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Full Length Research Paper

Phenotypic characterisation and molecular polymorphism of indigenous poultry populations of the species *Gallus gallus* of Savannah and Forest ecotypes of Benin

I. A. K. Youssao[1]*, P. C. Tobada[1], B. G. Koutinhouin[1], M. Dahouda[2], N. D. Idrissou[3], G. A. Bonou[1], U. P. Tougan[1], S. Ahounou[1], V. Yapi-Gnaoré[4], B. Kayang[5], X. Rognon[6] and M. Tixier-Boichard[6]

[1]Université d'Abomey-Calavi, Ecole Polytechnique d'Abomey-Calavi, Département de Production et Santé Animales, 01 BP 2009 Cotonou, Bénin.
[2]Faculté des Sciences Agronomiques / Université d'Abomey d'Abomey-Calavi, 01 BP 526 Cotonou, Bénin.
[3]Centre de Gestion Durable des Ressources Animales et Végétales (CGDRAV_ONG), BP 1096, Parakou, Bénin.
[4]Centre National de Recherche Agronomique, 01 BP 1740 Abidjan 01, Côte d'Ivoire.
[5]Department of Animal Science, Faculty of Agriculture, University of Ghana, Legon, Ghana.
[6]INRA,AgroParisTech, UMR1313 "Génétique animale et biologie intégrative", Centre de Recherches INRA, 78352 Jouy-en-Josas Cedex, France.

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The study of the phenotypic characterisation and molecular polymorphism of local chicken populations was carried out in Benin on 326 chickens of the Forest ecological area and 316 of the Savannah ecological area, all were 7 months old at least. The collection of blood for the molecular typing was achieved on 121 indigenous chickens of which 60 from the Savannah ecological area and 61 from the Forest ecological area. The genotyping was carried out for 22 microsatellite loci. Weight and body measures of the Savannah chickens were significantly higher ($P < 0.001$) than those of the Forest chickens. In the Savannah ecological area, the most frequent plumage colours were the black (22.15%), the white (19.62%), the coppery black (7.59%) and the golden partridge (7.59%). In the Forest area, the fawn (15.34%), the black (10.43%), the white (6.8%), the silver white (6.8%) and the golden partridge (6.75%) were the dominant feather colours. Thus, phenotypic characterisation showed significant differences between Savannah and Forest local chickens. The F_{ST} calculated between the Savannah and Forest populations revealed a low genetic differentiation and the dendogram showed that Savannah and Forest chickens were quite intermingled. In conclusion, local populations from Savannah and Forest area may be considered as ecotypes, but not as two distinct breeds.

Key words: Body weight, plumage colour, molecular polymorphism, local chickens, Benin.

INTRODUCTION

The genetic resources of poultry in West Africa are mainly represented by domestic local chickens (*Gallus gallus domesticus),* guinea fowls (*Numida meleagris*) and ducks (*Cairina* sp.). These local avian species are bred under traditional breeding system. The animals are bred for meat as well as for eggs; the divagation system is the rule and different species can be found intermingled. This type of breeding is described as family poultry farming and still constitutes the main part of poultry rearing in sub-

*Corresponding author. E-mail: issaka.youssao@epac.uac.bj. Tél: 00 229 97 91 20 74 / 95 28 59 88.

Saharan Africa. The size of the national poultry livestock of Benin was estimated in 2005 to be 16 690 000 heads of which 81.29% were local chickens (Bebay, 2006) for 6 000 000 inhabitants. In spite of this numerical importance, the local production of poultry meat is below the needs expressed by the consumers and this deficit has led to increased imports as well as to programmes of introduction of exotic breeds in Benin.

In 10 years, the quantum amount of poultry meat imported passed from 19361 tons metric in 1995 to 49634 tons metric in 2005 (FAO, 2008). Apart from this meat dependence toward foreign countries, certain diseases, which did not exist on the African continent, are present today in the majority of the West African countries in an endemic or epidemic form. Indeed, the Gumboro disease, the Marek's disease and the Newcastle disease are now present in Benin. Recently, the highly pathogenic avian influenza (HPIA) appeared in Africa in 2006 (Bebay, 2006) and in 2007 in Benin (Direction de l'Elevage, 2007) after having caused significant economic losses in several countries of Asia and Europe.

In addition, the crossings between large format cockerels of exotic breeds and local hens (small size) were realized in the majority of the countries of West Africa to increase body weight of progeny (FAO, 2004). These anarchistic crossings failed most often because of the lack of follow-up or the lack of adapted husbandry techniques. In the majority of cases, the females resulting from these crossings do not have, unfortunately, a good maternal ability (incubation, hatching, chicks' follow-up) (FAO, 2004).

From all that proceeds, it comes out that the local production must be encouraged to reduce imports and limit the spreading of new diseases. The improvement of this production must start with a better knowledge of the endogenous genetic resources, in order to utilize them for the development of the poultry sector in Africa. The purpose of this study was to characterise two ecotypes of *Gallus gallus* (the indigenous poultry populations of the savannah and the forest zones of Benin) in terms of morphobiometric, phaneroptic and genetic diversity.

MATERIALS AND METHODS

Areas of study

The study on the phenotypic characterisation of local chickens of the species *Gallus gallus* was carried out from October 2005 to May 2006 in the Forest and Savannah areas of Benin. The savannah area covers the departments of Borgou and Donga, while the forest zone includes the departments of Atlantic, Littoral, Mono and Couffo. In the department of Borgou, the survey was carried out in the Communes of Parakou, N'Dali, Kalale and Bembereke. In the Department of Donga, the investigations were done in the Communes of Djougou and Copargo. In the Atlantic and Littotal, the area of study was the Communes of Abomey-Calavi, Allada and Cotonou. In the Departments of Mono-Couffo, the area of study was the Communes of Lokossa and Aplahoue. The farmers identified for these investigations had a herd of at least 10 chickens during the survey. These Communes were retained in order to have a good spatial distribution of the local chicken populations in each area. Figure 1 locates the surveyed Communes.

The two departments of Savannah are characterized by a climate of Sudanese type. This climate is marked by one dry season (November to May) and one rainy season (June to October) with a yearly rainfall varying from 900 to 1000 mm and an average monthly temperature which varies between 26 and 28°C (ASECNA, 2007).

The Forest area of Benin has a subequatorial climate with 2 dry seasons and 2 rainy seasons. The rainfall mode is bimodal with a large and a small season of rains separated by dry seasons. The seasons of rains go from April to July and October to November. Average rainfall was 1200 mm per year, from 2000 to 2006 (ASECNA, 2007). The average monthly temperature varies between 27 and 31°C and the relative humidity fluctuates between 65% from January to March and 97% from June to July (ASECNA, 2007).

Animals

The study on the phenotypic characterisation of the local chicken populations of the species *G. gallus* was carried out on 642 adult chickens, of which 326 were from the Forest ecotype and 316 from the Savannah ecotype. All the birds (both sex) used in the study were seven months of age at least. Females should have laid eggs once at least. According to the farmer's declarations, the experimental birds came from farms that had no prior contact with exotic breeds. The breeding system can be characterized as extensive with random mating.

The blood collection for the molecular typing was performed on 121 indigenous chickens raised at the Experimental Farm of the "Ecole Polytechnique d'Abomey-Calavi", of which 61 samples originate from the Savannah and 60 from the Forest area. The blood was sampled from birds that were used for the phenotypic study.

Data-collection

Production system

A survey was carried out by direct discussion with the farmer. Questions were organized in 4 sections:

I. description of the farmer and the flock: farmer's education, age and sex, motivations for rearing local chickens, species raised and main productions;
II. origin of the flock: number of founder animals, how where they obtained (purchase, gift, inheritance);
III. management of the flock: individual identification, flock size, feeding system, mating plan, choice of breeding animals, incubation system, final use of the animals;
IV. health issues: mortality, time of occurrence and main causes of death, treatments.

Answers were collected using a pre-formatted Excel file, answer frequencies were calculated for each question and area of study. Possible differences between Savannah and Forest areas were analyzed with a Chi-square test.

Morphological and phenotypic traits

Body weight of each subject described was measured using a

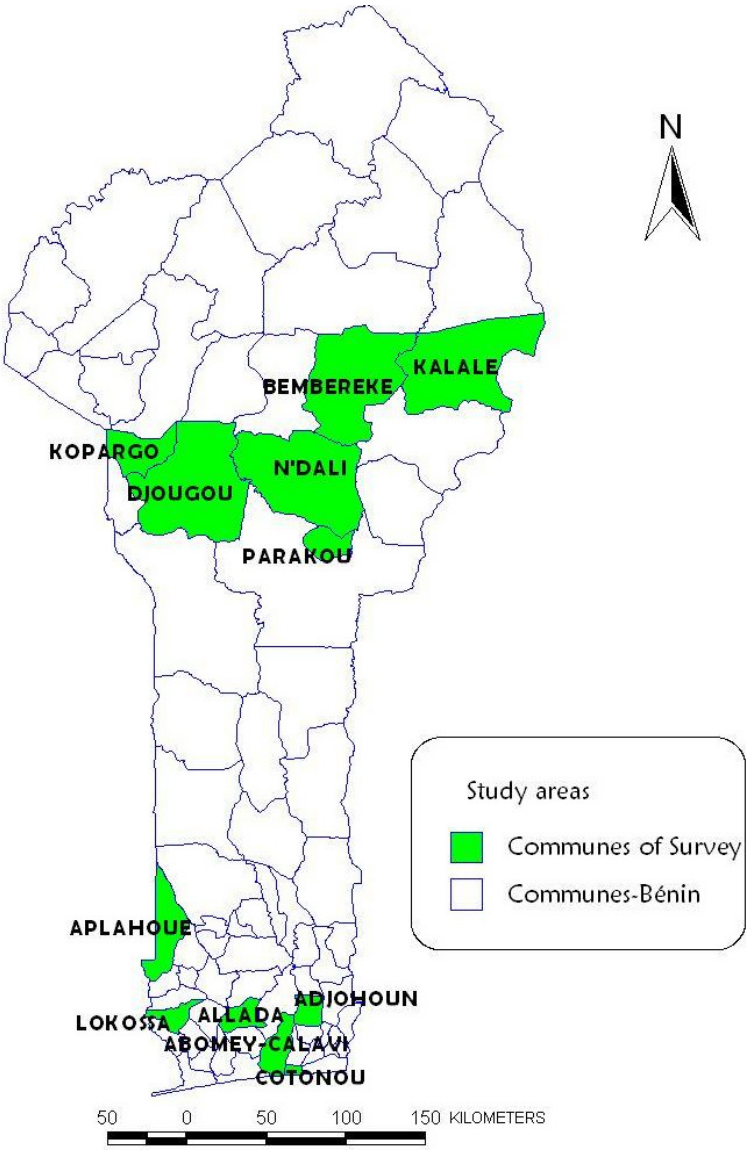

Figure 1. Areas of study.

mechanical balance of 2.5 kg of capacity with a precision of 20 g. The following body measures were then recorded using one ribbon meter: breast circumference, body length, shank length. The phaneroptic characters were determined by direct observation on each animal according to the nomenclature described by Coquerelle (2000). These criteria were: plumage colour and feather patterns, feathers' structure, feather distribution, shank colour and eyes colour.

Colour variants are controlled by major genes, where generally, one mutant allele is known as compared to the wild type. Only the *E* locus (black extension) includes several alleles which are associated to a various proportion of black pigments on the body. Since interactions between colour mutations are frequent, it is not always possible to identify the genotype pertaining to one locus for all individuals. Thus, the analysis was done on phenotypic frequency, which does not strictly correspond to an allelic frequency.

Data collection was completed by taking pictures for all animals under study. These photographs were brought to laboratory and were submitted to a second description in order to check the accuracy of the phaneroptic characters recorded on the site of study. The identification of major mutations with visible effects on the phaneroptic characters of local chickens was made according to Coquerelle (2000) and Bantam Club Français (2006).

DNA extraction and genotyping

Blood sampling was carried out at the level of the wing vein using a needle of 19G and preserved in a dry tube with heparin and of a capacity of 2 ml. Once conditioned, these tubes were preserved at -20°C in a refrigerated container of dry ice and were transported to the University of Legon in Ghana for the DNA extraction.

The DNA was obtained from 10 µl of the sampled blood, by an extraction with the Qiagen QIAamp kit in the laboratory of the Department of Animal Sciences of the Agriculture and Food Faculty of the University of Legon in Ghana. The genotyping was realized

by the GIE LABOGENA (Jouy-en-Josas, France), using 22 microsatellite markers previously selected for the AvianDiv European project (Hillel et al., 2003). Briefly, the genotyping procedure consisted in PCR amplification with fluorescent primers followed by migration on a capillary sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems) as described by Berthouly et al. (2008).

Statistical analysis

Morphological and phenotypic traits analysis

The statistical analysis was carried out with SAS software (Statistical Analysis System, 1989) and several procedures were used. The procedure *Proc freq* was used to determine the phaneroptic frequencies. These frequencies were calculated by ecotype (Savannah *vs* Forest) and were compared by the test of Chi-square.

A linear model was adjusted to the data of body measures and live weight. This model included the fixed effects of the ecotype and the sex. The interaction between the ecotype and the sex was taken into account in the model of variance analysis, as shown below:

$$Y_{ijk} = \mu + E_i + S_j + ES_{ij} + e_{ijk}$$

With
- Y_{ijk}: the weight or a body measure of the k^{th} hen of ecotype i and sex j;
- μ: general mean;
- E_i: fixed effect of the ecotype i (Savannah and Forest);
- S_j: fixed effect of the sex j (female and male).
- ES_{ij}: interaction between ecotype i and sex j;
- e_{ijk}: residual error.

The procedure of the generalized linear models (*Proc GLM*) was used for the analysis of the variance and the means were then calculated and compared by a t-test.

Molecular analysis

Allelic frequencies, observed heterozygoty (Ho) and mean unbiased estimates of gene diversity (He) (Nei, 1978), the average number of alleles (ANA) per locus and the average number of efficient alleles (Ae) per locus were calculated using the software GENETIX 4.4 (Belkhir et al., 2000). These parameters describe the importance of polymorphism within the populations. The GENEPOP 4.0 software was used to determine departure from Hardy-Weinberg equilibrium (Rousset, 2008), F-Statistics and pairwise genetic differentiation (Weir and Cockerman, 1984; Goudet, 2001). The multiple significance test was corrected using sequential Bonferroni procedure (Rice, 1989). The assignment of an individual to its supposed population, individuals were clustered according to the *DAS* (Jin and Chakraborty, 1994) genetic distance matrix, using the neighbor-joining (Saitou and Nei, 1987) method. Distance matrix and phenogram were computed using POPULATION 1.2.28 and TREEPLOT 0.7

RESULTS

Production system

The farmer's motivation for local chickens appeared to focus rather on meat quality and robustness of chickens in the Savannah, whereas tradition was the most frequent motivation declared in the Forest area. The owner of the animals was generally a farmer in the Savannah, but it could be craftsmen in the Forest. The surface of cultivated land per farmer was also greater on average in the Savannah. Women appeared more often to be in charge of poultry keeping in the Forest than in the Savannah.

The local chicken production system was familial and extensive in both areas. Only one parent was known (the hen) and rarely both (the male and the female parent). The incubation was natural according to all poultry breeders and less than 1% practiced occasionally the artificial incubation. The reproduction was not seasonal in the two areas and there was no evidence of a peak period for laying throughout. The average number of reproductive cocks was 3.42 in the Savannah and 2.95 in the Forest. The number of reproductive hens was more important ($P < 0.001$) in the Savannah (9.18) than in the Forest (5.45). The choice of the breeding animals (male and female) was made essentially at random in the two regions; it was very difficult for farmers to express any breeding objective, which could be considered in a breeding program involving selection within local popuations or crossing with exotic breeds or commercial lines.

The divagation was practiced by 92.11% of breeders in the Savannah of Benin and 94.12% of those of the Forest. The hen houses with frontage were rare and only used by 7.89% of breeders in the Savannah and 5.88 in the Forest; any permanent building was not found. The majority of the habitats were rudimentary and limited to a shelter used at night or during the rainy season; it did not comply with standard husbandry. The majority of the chicken breeders fed their animals by giving some cereals; the proportion of those giving some kitchen residues was more important ($P < 0.001$) in the Forest (76.47%) than the Savannah (9.65%) and the same tendency were observed on the use of termite in the poultry feeding.

In the Savannah as in the Forest, 85 to 88% of the breeders declared that majority of mortalities were observed between hatching and weaning. The causes of mortality varied with the age of animals and the region and could be grouped in four classes: accident, predator, disease and season. According to the interviewed breeders, the illnesses constituted the main reason of mortalities before the weaning, followed by the season, the predators and the accidents. It comes out from this study that the death rate was higher in the dry season than in the rainy season in the Savannah region of Benin whatever the age of the birds, whereas in the Forest region of the country, mortalities were noticed more often during the major rainy season. Veterinary treatments or vaccinations were more frequent in the Savannah than in the Forest. The reason for selling an animal was mainly

Table 1. Effect of ecotype, sex and the interaction between ecotype and sex on weight and body measurements of local chicken populations of the species *G. gallus* in Benin.

Parameters	Area		Sex		Interaction between area and sex				RSD	Significativity		
	Savannah	Forest	Female	Male	Savannah		Forest			Area	Sex	Interaction
					Male	Female	Male	Female				
Weight (g)	1197a	946b	965a	1177b	1309a	1085b	1046b	846c	233	***	***	NS
Breast circumference (cm)	34.2a	31.2b	31.3a	34.1b	35.9a	32.6b	32.4b	30.1c	2.4	***	***	*
shank length (cm)	13.4a	12.5b	12.0a	13.9b	14.7a	12.2c	13.3b	11.8d	1.1	***	***	*
Tarsus length (cm)	9.7a	8.5b	8.2a	10.0b	10.9a	8.6c	9.1b	7.8d	1.1	***	***	**
Body length (cm)	20.6a	19.2b	19.1a	20.7b	21.6a	19.6b	19.8b	18.7c	1.5	***	***	*

* : P < 0.05 ; ** : P < 0.01 ; *** : P < 0.001 ; R S D: residual standard deviation. Within rows, means followed by different letters, differ significantly with the threshold of 5%.

the need for meat in the Savannah, whereas it was the need of money in the Forest. Males are sold in priority, according to the market price in the Forest, whereas price was generally negotiated on an individual basis in the Savannah.

Live weight and body measures

The live weight and body measures of the animals of Savannah ecotype were significantly higher (P < 0.001) than those of the animals from the Forest area (Table 1). The difference between the average live weight of chickens of Savannah ecotype and those of the Forest was 251 g, in favour of chickens of Savannah ecotype (P < 0.001). The live weight and body measures varied according to the sex (P < 0.001) with higher values in males. The interaction between sex and area was significant (P < 0.01). The most important differences were obtained on live weight and tarsus length. Sexual dimorphism represented 20.6% of the female weight in the Savannah and 23.7% in the Forest, thus the dimorphism was slightly more noticeable in the Forest. For the tarsus, the difference between sexes was 26.9% of the value of the females in the Savannah and 16.8% in the Forest; thus, a more important dimorphism was observed for the tarsus length in the Savannah than in the Forest. In general, the females of the Savannah ecotype had live weights and body measures higher than those of the Forest (P < 0.01) and the same difference was observed in males. No significant difference was observed between females from the Savannah and males from the Forest for live weight, breast circumference and body length (Table 1).

Study of the phenotype

Plumage colour

A total of 22 colours of plumage was identified in the Forest and 18 in the Savannah. The identification was not possible for 6.96% of the animals in Savannah *vs* 6.24% in the Forest. Somen colours of feather were found in both sexes (self-coloured white, dirty white, ermined white, red ermined, fawn, black and grey), others only in males (golden black, coppered black, silver black, red with black tail and white with black tail) or in females (golden salmon, silver salmon, golden wheaten, wheaten and partridge colour). Table 2 shows the frequencies of observation of these various colours of feather per area. In the two ecotypes, the most frequent colours were the black and the white. The self-coloured white, black, coppered black and the golden wheaten dominated in the Savannah ecotype whereas the ermined, fawn, grey, white were found in the Forest ecotype (P < 0.05). The white with black tail, the golden salmon, the golden partridge with salmon breast and the silver partridge with salmon breast were exclusively met in the Forest ecotype. Feather colour and the corresponding mutations are described in Table 2 and the frequencies of the observed phenotypes are given in Table 3. The mutations *I* and *E* were the most frequent, as the wild phenotype for the shank colour (grey leg) with at least 45% of the chickens of the Savannah ecotype, while mutations *E* (black extended), *EB*

Table 2. Diversity of feather colours of local chicken *(Gallus gallus)* populations in Benin.

Sex	Feather colour	Mutations pertaining to the wild type	Benin Savannah (N = 316)			Benin Forest (N=326)			Benin	Test of Significance between areas
			Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	(%)	
Phenotype found in the two sexes without feather drawing	Self-coloured White	*I E*	2.53	17.09	19.62	1.23	5.52	6.75	13.08	***
	Dirty white	*I* (heterozygous) and *E*	1.27	3.16	4.43	0.92	2.76	3.68	4.05	NS
	Ermined white	*S CO EY*	0.63	1.27	1.9	1.53	5.21	6.75	4.36	**
	Ermined red	*CO EY*	0.95	2.53	3.48	1.23	3.68	4.91	4.21	NS
	Fawn	*CO EB*	0.95	3.48	4.43	5.21	10.12	15.34	9.97	**
	Black	*E*	4.75	17.41	22.15	3.07	7.36	10.43	16.20	***
	Grey	*E BL*	0.32	0.32	0.64	1.84	3.07	4.91	2.81	**
Phenotype found in the cocks group without feather drawing	Golden§ black		4.11	0	4.11	5.52	0	5.52	4.83	NS
	Coppery black	*ER*	7.59	0	7.59	3.07	0	3.07	5.29	*
	Silver black	*ER S*	3.16	0	3.16	5.52	0	5.52	4.36	NS
	Red with black tail	*DB*	4.43	0	4.43	3.07	0	3.07	3.74	NS
	White with black tail	*DB S*	0	0	0	1.23	0	1.23	0.62	*
Phenotype found in the hens group without feather drawing	Golden salmon		0	0	0	0	2.45	2.45	1.24	**
	Silver salmon	*S*	0	3.8	3.8	0	1.84	1.84	2.80	NS
	Golden wheaten	*EY*	0	1.27	1.27	0	0	0	0.63	*
	Wheaten (light without black)	*EY I*	0	1.9	1.9	0	1.84	1.84	1.87	NS
	Partridge	*EB*	0	1.27	1.27	0	1.23	1.23	1.25	NS
	Silver Partridge	*EB S*	0	1.27	1.27	0	3.68	3.68	2.49	NS
	Golden Partridge	*EB*	0	7.59	7.59	0	6.75	6.75	7.16	NS
	Golden partridge with salmon breast [#]	*EB*	0	0	0	0	3.07	3.07	1.56	**
	Silver partridge with salmon breast	*EB S*	0	0	0	0	2.45	2.45	1.24	**
	Unknown [##]		2.85	4.11	6.96	2.15	3.37	5.52	6.23	NS

§ golden stands for the wild-type allele at the S locus ; [#] salmon breast indicates the wild-type allele for CO locus in females; [##]: have nether been identified before; NS: not significant; *: $P < 0.05$; *: $P < 0.01$; **: $P < 0.001$.

(brown) and *ID* (white leg) were the most observed in chickens of the Forestern ecotype, with a respective frequency of 31, 33 and 38%.

Feather pattern

Feather pattern of 386 chickens of which 192 chicken of the forest ecotype and 194 of the savannah ecotype were recorded. The frequency of the feather patterns of local chicken population

Table 3. Phenotype and corresponding allele frequency per area.

Allele	Frequency of phenotypes		
	Savannah (%)	Forest (%)	Benin (%)
E	59.57a	30.68b	44.90
ID wild type	48.48a	38.36b	43.34
I	45.57a	19.02b	32.09
EB	14.56a	32.52b	23.68
CO	9.81a	27b	18.54
S	10.13a	21.47b	15.89
wild type allele at *CO* locus	11.55a	18.02b	14.84
EY	13.4a	15.34a	14.39
wild type allele at *E* locus	12.15a	12.5a	12.33
MO	20a	3.06b	11.40
ER	10.75a	8.59a	9.65
W	9.9a	0.00b	4.87
DB	5.46a	4.3a	4.87
B	1.82a	4.3a	3.08
BL	0.64a	4.91b	2.81
PG	1.03a	2.08a	1.56

Within rows, means followed by different letters differ significantly ($P < 0.05$).

are shown in Table 4. The lack of feather patterns was higher ($P < 0.001$) in the Forest than in Savannah (79.17% *vs* 42.27). On the whole, 7 drawings of plumage were identified: the cuckoo with black foundation, the golden cuckoo, the mottled salmon, mottled black, the silver autosomal barring, the multiple lacing and the thousand-flowers. Some feathers exhibited several drawings at the same time, with a low frequency for both ecotypes. The mottling phenotype, either black or salmon or thousand-flowers, was more frequent in the Savannah than in the Forest, whereas the cuckoo pattern was slightly more frequent in the Forest than in Savannah (6.25 *vs* 1.03%). Apart from these drawings of plumage, the autosomal barring was rarely observed in the two areas and the multiple lacing was not observed in the Savannah, while it was rare in the Forest.

Shank colour

The colour of the legs was identified on 326 birds from the Savannah and 316 of the Forest. Table 5 presents the various colours encountered with the corresponding mutation and their frequency per area. Among these colours, the white legs (mutation at the locus *ID*) presented the highest frequency of occurrence and were significantly more numerous ($P < 0.001$) in the Forest (49.1%) than in Savannah (30.9%) area. This colour was followed in term of frequency, by the grey (wild type on the locus *ID*) and the black (locus *E*) which were more frequent in the Savannah than in the Forest ($P < 0.05$) with a difference of almost 10% for each of the two colours. Finally, yellow legs (locus *W*) were found in a small proportion in the two areas without any significant difference.

Eyes colour

The orange (wild-type colour) was met with a higher frequency in the Forest than in Savannah (72.7 *vs* 37.6%). It was followed respectively by the Brown (locus *Br*) and the red, largely more represented in the Savannah (37.6 and 24.2%) than in the Forest (17.5 and 9.1%). Finally the pearl eyes were rare in the Forest (0.65%) and the grey eyes (locus *FM*) were rare in Savannah (0.61%).

Earlobe colour

Six earlobe colours were observed. The white earlobe was the most represented with a proportion of 60.8% in the Forest ecotype against 45.1% in the Savannah ecotype. It was followed by the bluish white earlobe, more represented in the Savannah, with a difference of 10% as compared to the Forest. The same tendency was observed between the two areas for the sandy earlobes which presented a difference in percentage of 8.8. The red earlobes came then with 12.7% in the Forest against 5.5% in the Savannah. The grey earlobes were found in both areas at a low frequency (7.3 in the Savannah and 3.8 in the Forest). Finally the yellow earlobes were only

Table 4. Frequency of feather patterns occurrence of local chickens in Benin according to the sex and ecological areas

Feather drawings	Mutations	Savannah Benin (N = 194)			Forest Benin (N=192)			Benin (%)	Test of significativity per area
		Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)		
Cuckoo (black foundation)	*B E*	0.52	0.52	1.03	1.04	5.21	6.25	3.63	**
Golden cuckoo	*B EY*	2.06	0.00	2.06	2.08	0.00	2.08	2.07	NS
Thousand flowers	*MO wild type E*	3.61	3.61	7.22	1.04	0.00	1.04	4.15	**
Mottled salmon	*MO EY*	0.00	6.19	6.19	0.00	1.04	1.04	3.63	**
Mottled black	*MO E*	9.79	27.32	37.11	1.56	4.17	6.25	21.76	***
Silver autosomal barrure	*PG DB S*	1.03	0.00	1.03	0.00	0.00	0.00	0.52	NS
Multiple lacing	*PG*	0.00	0.00	0.00	0.00	2.08	2.08	1.03	*
Mixed pattern	-	1.03	2.06	3.09	0.52	1.56	2.08	2.59	NS
Whithout drawing	-	10.31	38.14	42.27	20.83	60.42	79.17	60.62	***

NS : not significant; * : P < 0.05 ; * : P < 0.01 ; ** : P < 0.001.

Table 5. Shank Colour of local chickens according to the area and the sex.

Shank colour	Allele	Savannah Benin (N = 316)			Forest Benin (N=326)			Benin (%)	Test of significativity per area
		Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)		
White	*ID*	7.58	23.33	30.91	12.89	36.16	49.06	40.13	***
Yellow	*W*	0.91	3.33	4.24	1.26	4.40	5.66	4.96	NS
Grey-blue	*ID* Wild type	12.42	36.06	48.48	10.06	28.30	38.36	43.34	**

NS: not significant; * : P<0.05 ; **: P<0.01 ; *** : P<0.001.

observed in the Savannah with a very low proportion of 0.61%.

Rare mutations

H locus) were found at a frequency of 1.2% in the Savannah and 1.86% in the Forest. The pea comb mutation (*P* locus) was rare in both areas (0.61% in the Savannah and 1.3% in the Forest). The Single comb without micro crest was also found in the Forest (0.63%) area. No significant difference was observed for the spur proportion between males of the Savannah ecotype (35.63 %) and males of the Forest ecotype (27.37%). Of the 316 animals of Savannah ecotype chicken, 7 cases of polydactyl were recorded whereas no polydactyl chickens were found within the 326 chicken of the forest ecotype recorded.

Molecular polymorphism

The average allele number per population and per locus was 5.73 for the Savannah and 5.91 for the Forest (Table 6). The average efficient allele number was 2.18 and 2.24, respectively for chickens

Table 6. Summary of measurement parameters of within or between populations diversity and individual assignation of the 121 chicken.

Ecotype	H_e	H_o	ANA	A_e	F_{IS}	HWED
Savannah	0.542 ± 0.152	0.536 ± 0.151	5.73	2.18	0.012	0
Forest	0.554 ± 0.167	0.568 ± 0.181	5.91	2.24	-0.024	1[d]

H_e, expected heterozygosity; H_o, observed heterozygosity; ANA, average number of present allele per locus ; Ae : average number of effective alleles per locus; HWED, Hardy-Weinberg equilibrium departure. [d]Number of loci with heterozygote deficiency (after Bonferroni correction).

of the Savannah and those of the Forest. The average effective allele number was definitely smaller than the total number of alleles, which reveals a heterogeneous distribution of the allelic frequencies.

The heterozygoty rate (observed and expected) was rather high (Table 6) and the FIS values ranged from -0.024 (Forest) to 0.012 (Savannah). After Bonferroni correction, only one locus exhibited significant deficit (locus MCW330, $P < 0.01$) for the Forest sample and no heterozygote excess was found. Although a low F_{ST}-value (0.009), both samples were found significantly differentiated ($P < 0.001$). Figure 2 showed the neighbor-joining tree constructed from the pairwise distances between all the individuals. The forest and Savannah samples exhibited a partial overlapping pattern.

DISCUSSION

Live weight and body measures

The weight and body measures of Savannah chickens were significantly higher than those of Forest chickens. This difference could be due to the breeding system, the climate or the genetic background. The results of the survey suggested that farmers from the Savannah were more involved in agriculture and breeding than those in the Forest, for whom poultry keeping was more a cultural tradition. For instance, farmers from the Savannah used veterinary treatments more often and paid attention to the body weight of males in order to sell them better. Nevertheless, both areas showed the same divagation system and the lack of a declared selection objective.

Similar differences between Savannah and Forest have been found for barymetric measures of other animal species. Regarding ruminants, the zebus, located in the zone of Malanville in Benin, have weights and body measures higher than the Borgou breed, located in the Departments of Borgou, Donga and the “Collines” in Benin (Domingo, 1976; Youssao, 2008). In the same way, the Borgou breed has higher weight and body measures than the “lagunaire” breed located in the Forest zones of Benin. The same tendency is observed with other animal species (sheep, goat and others) (Youssao, 2008).

The live weight and body measures of males were significantly higher than those of females. The same tendency was observed by Godonou (2002) and Dossou (2005) in local chickens. The sexual dimorphism of the chicken becomes in general more noticeable with advanced age. Thus, the result of a larger dimorphism for shank length in the Savannah is logic but the slightly lower dimorphism on live body weight in the Savannah is more surprising. However, body weight can be modified by variable nutritional conditions at the time of weighing, whereas the tarsus length is a more stable indicator of format of adult animals.

In the Wet Dense Forest with Bimodal Pluviometry of Cameroun, on the whole of the studied Provinces, the average body weight was 1535 g for cocks and 1220 g for hens (Fotsa et al., 2008). Local chickens of the “High lands” of the West Cameroun, where the climate is of sudano-guinean type, exhibited an adult weight of 1676 g for males against 1278 g for the female (Keambou et al., 2008). However, the body measures carried out on the animals of the West by Keambou (2006) showed that the tarsus was shorter but thicker than those of the animals of the wet forest. Thus, local populations of Cameroun exhibited higher body weight and body measures than those of Forest Benin, where pluviometry is also bimodal or of Savannah Benin where the climate is of sudanese type. Thus, climatic differences cannot be the only reason for a lower body weight of local chickens in Forest Benin. Consequently, it would be interesting to compare on station the growth performance of chickens from each ecotype in the same conditions, in order to test whether the difference in live weight could be due to climate, to the breeding system or to the genetic background.

Morphological mutations

An important phenotypic diversity was observed for both ecotypes. It must be recalled that some phenotypes cannot be observed in the presence of another one; for example, it is not possible to know if a self-coloured white animal carries the *CO* mutation or a mutation of feather

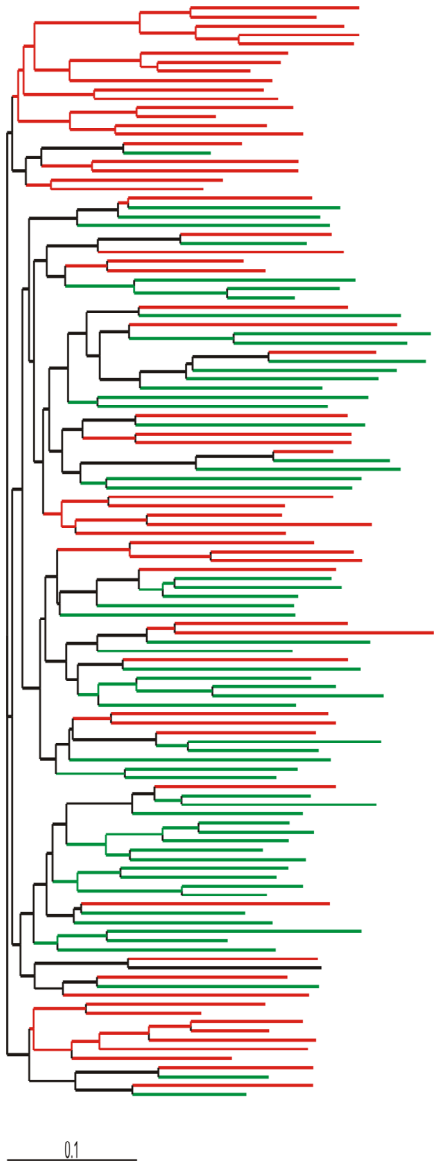

Figure 2. Neighbour-joining tree derived from DAS distances, depicting the genetic relationships of 121 local chickens belonging to local populations from forest (red line) and savannah (green line) samples. Savannah and Forest genetic distances were based on 22 microsatellite markers. § Green lines: Savannah ecotype ; § Red lines: Forest ecotype.

drawing because the white can be due to the *C* mutation which inhibits any colour. Generally, the high variability of plumage colour is considered as a consequence of domestication which increases the survival of mutant phenotypes. The maintenance of this high variability indicates the absence of selection on a preferred phenotype, showing that the population is not standardized (Lauvergne et al., 1993). Furthermore,

the cultural value of various plumage colours has already been reported for village chickens in Africa (Gueye, 1998; Gueye, 2002; Benabdeljelil and Bordas, 2005).

The present data showed differences in frequency of some plumage colours and shank colours between the Savannah and the Forest ecotypes, but the corresponding mutations are not usually associated with a significant effect on body weight in temperate climate. The relationship between such frequency differences and geographical areas is not easily explained, but it seems to occur in other African countries (Fotsa, 2008).

Indeed, this difference in distribution of the feather colours was also observed in two different agro-ecological zones in Senegal (Missohou et al., 1998). Thus, in Dahra (agro-ecological zone, with weak pluviometry of Senegal), the fawn (26%), the red (9%), the white and fawn (7.4%) and the golden red (7.2%) plumage colours were observed, while in Kolda (ecological zone with large pluviometry), the colours observed were the white (16.4 %), the ermined fawn (9.6%), the thousand-flowers (9.5 %) and the white and fawn (8.9%). By comparing the two studies, it comes out that the fawn and the white were most frequent respectively in the agro-ecological zones with strong and weak pluviometry of Senegal, whereas in Benin, the frequently encountered plumage in the Savannah ecotype were the self-coloured white and the black plumage and the fawn and black colours in the Forest ecotype. The high frequency of the white colour in the savannah area is attributable to their frequent use at the time of religious sacrifices. Following the declarations of the villagers, the high frequency of white chickens in the savannah area is mainly attributable to their preference for this colour for religious purposes. The price of white hens in the savannah zones is accordingly higher than that of multicolored hens. In Benin, the frequency of hens with uniform black plumage colour or those with black plumage background (mottled, coppered and golden), is higher as that reported by Missohou et al., (1998) in Senegal. On the other hand, the partridge colour and partridge with black background (silver, golden, silver with salmon breast, golden with salmon breast) presented the same proportions in Benin and in Senegal, regarding the agro ecological zones with weak pluviometry (Savannah Benin and Dahra with respective proportions of 10.13 and 8%) as well as those with large pluviometry (Forest Benin and Kolda with respective proportions of 13.67 and 13%). According to Pedersen (2002), in Zimbabwe, the colours fawn (26%), black (23%) and grey (23%) were found in the area of Sanyati (ecological zone with weak pluviometry).

According to Dossou (2005) and Youssao et al. (2007), the ermined fawn, the fawn, the black and the dirty white were the most common colours observed within the 311 chickens recorded in the Commune of Abomey-Calavi with frequencies ranging from 8.31 to 10.30%. These results are consistent with the observations made in the Forest area of the present study. According to Akouango et al. (2004), the various phenotypes met in Congo are the ermined fawn (37.25%), the negro black (16.7%), the golden (15.16%), the fawn (10.16%), the mottled (7.84%), the silver wheaten (7.6%) and the Pile (5.3%). By comparing the results of Akouango et al. (2004) with those of this study, it comes out that the fawn and the Black (self-coloured or with black foundation) are the most dominant. Apart from a mixed type colour, all the plumage colours observed by Akouango et al. (2004) were present in Benin. On the other hand, some colours such as: the silver, the dirty white, the silver or golden black and the salmon were not described by Akouango et al. (2004).

Report by Dossou (2005) in the Commune regarding shank colour, a high proportion of white legs (44.8%) were previously observed of Abomey-Calavi. In Senegal, the white was also dominating with a proportion of 56.5% (Missohou et al., 1998). Although yellow legs were found in a small proportion in Benin, it was more or less popular in Cameroun with proportions varying from 31.38 to 37.89% according to the area (Center, South and East of Cameroun) (Fotsa et al., 2008) and from 39 to 46% according to the zones (rural and urban) within area of Cameroun (Keambou et al., 2008). The strongest proportion of yellow legs observed in urban zone could be due to the introgression of exotic breeds, such as Rhode Island Red or White Leghorns, which have yellow legs. In the present study, the animals used are all chosen in farms where no crossing was declared. In Senegal, according to Missohou et al. (1998), the white earlobes were also frequent (78%). On the other hand, in Cameroun, the dominant colour of the earlobes were red (40.3%) and white (35.6%) (Keambou et al., 2008).

Molecular polymorphism

The indigenous chicken populations of the ecological areas analyzed in this study showed a higher level of genetic diversity than what is generally described for commercial lines (Hillel et al., 2003; Granevitze et al., 2007). This result is consistent with what is generally observed in populations raised without a selection programme in contrast to what can be observed in commercial lines or standardised breeds (Wimmers et al., 2000; Berthouly et al., 2008; Muchadeyi et al., 2007). Only one case of a deficiency in heterozygotes was observed. This situation is relatively different from what can be observed in many local populations (Berthouly et al., 2008; Granewitz et al., 2007; Muchadeyi et al., 2007) where a Wahlund effect takes place, due to a fragmentation of the population in small sub-units having few or no exchanges of reproducers between them. With regard to morphometric and phaneroptics traits, the results from this study reveal some significant differences between the Savannah and the Forest chicken popula-

tions in Benin, suggesting that their genetic background was partly different. Similar results were also reported by Bonou (2006) on phenotypic characteristics of local chicken of Benin.

Conclusion

The present study showed that the local chicken populations of the species *G. gallus* of the two great climatic areas of Benin have a remarkable heterogeneity of their phenotypic characters. The distribution of plumage colour showed some differences between the Savannah and the Forest Benin, which probably reveal effects of climate as well as cultural preferences of the breeders. Although the Savannah and the Forest populations did not differ greatly on the basis of molecular polymorphism, they exhibited marked differences in body weight. They are theoretically many causes of variation of body weight of chickens, which could be addressed in further complementary investigations. Thus, it would be interesting to compare the growth performance of the Savannah ecotype chickens to those of the Forest ecotype in a controlled environment in station in order to test whether the difference of the live weight between those two ecotypes chickens is due to the climate, the production system or the genetic background. Furthermore, since the growth performance of local chicken was quite low, performance testing will be necessary to assess the possibility of further improvement of local chickens. A global strategy should also take into account the improvement of the production system in village conditions.

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Abreviation

HPIA, Highly Pathogenic Avian Influenza; **FAO,** Food and Agriculture Organization; **ASECNA,** Agence pour la Sécurité de la Navigation Aérienne; **SAS,** Statistical Analysis System.

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