Research Article

Assessment of genetic diversity of native chicken (*Gallus gallus domesticus*) population in Central African Republic

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Abstract

In the Central African Republic (CAR), poultry production primarily relies on indigenous chicken *Gallus gallus domesticus* as a raw genetic resource that has never been characterised despite its great genetic and economic potential in rural and periurban areas. The present study assessed the genetic diversity of native chicken in CAR using 16 microsatellite markers. Blood samples were randomly collected on FTA filter cards from 205 adult native chickens from two agroecological zones out of four in CAR, including 102 from the forest and 103 from the savannah. Sixteen microsatellite markers could amplify, giving a coverage rate of 61.54% of the SSR panel used. A low but positive Fis mean value (0.16) suggested a low inbreeding and a weak reduction in Heterozygosity of individuals due to non-random mating within each population. Overall genetic diversity (0.54), Heterozygosity (0.45) and allele frequency (0.61) confirmed a moderate to high genetic variation within the CAR indigenous chicken population. The molecular variance was more significant within individuals (81%) than among individuals (16%) and between populations (3%). Evanno's method shows that the likely number of sub-populations is 2 as influenced by the agroecological zone while Structure showed admixture between the two populationsdespite the low observed inbreeding. These results suggest a weak structuring of the CAR native chicken population and great possibilities for selection and genetic improvement. However, SSR markers are neutral and further studies need to be done using functional markers to detect functional and productivity genes relevant to disease resistance, environmental resilience and production traits.

Keywords: Genetic diversity, microsatellite-markers, native chicken, forest-and-savannah ecotypes, Central African Republic.

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Résumé

En République Centrafricaine (RCA), la production avicole repose principalement sur la poule locale dite indigène (*Gallus gallus domesticus*). Cette ressource n'a jamais été génétiquement caractérisée malgré l'énorme potentiel économique découlant de son exploitation en milieu rural et périurbain. Cette étude avait pour but d'évaluer la diversité génétique de la poule locale en RCA par génotypage à l'aide de 16 marqueurs microsatellites. Des échantillons de sang ont été prélevés au hasard sur 205 sujets adultes provenant de deux des quatre zones agroécologiques de RCA, dont 102 en zone forestière et 103 en savane. Les résultats ont révélé que 16 marqueurs microsatellites ont été amplifiés avec un taux d'amplification de 61,54 % des marqueurs recommandés pour l'espèce poule. La valeur moyenne du Fis a été faible et positive (0,16) suggérant une faible consanguinité et une faible réduction de l'hétérozygotie. La diversité génétique globale (0,54), l'hétérozygotie moyen (0,45) et la fréquence allélique élevée (0,61) ont confirmé une variabilité génétique modérée à élevée au sein de la population de poule locale de la RCA. La variance moléculaire au sein de l'individu a été très élevée (81 %) qu'entre individus (16 %) et entre populations (3 %). La méthode d'Evanno montre que le nombre probable de sous-populations est de 2, tandis que la structure génétique a confirmé un brassage de deux sous populations et une faible consanguinité observée. Ces résultats suggèrent une faible structuration de la population de poules originaires de RCA et une possibilité de sélection et d'amélioration génétique. Cependant, les microsatellites étant des marqueurs neutres, des études supplémentaires doivent être réalisées à l'aide des marqueurs fonctionnels pour détecter les gènes utiles en vue de l'amélioration des performances de production et d'adaptabilité.

Mots clés : diversité génétique, marqueur microsatellite, poule locale, forêt, savane, République Centrafricaine.

Introduction

The poultry sector in Central Africa is almost 100% dependent on breeding stock imports for commercial production. This dependence has been demonstrated to be a setback for food self-determination in a global crisis context. Indeed, world agri-food systems have been affected by international security and sanitation crises, resulting in severe economic losses and inflation, according to FAO (2022). These crises include the ongoing Russo-Ukrainian and Israelo-Palestinian wars and the COVID-19 pandemic, severely impacting the economies and food security of countries with extraverted economies and livestock-food systems, including poultry production. In this context, the biodiversity of native livestock breeds is crucial not only for food security and poverty alleviation but also for socio-cultural resilience and climate change mitigation (FAO, 2015). Native breeds of livestock serve as a reservoir for genetic improvement of production performances and adaptability traits, such as growth, egg production, heat tolerance (Hako *et al.*, 2009 and 2023), and superior immune response to diseases (Hako *et al.*, 2015 and 2021).

Among African indigenous livestock breeds, using microsatellite markers, the tremendous genetic diversity of indigenous chicken populations has been confirmed in Cameroon, a country neighbouring the Central African Republic (CAR). Indigenous chickens are typically colourful and phenotypically diverse. They are reared in the backyard, also known as the low input/output production system (Fotsa et al., 2011; Keambou *et al.*, 2014). These chickens are dual-purpose, with lightweight and small-sized eggs. A similar situation is observed in CAR, where native chicken is reared among other livestock species

(Bembidé *et al.*, 2013). The native chicken is a crucial source of household food security and income, particularly for poorer households that cannot keep other livestock such as cattle, sheep, and goats. It is genetically well-suited for adaptation to open environments and breeding projects in African conditions (Romanov and Weigend, 2001). However, keeping local chickens has become challenging due to rapid demographic growth and urbanisation. From 1960 to 2019, the urbanisation rate increased, and the overall rural population in sub-Saharan Africa decreased from 85.31% to 59.23%. A similar trend has been observed in CAR, with a growing population from 1.5 million to 4.7 million from 1960 to 2019, leading to a decrease in the rural population from 90% to 58% of the national population in the same period (FAO, 2022).

In 2022, the national chicken population was estimated at 7,138,000 million heads, representing 82% of the country's poultry population. With 1.17 g of protein and 7.12 g of meat per capita daily, chicken production cannot satisfy the growing national demand estimated at 5.58 million inhabitants. The government has resorted to importing commercial chicken breeds and strains to meet the increasing demand for chicken meat and eggs, including 2086.88 tonnes of eggs for USD 1,770,510 and 177,000 live birds as breeding stock for USD 122,000 (USD1 = XAF 629 in June 2022) (FOA, 2023). However, this overreliance on imports for commercial poultry production is not a sustainable option for preserving chicken genetic diversity. The gradual replacement of native breeds with imported ones has already contributed to a poultry biodiversity crisis in sub-Saharan Africa, with 37 native breeds already extinct, as reported by the FAO in 2007. Although imports can address immediate gaps in supply, they are the primary source of foreign gene introgression within national gene pools. A more effective and sustainable solution in the medium and long term is to explore and genetically improve indigenous chicken breeds in the Central African Republic (CAR). This aligns with the African Union Agenda 2063, which prioritises preserving and valorising African livestock breeds heritage to secure social, cultural, economic, and food self-determination and a resilient food system. Genetic characterisation of native chicken breeds in CAR can provide valuable insights into genetic diversity, informing future research and genetic improvement strategies. While a phenotypic characterisation of indigenous chickens in CAR has already been conducted, this research aims to complement it by assessing the genetic diversity of two ecotypes of native chicken breeds in CAR.

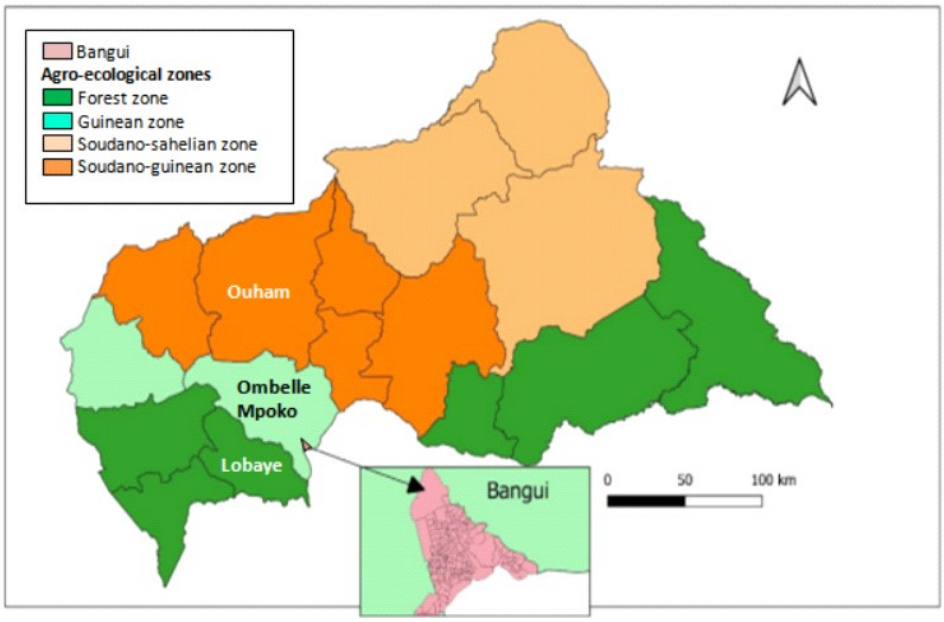
Materials and methods

Field study location

Samples were obtained from two administrative divisions covering two of the four agroecological zones in the Central African Republic, as shown in Figure 1. The first division, called Ouham, is situated in the north in the Soudano-Guinean zone (Savannah) and covers an area of 50,250 km2, with GPS coordinates of 7° 02 03 N, 18° 02 03 E. It shares its northern border with Cameroon, potentially allowing gene-flow exchange between Cameroon and CAR's native chickens. The second division, called Lobaye, is located in the south in the Forest zone, covering an area of 19,235 km2, with GPS coordinates of 4° 02 03 N, 18° 02 03 E. It shares its borders with the Republic of Congo and the Democratic Republic of Congo, which could be potential routes for in and out-migration of native chicken gene pools. The total area covered by the sample collection was 69,485 km2. These two divisions were selected based on their accessibility and the importance of poultry production in these areas at the time of the study. These administrative divisions are located in the north and south locations of the country's cross-section. The Ombelle Mpoko division, a 33,835 km2 gap between the two sampled areas, was

considered a buffer zone to compare the effect of the agroecological zone on the genetic diversity and explore the possible uniqueness of both sub-populations. The environmental difference between both sites was considered a potential factor affecting the variation in genetic diversity.

The poultry sector is the second-largest in meat production after cattle, with native chicken species being reared in up to 90% of rural and periurban households in the sampled administrative divisions (Bembide *et al.*, 2013).



Source: Adapted from FAO, 2021

Figure 1: Map of the Central African Republic showing the two sampled administrative divisions (Ouham and Lobaye) and the tampon zone (Ombelle Mpoko)

Animal material

The study utilised adult local chickens of both sexes, previously described by Bembidé *et al.* (2013). On average, the hens weighed 1,176±206 g, and the roosters weighed 1,514296 g. These chickens are endowed with a variety of stunning colours, out of which white is the most prevalent (18%), followed by wild type (13.4%), wheaten (11.1%), golden red (6.7%), bared (4.9%), and various mixes (45.9%). The plumage structure and distribution of these chickens include 75% of birds having a typical feathered appearance, while 10% have a crested head, 7% have a naked neck, 5.9% have feathered shanks, and 2.1% are frizzled.

Sample collection and DNA extraction

A total of 205 adult native chickens were randomly sampled from November 2011 to February 2012, with 102 being from the Forest zone, and 103 from the Soudano-guinean (Savannah) zone. To collect the blood samples, a drop was taken from the wing vein of each chicken and placed onto Whatman FTA™ filter cards (Whatman International Ltd). The filter cards were then air-dried under shade for 30 to 45 minutes and kept individually in labelled envelopes until DNA extraction. Genomic DNA was extracted using a boiling method, as described by Smith and Burgoyne (2004).

PCR amplification and genotyping

Sixteen microsatellite markers labelled with fluorescent dyes were used for the experiment based on their high polymorphism and genome coverage, as recommended by FAO (2011). To avoid interactions and mispairing during amplification, each set of 3 to 4 markers was made

of primers with different dyes and located on other chromosomes. Optimal PCR conditions were determined using gradient PCR Techne Plus®. A volume of 15 µl containing 25ng/µl of target DNA, 2X buffer, 0.8µM dNTPs, 0.4u/iL DreamTaq DNA polymerase, and 0.2µM of each forward and reverse primer was used for multiplex PCR reactions. The remaining primers which did not amplify in sets were amplified individually in a 10 µl volume reaction with 25ng/µl of target DNA, 1X buffer, 0.2µM dNTPs, 0.08u/iL DreamTaq DNA polymerase, and 0.2µM of each forward and reverse primer. Thermal cycling was performed in a GeneAmp® PCR system 9700 thermal cycler (Applied Biosystem) with a touch-down PCR program. The program started with 10 minutes at 94°C followed by ten cycles consisting of 1 min 15 sec at 94°C, 1 min 15 sec at 62°C, and 1 min 15 sec at 72°C. Then, 15 cycles of 1 min 15 sec at 94°C, 1 min 15 sec at 60°C, and 1 min 15 sec at 72°C were performed, followed by 15 cycles of 1 min 15 sec at 94°C, 1 min 15 sec at 58°C, and 1 min 15 sec at 72°C. Finally, a final extension step of 20 min at 72°C was conducted. The samples were analyzed on an ABI PRISM 377 Sequencer and GeneScanTM-500 LIZ® (Applied Biosystem) was used as an internal size standard. The GeneMapper version 4.1 determined the fragment sizes in base pairs.

Data analysis

The alleles were scored using GeneMapper v.4.1 software. The total allele numbers, the allele frequencies, the average number of alleles per locus, the Expected and Observed Heterozygosities (He, Ho), the Polymorphism Information Content (PIC), the Analysis of Molecular Variance, and Wrigth F-statistics (differentiation coefficient F_{ST}, within population inbreeding coefficient F_{IS} and among population inbreeding F_{IT}) were performed using Arlequin V.1.1 and GenAlex V.6.3 (Peakall and Smouse, 2006). The Evanno method (Evanno *et al.*, 2005) of estimating the more likely number of clusters was implemented, according to Dent Earl and Bridgett (2011). The algorithm implemented in STRUCTURE was used to cluster individuals based on multilocus genotypes (Pritchard *et al.*, 2000). The analysis involved an admixture model with correlated allele frequencies. The model was tested using 20,000 iterations (burn-in phase) and then 50,000 iterations for 2 = K = 6 with 100 runs for each K value, where K was the number of assumed clusters to be examined. A pairwise comparison of the 100,000 solutions was carried out. Solutions with over 95% similarity were considered identical.

Results

Genetic diversity parameters

Allele frequencies, allele numbers, genetic diversity, heterozygosities, and the polymorphism information content (PIC) of the native chicken of CAR are shown in Table 1. The polymorphism information contained was higher than 20%, indicating that all markers used were informative and qualified for the study of the genetic diversity of the CAR native chicken population, except MCW0248, with PIC=17.27%. Overall genetic diversity parameters show a moderate to high genetic variation within the CAR indigenous chicken population as confirmed by the mean heterozygosity (0.451), the genetic diversity (0.544) and allele frequency (0.609). Only 16 of the 26 markers were amplified, giving 61.54% coverage. Fourteen loci over 16 are in Hardy-Weinberg disequilibrium ($P < 0.001$ for 11 of them) with unbiased heterozygosities ranging from 0.36 to 0.72. Two alleles were in Hardy-Weinberg equilibrium and presented a low unbiased heterozygosity (0.21 and 0.23). The moderate to high genetic variation observed is a characteristic of outbreed populations with random mating as the dominant reproduction system.

Table1. Diversity parameters at the 16 microsatellite markers of CAR native chicken

Marker	Allele Frequency	Allele Number	Genetic diversity	UHe	PIC	P-value	Signif. HWE
MCW0034	0.45	11	0.74	0.72	0.71	0.00	***
MCW0067	0.64	5	0.53	0.47	0.48	0.00	***
LEI0166	0.76	4	0.39	0.36	0.36	0.00	***
MCW0014	0.80	7	0.34	0.21	0.32	0.00	Ns
MCW0206	0.49	6	0.64	0.56	0.58	0.00	***
MCW0069	0.52	9	0.63	0.60	0.57	0.00	***
MCW0104	0.84	16	0.29	0.24	0.29	0.19	ns
MCW0183	0.54	9	0.65	0.41	0.62	0.00	***
ADL0278	0.50	5	0.62	0.62	0.55	0.00	***
LEI0234	0.25	15	0.86	0.65	0.85	0.00	***
MCW0020	0.53	4	0.63	0.46	0.58	0.00	***
MCW0103	0.77	2	0.35	0.30	0.29	0.00	*
MCW0016	058	8	0.61	0.61	0.58	0.02	*
MCW0248	0.90	6	0.18	0.18	0.17	0.00	***
MCW0037	0.44	3	0.61	0.50	0.52	0.00	***
MCW0111	0.64	7	0.50	0.33	0.43	0.00	**
Mean	0.61	7.69	0.54	0.45	0.50		

*P < 0.05, **P < 0.01, ***P < 0.001

The analysis of allelic patterns across populations revealed a range of similarities between the Savannah and Forest agroecological zones. Still, the Savannah had a slight advantage in private alleles compared with the forest compared with the forest for the number of (6.9 and 6.5) and the number of private alleles (1.23 and 0.8), respectively. The Shannon Index (I), the number of effective alleles (Ne), and unbiased heterozygosity (He) also showed similar patterns. Despite the high number of alleles per locus (3 to 16, as illustrated in Table 1), rare alleles were found between the two agroecological zones. However, the similarity between the two zones was high, and no common allele presented a frequency of less than 50% in Figure 2.

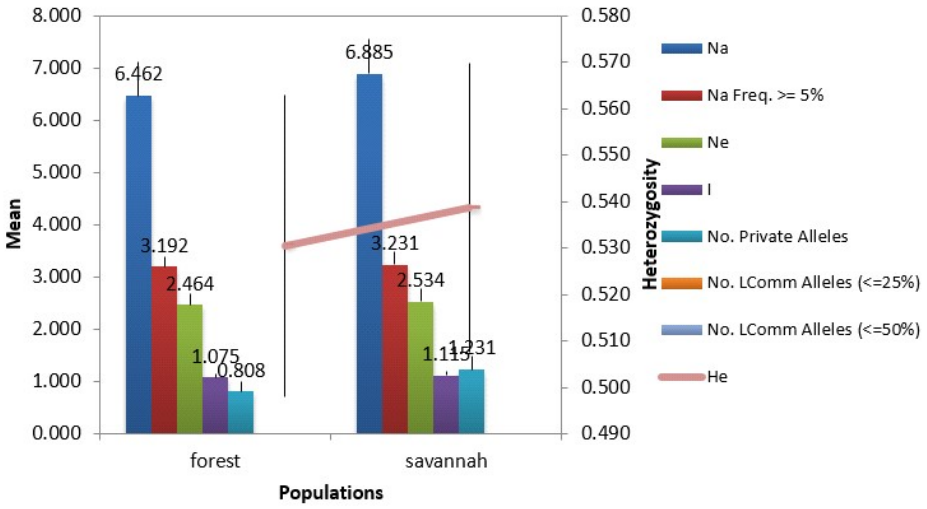


Figure 2: Allelic patterns across native chicken populations of CAR

According to the analysis of molecular variance displayed in Figure 3, a higher amount of significant variation is seen within individuals (81%) compared to individuals within populations (16%). Furthermore, more variation is observed among individuals within populations than among populations (3%). Based on this observation, it can be inferred that the native chicken of CAR is outbreeding; therefore, outcrossing or random mating is the dominant mode of reproduction.

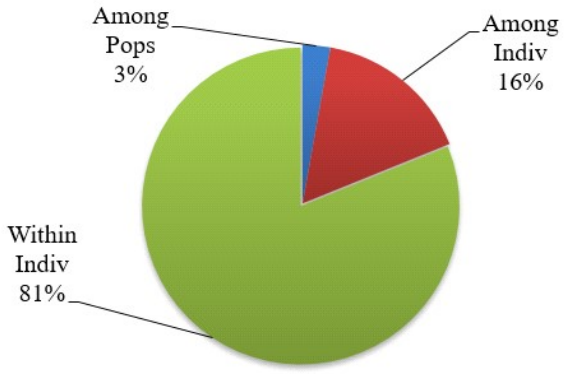


Figure 3: Analysis of Molecular Variance

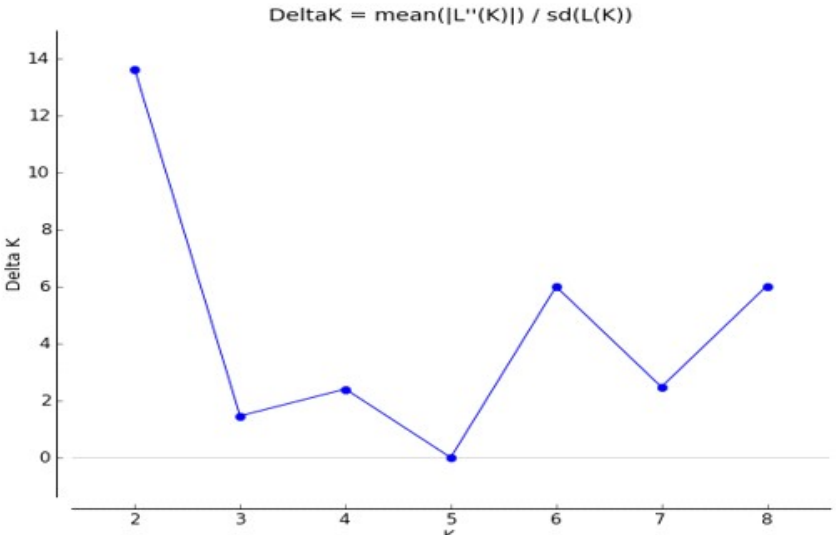


Figure 4a: Evanno method showing the more likely number of cluster or populations (Peaks at k = 2

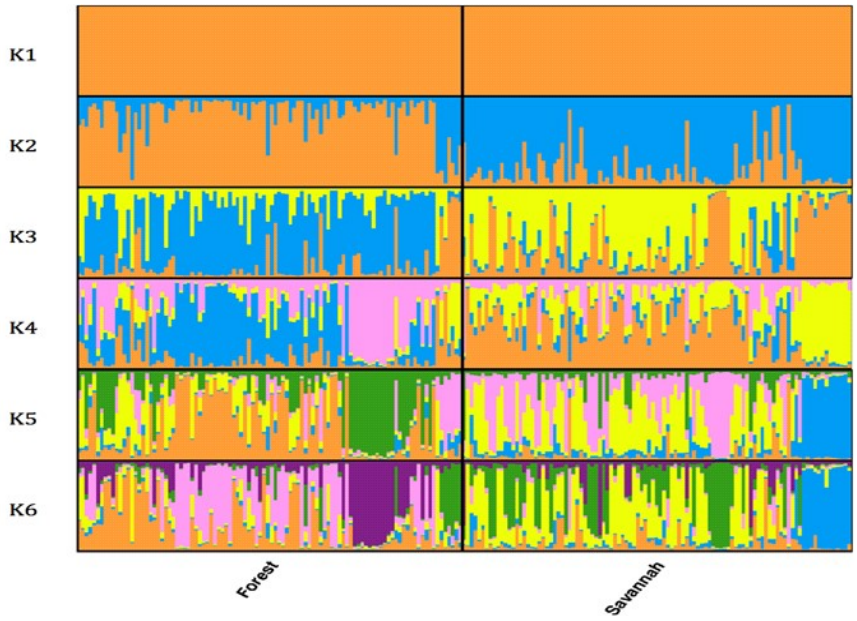


Figure 4b: Cluster assignment assessed with structure showing admixture between the savannah and the forest chicken populations.

Inbreeding information

Based on the data presented in Table 2, the average value of Fis is low at 0.16, indicating a low level of inbreeding within individuals compared to the population. Alternatively, this could suggest a weak reduction in heterozygosity of individuals due to non-random mating within each ecotype. On the other hand, Fst estimates genetic differentiation among populations, with an average value of 0.02, indicating weak genetic differentiation due to admixture between the studied populations. However, despite the weak differentiation evidenced by the F-statistics, the structure (Figure 4b) visually displays an influence of the agroecological zone on the genetic diversity despite the high level of admixture observed.

Table 2. F-Statistic

F-Statistic	F_{is}	F_{it}	F_{st}
Min.value	-0.03	-0.02	0.00
Max.value	0.39	0.42	0.21
Mean Value	0.16	0.17	0.02

Discussion

According to the overall analysis of the results, there is a weak genetic differentiation, structuring, and high genetic diversity of the two ecotypes of native chickens of CAR. This suggests that random mating is the dominant reproduction practice. The findings are consistent with a survey report by Bembide *et al.* (2013), which stated that the low-input/output production system is prevalent in CAR. This system involves chickens either not housed or poorly housed, with poor management and reared on free-range with unrestricted movements. Furthermore, traders transport the chickens from one locality to another and from one market to another.

Various studies have been conducted in countries such as Cameroon, Burkina Faso, Kenya, and Bangladesh using the same set of 30 microsatellite

markers. Similar analyses were performed in Cameroon and the Central African Republic (CAR) under the same conditions. However, only 16 microsatellite markers could be amplified in CAR's native chickens, which provided coverage of 53.3%. The coverage was lower than the 83.33% coverage obtained in Cameroon (Keambou *et al*., 2014) and also lower than the 66.7% coverage obtained by Yacouba *et al.* (2022) in Burkina Faso and the 60.0% coverage obtained by Noah *et al.* (2017) in Kenya. The coverage was, however, similar to that obtained in Bangladesh by Rashid *et al.* (2020).

The indigenous chickens of CAR were found to have only one identical marker (MCW0104), making them only 6.25% similar to those from Bangladesh. However, the level of identity increased with proximity, with six alleles (6.25%) being identical to those found in chickens from Kenya (MCW0034, MCW0067, MCW0103, MCW0111, MCW0183, and ADL0278) and 16 alleles (64% similarity) being identical to those found in chickens from Cameroon. These results demonstrate the unique nature of the indigenous chicken populations in CAR despite the evidence of possible admixture due to gene pool exchanges between CAR and Cameroon.

The average number of alleles per marker obtained in this study was 7.67, which is higher than that reported by Fotsa *et al.* (2011) (7.09) and that reported for local chicken in Burkina Faso (6.66), Iran (5.4), China (3.8), Egypt (7.3) and Vietnam (5) (Osei-Amponsah *et al*., 2010; Mohammadabadi *et al*., 2010; Cuc *et al*., 2010; Eltanany *et al*., 2011). However, it is lower than that reported in Cameroon (Keambou *et al*., 2014), Pakistan (Babar *et al*., 2012), Ethiopia (Nigussie *et al*., 2011), and Bangladesh (Rashid *et al*., 2020). Nonetheless, it falls within the same range as that reported in Ghana (7.8) (Liu *et al*., 2008). The allele pattern can identify genetic diversity and uniqueness in a population. A higher number of different alleles indicates greater genetic diversity. Private alleles are unique to a particular population, confirming their distinctiveness. CAR's Forest and Savannah ecotypes are exceptional, with the latter possessing more private alleles.

The overall allele frequency (0.61), genetic diversity (0.54), and heterozygosity (0.45) confirmed moderate to high genetic variation within the CAR indigenous chicken population. A mild to high heterozygosity is evidence of high gene diversity and low population consistency (Chen *et al*., 2004), as confirmed by the low population differentiation (F_{ST} =0.02). These results are consistent with previous reports (Noah *et al*. (2017); Yacouba *et al*. (2020), Rashid *et al*., 2020). The polymorphic information content (PIC) values ranged from 0.17 to 0.84, which is higher than the range reported in Chinese local chicken (0.30 to 0.49) (Eltanany *et al*., 2010) and comparable to those reported in Cameroon local chicken by Fotsa *et al.* (2011) and Keambou *et al*. (2014), in Burkina Faso (Yacouba *et al*., 2020), and Rashid *et al.* (2020) in Bangladesh. According to Bostein *et al.* (1980), a PIC value greater than 0.5 indicates a highly informative locus, a value between 0.25 and 0.5 indicates a reasonably informative locus and a value less than 0.25 indicates a slightly informative locus. This suggests that 30% of the used SSRs are highly informative, and 66% are reasonably informative for the diversity study of CAR indigenous chicken. However, the SSR marker MCW0248, with a PIC value of 0.17 and genetic diversity of 0.18, is not informative enough and should be excluded from diversity studies in native CAR chicken. The Hardy-Weinberg equilibrium observed at loci MCW0014 and MCW0104 and the low unbiased heterozygosity observed for them (less than 0.25) may be due to a selective pressure on both markers.

The study shows that the AMOVA is higher within the population than among individuals and populations. This finding is consistent with other studies conducted on local chicken populations, such as those reported by Nigussie (2011) in Ethiopia, Keambou *et al.* (2014) in Cameroon, Shabatzi *et al.* (2007) in Iran, Rashid *et al.* (2020) in Bangladesh, Yacouba et al. (2020) in Burkina Faso, and Noah et al. (2017) in Kenya. It is hypothesised that the variance within the population is higher than among natural unselected populations. The low but positive mean Fis value (0.16) suggests low inbreeding within individuals relative to the population or a weak reduction in heterozygosity of individuals due to non-random mating within each population. According to Tixier-Boichard *et al.* (2009), many farmers prefer particular phenotypes motivated by product quality, environmental adaptation, and cultural uses. This is also true in the Central African Republic, where farmers select traits such as egg production rate, growth rate, disease resistance, and cultural goals (Bembide *et al.*, 2012). Even if there is a minimal attempt for selection by farmers, the Structure analysis confirmed a high intensity of admixture between Central African Republic native chickens. The Evanno method showed many peaks, but the prominent break in slope of its evolution indicates that the absolute peak is at K=2 (Evanno *et al.*, 2005). Thus, sub-populations equivalents to distinct ecotypes of the same population are observed in Central African Republic native chickens.

Conclusions

This study has found that the native chicken population in the Central African Republic (CAR) shows a weak differentiation. In other words, the chickens from savannah and forest agroecological zones are not genetically distinct populations. Instead, they form a single population whose genetic diversity is influenced by environmental factors, leading to distinct ecotypes. The research has also revealed a high level of individual molecular variance, indicating a great potential for selective breeding and genetic improvement programs. However, further studies are required, including all four agroecological zones and using functional markers to identify the relevant genes for improving adaptability traits and economically essential characteristics.

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