007. Global plan of action for animal genetic resources and the Interlaken declaration. Proceeding of the International Technical Conference on Animal Genetic Resources for Food and Agriculture Interlaken, September 3-7, 2007 Food and Agriculture Organization of the United Nations 1-37.
8. Mboumba, S., G.D. Maganga, M.A. Ndzighe and T.C. Keambou, 2020. Morphobiometric characterization of the local chicken from two regions of Gabon. J. Interdiscip. Res. Sci., 1: 26-34.
9. Habimana, R., T.O. Okeno, K. Ngeno, S. Mboumba and P. Assami *et al*., 2020. Genetic diversity and population structure of indigenous chicken in rwanda using microsatellite markers. PLOS ONE, Vol. 15, 10.1371/journal.pone.0225084.
10. Keambou, T.C., B.A. Hako, S. Ommeh, C. Bembide and E.P. Ngono *et al*., 2014. Genetic diversity of the Cameroon indigenous chicken ecotypes. Int. J. Poult. Sci., 13: 279-291.
11. Smith, L.M. and L.A. Burgoyne, 2004. Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper. BMC Ecol., 4: 4-4.
12. FAO, 2011. Draft guidelines on molecular genetic characterization of animal genetic resources. Commission on Genetic Resources for Food and Agriculture, Thirteenth Regular Session, Rome, Italy. http://www.fao.org/docrep/meeting/022/am652e.pdf.
13. Guo, X. and R.C. Elston, 1999. Linkage information content of polymorphic genetic markers. Hum. Heredity, 49: 112-118.
14. Liu, K. and S.V. Muse, 2005. PowerMarker: An integrated analysis environment for genetic marker analysis. Bioinformatics, 21: 2128-2129.
15. Peakall, R. and P.E. Smouse, 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics, 28: 2537-2539.
16. Nei, M., 1972. Genetic distance between populations. Am. Naturalist, 106: 283-292.
17. Yeh, F.C., R.C. Yang, T.B.J. Boyle, Z. Ye, J.M. Xiyan, R. Yang and T.J. Boyle, 2000. PopGene32, Microsoft Windows-based freeware for population genetic analysis. Version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada. https://www.scienceopen.com/document?vid=2d45ad78-b140-4b66-b80f-2c9f513ec997.
18. Perrier, X. and J.P. Jacquemoud-Collet, 2006. DARwin software. Genetic Improvement of Vegetatively Propagated Crops. http://darwin.cirad.fr/Home.php.
19. Pritchard, J.K., M. Stephens and P. Donnelly, 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.
20. Evanno, G., S. Regnaut and J. Goudet, 2005. Detecting the number of clusters of individuals using the software structure: A simulation study. Mol. Ecol., 14: 2611-2620.
21. Earl, D.A. and B.M. vonHoldt, 2012. Structure harvester: A website and program for visualizing structure output and implementing the Evanno method. Conservation Genet. Resour., 4: 359-361.
22. Kaya, M. and M.A. Yildiz, 2008. Genetic diversity among Turkish native chickens, denizli and gerze, estimated by microsatellite markers. Biochem. Genet., 46: 480-491.

23. Yacouba, Z., H. Isidore, K. Michel, G.B. Isidore and T. Boureima *et al*., 2022. Genetic diversity and population structure of local chicken ecotypes in Burkina Faso using microsatellite markers. Genes, Vol. 13, 10.3390/genes 13091523.
24. Fotsa, J.C., D.P. Kamdem, A. Bordas, M. Tixier-Boichard and X. Rognon, 2011. Assessment of the genetic diversity of Cameroon indigenous chickens by the use of microsatellites. Livest. Res. Rural Dev., Vol. 23.
25. Bodzsar, N., H. Eding, T. Revay, A. Hidas and S. Weigend, 2009. Genetic diversity of Hungarian indigenous chicken breeds based on microsatellite markers. Anim. Genet., 40: 516-523.
26. Dana, N., 2011. Breeding programs for indigenous chicken in Ethiopia: Analysis of diversity in production systems and chicken populations. Ph.D. Thesis, Wageningen University, The Netherlands.
27. Clementino, C.S., F.J.V. Barbosa, A.M.F. Carvalho, R.A.R. Costa-Filho and G.R. Silva *et al*., 2010. Microsatellite DNA Loci for population studies in Brazilian chicken ecotypes. Int. J. Poult. Sci., 9: 1100-1106.
28. Li, Y.C., A.B. Korol, T. Fahima, A. Beiles and E. Nevo, 2002. Microsatellites: Genomic distribution, putative functions and mutational mechanisms: A review. Mol. Ecol., 11: 2453-2465.
29. Putman, A.I. and I. Carbone, 2014. Challenges in analysis and interpretation of microsatellite data for population genetic studies. Ecol. Evol., 4: 4399-4428.
30. Cheng, H.W., 2010. Breeding of tomorrow's chickens to improve well-being. Poult. Sci., 89: 805-813.
31. Loukou N.E., C.V. Yapi-Gnaoré, T. Gnénékita, Y. Coulibaly and X. Rognon *et al*., 2009. Evaluation de la diversité des poulets traditionnels de deux zones agroecologiques de Cote d'Ivoire a l'aide de marqueurs microsatellites. J. Anim. Plant Sci., 5: 425-436.
32. Jordana, J., P. Alexandrino, A. Beja-Pereira, I. Bessa and J. Cañon *et al*., 2004. Genetic structure of eighteen local south European beef cattle breeds by comparative *F*-statistics analysis. J. Anim. Breed. Genet., 120: 73-87.
33. Mohammadabadi, M.R., M. Nikbakhti, H.R. Mirzaee, A. Shandi, D.A. Saghi, M.N. Romanov and I.G. Moiseyeva, 2010. Genetic variability in three native Iranian chicken populations of the Khorasan province based on microsatellite markers. Russ. J. Genet., 46: 505-509.
34. Holsinger, K.E. and B.S. Weir, 2009. Genetics in geographically structured populations: Defining, estimating and interpreting $F_{ST}$. Nat. Rev. Genet., 10: 639-650.
35. Ozdemir, D. and M. Cassandro, 2018. Assessment of the population structure and genetic diversity of Denizli chicken subpopulations using SSR markers. Ital. J. Anim. Sci., 17: 312-320.
36. Muchadeyi, F.C., H. Eding, C.B.A. Wollny, E. Groeneveld and S.M. Makuza *et al*., 2007. Absence of population substructuring in Zimbabwe chicken ecotypes inferred using microsatellite analysis. Anim. Genet., 38: 332-339.
37. van Marle-K-sterl, E., C.A. Heferl, L.H. Nelll and M.A.M. Groenen, 2008. Genetic diversity and population structure of locally adapted South African chicken lines: Implications for conservation. S. Afr. J. Anim. Sci., vol.38.



.