



Genetic diversity and phylogenetic relationships within local pigs in southern Benin

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Abstract

The current context of climate change requires the conservation of local zoogenetic resources already very well adapted to the traditional breeding system, rough feeding, and heat and cold stress. This study assessed genetic diversity in local pigs in southern Benin, as a prerequisite for their sustainable use and sustainable management in Benin. A total of 69 individuals including 54 local pigs, 7 Large-White, and 8 hybrids (local pigs × Bush-pig) were genotyped by using 17 microsatellite markers. On the average, 8.94 alleles were detected per locus. Average expected and observed heterozygosities were respectively 0.51 and 0.46. Polymorphic information content was 0.61, and genetic diversity was 0.53. A phylogenetic tree gathered local pigs into three genetic clusters. Genetic structural analyses revealed introgression of Large-White's genes into the local pig's genome. Three groups were identified: hybrids (subpopulation 1), a mixture of Large-White and local pigs (subpopulation 2), and only local pigs (subpopulation 3). Symmetrical allelic distances were higher between subpopulations 1 and 2 (0.787) and then 1 and 3 (0.713). The same trend was detected for genetic distances between pairs of subpopulations. Genetic differentiation between subpopulations 2 and 3 was very weak as a consequence of high gene flow (10.82). Molecular variance analysis showed that 77% of genetic diversity within populations was related to variability between the individuals. These results showed that local pigs in southern Benin are threatened by genetic erosion and suggest prompt actions to implement sustainable conservation strategies.

Keywords Benin · Conservation · Introgression · Local pigs · Microsatellite markers · Molecular genetics

Introduction

Africa harbors a great diversity of livestock, among which over 400 species are traditionally exploited by local populations. Many studies on the diversity of various pig breeds have been reported in the literature in recent years (Traspov et al. 2016; Mujibi et al. 2018; Qiao et al. 2019; Hlongwane et al. 2020; Nicodemus et al. 2020; Quan et al. 2020; Zhao et al. 2021a). This diversity plays an important role to overcome some of the greatest challenges such as climate change, poverty, and disease resistance as reported about the Chinese local pigs by Zhao et al. (2021a,b).

Existing data suggest that indigenous pigs, also called runner pigs of Africa or local pigs, originate from different domestication centers, as opposed to the international commercial breeds (Mujibi et al. 2018). It has been also suggested that the genetic improvement of the local pigs may make a significant contribution to food security not only in Africa but also in other tropical environments (Mujibi et al. 2018). Indeed, local pigs have many excellent characteristics such as organoleptic quality of meat, high resistance to disease, and good adaptability to the local environment (Ma et al. 2013; Djimènou et al. 2018).

Breeding and production of pigs ensures self-employment for thousands of families that depend directly or indirectly on incomes resulting from this activity. In addition, it constitutes a potential source of income that is easily mobilized for medical care, children schooling, food safety, and various unforeseen expenditures (Mopaté 2008; Djimènou et al. 2017a). Moreover, it provides manure that is more concentrated and richer in fertilizing elements than that provided

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by the cattle and is, therefore, preferred by farmers. Finally, as a prolific omnivorous species with a short reproduction cycle, pigs ensure a good valorization of agricultural by-products and fodders in feeding (Djimènou et al. 2017b). Thus, in contrast to cattle, the investments for pig breeding yield quick profits. In Benin, the breed mostly used for pig production is the local breed (Ayssiwèdé 2004; Djimènou et al. 2017a).

In Benin, local pigs are important zoogenetic resources generally used for their economic, sociocultural, and nutritional status (Ayssiwèdé 2004; Youssao et al. 2008a,b, 2009; Houndonougbo et al. 2012; Djimènou et al. 2017a). The meat of local pigs is an essential ingredient of many traditional dishes and is highly appreciated by the local populations (Youssao et al. 2004, 2008b). The consumption of pig meat ranks second in meat consumption after consumption of ruminants in southern Benin, whereas at a national level, it ranks third after consumption of cattle and poultry (Dognon et al. 2018).

Local pigs (Fig. 1) have a great capacity for environmental adaptation and resistance to various diseases, contrary to the exotic pigs, which are susceptible to heat stress and tropical diseases (Agbokounou et al. 2016). Thus, local genetic resources play a great role in the current context of climate change where adaptation, an attribute of local resources, constitutes a major problem for exotic breeds in the tropical areas (Naves et al. 2011). New breeding practices contribute to the alteration of the diversity of animal genetic resources through the creation of new genetic types that are often not adapted to the environment (FAO 2007). Unfortunately, most of these changes are not beneficial (Woolliams et al. 2008) and many animal species are now threatened with extinction, while others are liable to suffer from an inefficient use or loss due to crossbreeding (FAO 2007). Therefore, to face future challenges in the agricultural and food industries, specific



Fig. 1 African local pig in southern Benin

efforts will be urgently needed to conserve animal genetic resources (Adeola and Omitogun 2012).

In Benin, no other studies have been conducted related to the genetic diversity of the local pigs, except for the recent phenotypic study performed by Djimènou et al. (2018). This study showed that the local pigs of southern Benin were morphologically structured into three morphotypes according to a size gradient. However, more research will be required for an in-depth knowledge of the genetic diversity within local pig populations. Thus, the present study was undertaken to better explain the phenotypic diversity observed in the local pigs. In this study, microsatellite markers were used to determine the genetic diversity and relationships within the local pigs in southern Benin and assess the genetic differentiation among subpopulations. The information generated from this study may be useful for the genetic improvement and sustainable management of the local pigs in Benin.

Material and methods

Study area and sampling method

The study was conducted in all seven administrative departments of southern Benin (Fig. 2). A total of sixty-nine (69) blood samples were collected from individuals from each of the three (3) breeds included in the study: 54 local pigs, 7 Large-White pigs, and 8 hybrids (local pigs \times Bush-pig). Bush-pig was a wild swine, *Potamochoerus porcus*, member of the pig's family. The selection of individuals (one per herd) for sampling was performed by exclusion of the highly related animals (no full- or half-sibs), and prioritization of the adult individuals. All hybrids were sampled in the only available farm in Abomey-Calavi, located in the Atlantic Department. Hybrids were included in the study to distinguish the genetic relationships between them and the local pigs. Large-White pigs were also included as reference for comparative purposes, especially for the genetic admixture assessment.

For each animal, blood samples were collected (5 to 10 ml) following standard routine monitoring procedures and guidelines, as recommended by the Food and Agriculture Organization of the United Nations (FAO 2011). Collected blood samples were stored at 4 °C until DNA extraction. To limit cross-contamination between farms, all necessary biosafety precautions were taken including using sodium hypo-chloride to sterilize the feet and hands of samplers before entering each herd.

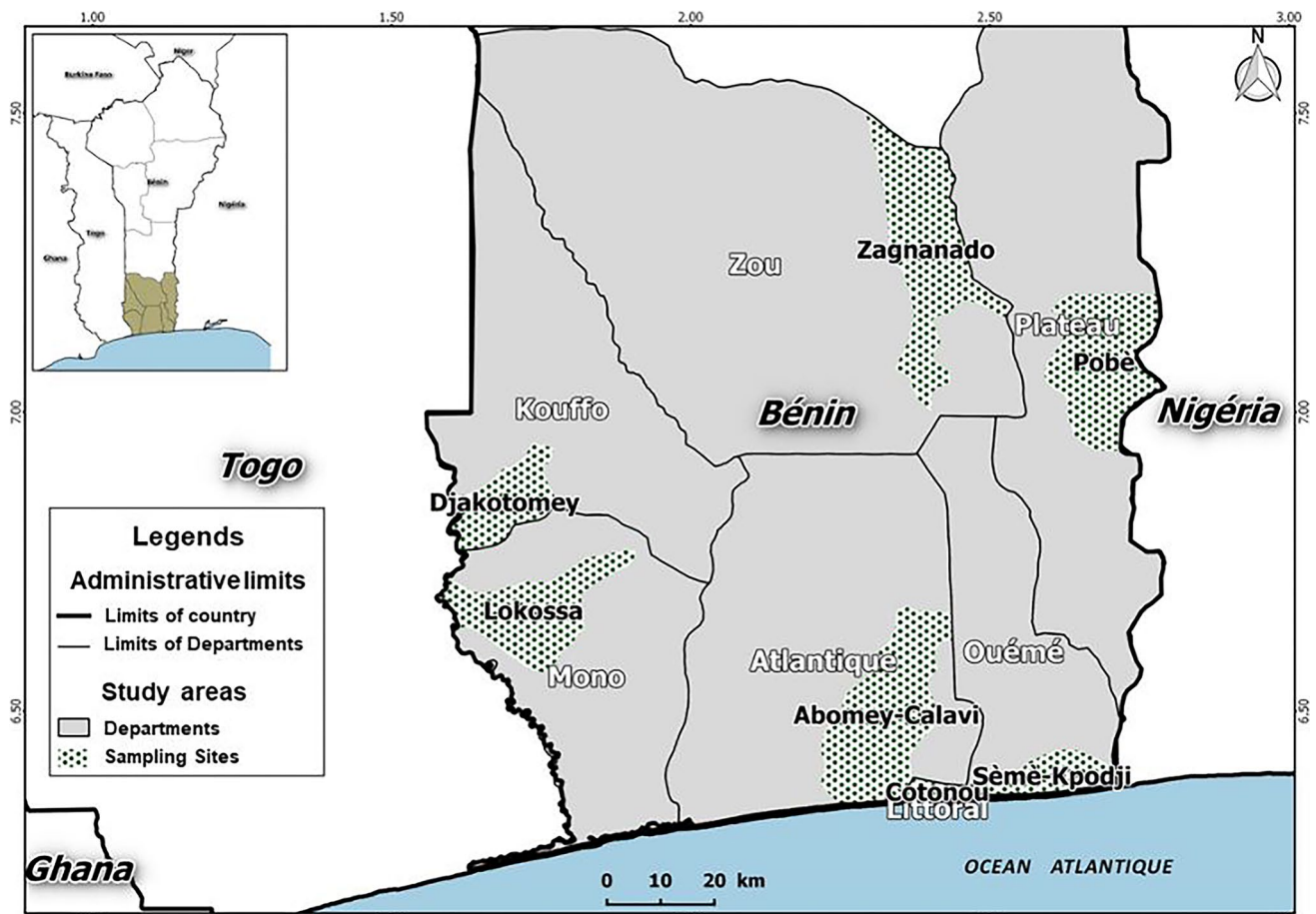


Fig. 2 Study areas in southern Benin

DNA extraction

DNA was extracted from total blood of individual pigs in the genotyping laboratory of the International Center for Research and Development on Livestock in Sub-humid Zone (CIRDES) at Bobo-Dioulasso in Burkina Faso. The Maxwell® Promega blood kit (Madison, WI, USA) was used for DNA extraction according to the manufacturer's instructions (<http://france.promega.com/>). To ensure the effectiveness of the DNA extraction technique, the first 10 extracted DNA samples were checked by agarose (1%) gel electrophoresis. Then, the purity and concentration of DNA samples were evaluated using a spectrophotometer (NanoDrop®, Thermo Fisher Scientific Inc., Wilmington, USA). DNA samples were stored at -20°C .

Genotyping using microsatellite markers

Nineteen (19) microsatellite markers were selected from thirty (30) recommended by the FAO (2011) for genetic diversity studies in pig (Supplementary Table 1). Marker selection was based on their chromosomal localization,

distribution in the genome, and the degree of polymorphism of each microsatellite locus (De Meeûs 2012).

To ensure successful PCR, the annealing temperature of each microsatellite marker was optimized, which resulted in a temperature range of 50 to 60 $^{\circ}\text{C}$. Within this temperature range, 17 microsatellite markers, out of the 19 selected, were successfully amplified using the Thermocycler BIOM-ETRA® TGradient version 4.20 gr., and, therefore, used for genotyping.

All 69 pigs were genotyped using the 17 optimized microsatellite markers. The microsatellites CGA and So386 respectively located on chromosomes 1p and 11 were not optimized in the lab conditions. PCR products were separated on a 6.5% polyacrylamide gel in a Sequencer Li-Cor® DNA Analyzer, Model 4300, following the manufacturer's instructions. The fragment length of PCR products was determined using the SAGA^{GT} Generation 2.0 software.

Statistical analyses

Null alleles were detected using the Micro-Checker 2.2.3 software and Brookfield 1 (Oosterhout et al. 2004). To

estimate the level of genetic diversity and differentiation in the studied populations, various parameters were computed. The number of alleles (N_a), the polymorphism of loci, the allelic richness (R_t), and the inbreeding coefficient (F_{IS}) per locus were calculated with the FSTAT 2.9.3.2 software (Goudet 2002). The polymorphic information content (PIC) values were estimated per locus using the Cervus 3.0.6 software (Kalinowski et al. 2007), whereas values of expected (H_e) and observed heterozygosity (H_o), and genetic diversity (H_s), were calculated using the Genetix 4.0.5.2 software (Belkhir et al. 2004). A Hardy–Weinberg equilibrium (HWE) test was performed using the Genepop 4.2.2 software (Rousset 2008).

Phylogenetic relationships among individuals were revealed with the Population 1.2.3.2 software (Langella 1999) using neighbor-joining and inferred distances of Cavalli-Sforza and Edwards (1967). The phylogenetic tree was constructed with the FigTree application version 1.4.2 (Rambaut 2014) using a JAVA application environment. To visualize the distribution and assignment of individuals, a factorial analysis was performed using the Genetix 4.0.5.2 software (Belkhir et al. 2004).

To infer population structure, a Bayesian-based assignment approach, available in STRUCTURE software program version 2.3.4 (Hubisz et al. 2009; Falush et al. 2003; Pritchard et al. 2000), was used to detect the number of genetic populations (clusters or K) in the dataset. The model used for simulation was based on an assumption of admixed ancestry and correlated allele frequencies. To estimate the true number of subpopulations, the parameter $Pr(X|K)$ was applied, where K is a value between 2 and 6. Ten (10) independent runs for each K were performed. All runs were performed with a burn-in period of 10,000 steps, followed by 100,000 MCMC (Markov chain Monte Carlo) iterations. The simulation results of the runs generated by the STRUCTURE were submitted to the STRUCTURE HARVESTER (Earl and VonHoldt 2012) to detect the true number of clusters (K) using statistics based on the rate of change in the log probability and the modal distribution of Delta K (ΔK) values (Evanno et al. 2005).

The number of private alleles within each subpopulation was counted according to the definition suggested by Foulley and Ollivier (2006). The symmetrical allelic distances between pairs of subpopulations were calculated according to the formula $(a+b)/(a+b+c)$ proposed by Foulley and Ollivier (2006) where a indicates the number of private alleles of subpopulation I, b indicates private alleles of subpopulation J, and c indicates common alleles of both subpopulations I and J.

Chord distances by Cavalli-Sforza and Edwards (1967) were estimated per subpopulation pair using Genetix software version 4.05.2 (Belkhir et al. 2004). The genetic differentiation between subpopulation pairs was estimated

according to the models of Weir and Cockerham (1984), Reynolds et al. (1983), and Slatkin (1995) with Arlequin software vs 3.5.1.3 (Excoffier and Lisher 2010). An analysis of molecular variance (AMOVA) was performed to assess the genetic variability within and between populations using Arlequin software version 3.5.1.3. A bottleneck analysis was performed to check whether the local African pig population in southern Benin underwent a recent bottleneck by using the Bottleneck-1.2.0 software.

Results

Genetic variability

Over the 17 studied loci, 152 alleles (N_a) were revealed with an average of 8.94 alleles per locus (Table 1). The number of alleles ranged from 3 for So215 up to 23 for So005. The number of individuals for which loci were expressed was, on average, 65.35 ± 4.17 individuals per locus, varying from 42 for locus So355 to 69 for loci So155, So026, SW240, SW72, and SW951. The allelic richness (R_t) varied between 1.54 for So215 and 5.24 for So005, with an average of 3.63 ± 0.73 per locus. Seven (7) loci (SW122, So005, So026, So226, SW355, SW24, and SW911) demonstrated null alleles. The expected heterozygosity (H_e) varied between 0.10 for So215 and 0.74 for So226, with an average of 0.51 ± 0.14 per locus, whereas the observed heterozygosity (H_o) varied from 0.08 for So215 to 0.83 for So101, with an average of 0.46 ± 0.18 per locus. For all loci, the observed heterozygosity (H_o) was lower than the expected heterozygosity (H_e), with a 5% heterozygote deficiency. The same trend was observed in 10 loci not showing null alleles, but these loci exhibited a 4% heterozygote deficiency (Table 1).

Genetic diversity (H_s) varied from 0.18 for So227 to 0.77 for So226, with an average of 0.53 ± 0.13 per locus. The inbreeding coefficient (F_{IS}) ranged from -0.21 for So101 to 0.85 for So355, with 0.13 ± 0.14 on average, at a 95% confidence interval (CI). Fifteen (15) loci demonstrated positive values ($F_{IS} > 0$) and two (So101 and SW951) showed negative values. The polymorphic information content (PIC) varied from 0.11 for So215 to 0.81 for So005, with 0.61 ± 0.15 as the average. A Hardy–Weinberg Equilibrium (HWE) test attested that 10 loci were in HWE, whereas another seven loci revealed a strong HWE deviation. As a result, the whole population was not in HWE ($p < 0.001$).

Phylogenetic relations

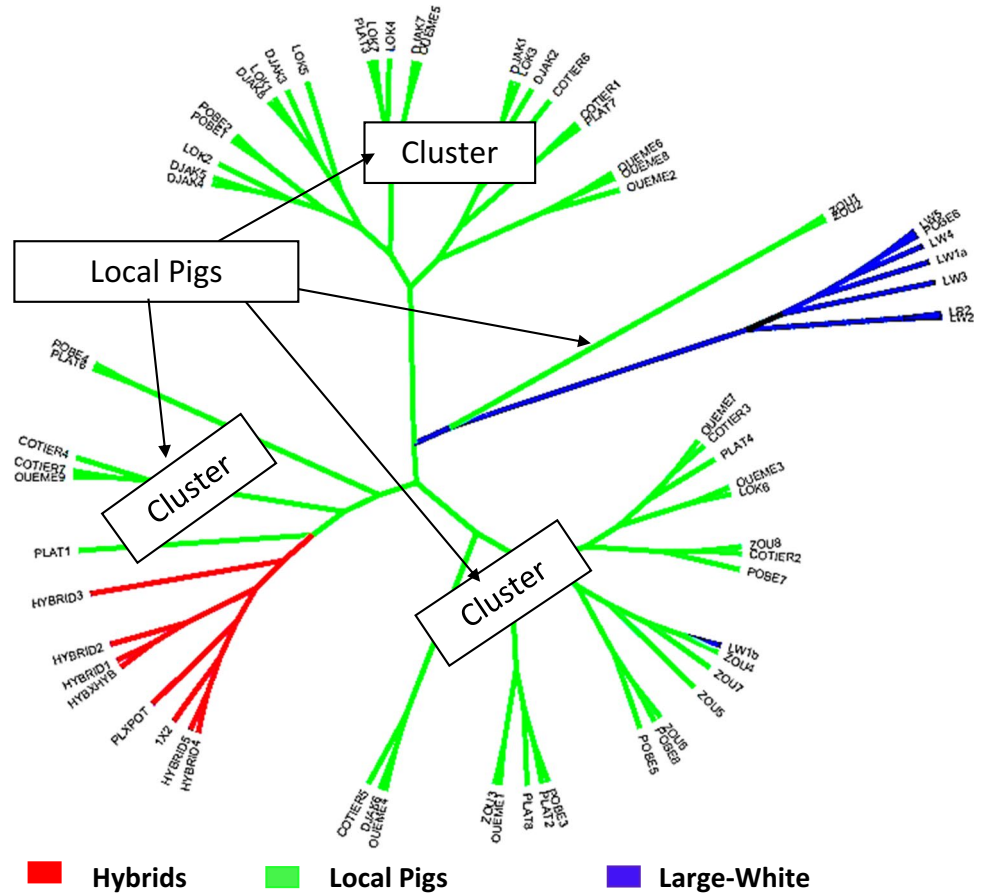
Figure 3 shows a phylogenetic tree revealing the links among individuals based on genetic distances. The tree isolated hybrids from the other populations as a separate genetic entity. The same trend was observed for individuals

Table 1 Parameters of genetic variability within the global population

Locus	Allele range (bp)	Θ (°C)	N	Na	Rt	Null allele frequency	He	Ho	Hs	F _{IS} (WC)	PIC	HWE test (chr ²)
So101	214–244	55	68	10	3.75	-0.04	0.60±0.07	0.83±0.11	0.61	-0.21	0.67	11.59 ns
So155	160–178	55	69	10	4.17	0.04	0.58±0.24	0.58±0.22	0.60	0.03	0.73	11.85 ns
SW122	134–148	55	65	8	3.85	0.12	0.57±0.23	0.39±0.26	0.61	0.27	0.68	20.65**
SW857	162–178	50	68	8	3.60	0.03	0.57±0.13	0.58±0.05	0.59	0.07	0.64	4.08 ns
So002	207–257	60	66	12	3.7	0.02	0.58±0.10	0.71±0.19	0.59	0.02	0.61	14.01*
So005	222–288	50	68	23	5.25	0.08	0.58±0.37	0.50±0.33	0.58	0.13	0.81	7.38 ns
So026	107–125	55	69	7	3.07	0.06	0.42±0.20	0.36±0.15	0.44	0.18	0.52	12.83*
So090	246–266	52	68	8	3.68	0.05	0.54±0.22	0.54±0.19	0.56	0.02	0.69	4.55 ns
So215	170–206	60	66	3	1.54	0.04	0.10±0.09	0.08±0.05	0.11	0.31	0.11	4.99 ns
So226	184–234	50	68	10	4.611	0.09	0.74±0.05	0.68±0.07	0.76	0.15	0.79	16.36*
So355	162–288	60	42	10	4.83	0.39	0.70±0.13	0.09±0.12	0.75	0.85	0.79	Infinity***
SW24	114–142	50	60	9	3.89	0.06	0.59±0.11	0.60±0.04	0.62	0.11	0.68	10.42 ns
SW240	113–137	55	69	10	4.44	0.04	0.68±0.13	0.69±0.09	0.70	0.05	0.76	16.36*
SW72	120–136	50	69	7	3.11	0.05	0.47±0.16	0.47±0.15	0.48	0.05	0.56	8.41 ns
SW911	166–186	52	59	7	3.97	0.11	0.48±0.32	0.37±0.25	0.52	0.25	0.71	13.52**
SW951	142–156	50	69	5	2.45	0.01	0.25±0.22	0.26±0.22	0.27	-0.02	0.39	6.57 ns
So227	146–270	58	68	5	1.84	0.01	0.16±0.11	0.16±0.10	0.18	0.04	0.22	4.14 ns
Mean (±SD)		-	65.35±4.17	8.94±2.64	3.63±0.73	-	0.51±0.14	0.46±0.18	0.53±0.13	0.13±0.14	0.61±0.15	Infinity***

Θ annealing temperature (°C), N number of individuals in which the locus was expressed, Na number of alleles, Rt allelic richness, He expected heterozygosity, Hs observed heterozygosity, H_s genetic diversity, F_{IS} inbreeding coefficient, PIC polymorphism information content, HWE Hardy–Weinberg equilibrium, SD standard deviation
 ns: no HWE deviation; *: HWE deviation at threshold of 5%; **: HWE deviation at threshold of 1%; ***: HWE deviation at threshold of 0.1%

Fig. 3 Phylogenetic tree of the pig populations showing different clusters of the local pigs in southern Benin

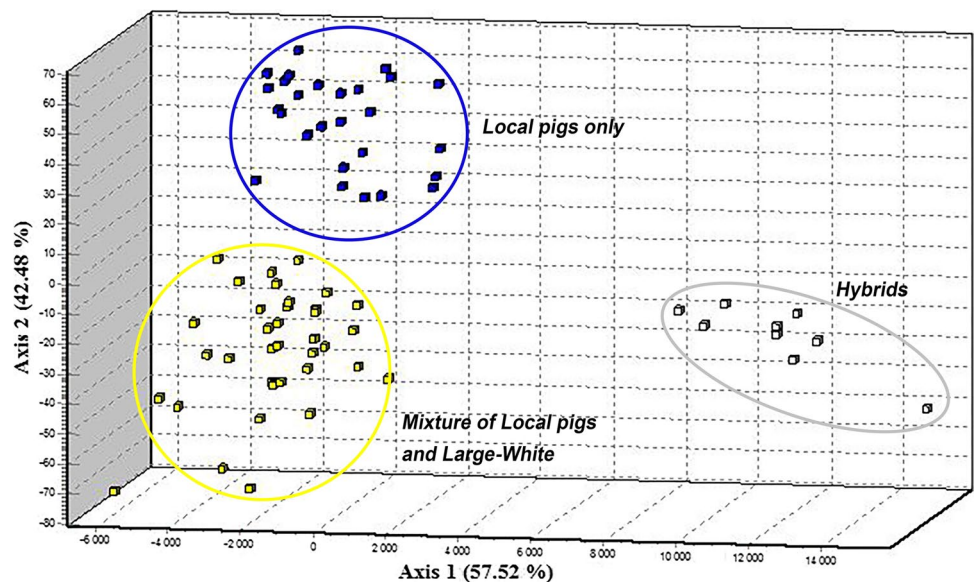


from the Large-White breed with the exception of one individual who was grouped with the local pigs. The local pigs were divided into four genetic branches, which were interconnected at the root of the phylogenetic tree.

Factorial correspondence analysis

The outcome of the factorial correspondence analysis (Fig. 4) indicated that the first factorial axis conserved 58% of the total inertia while the second axis conserved 42%

Fig. 4 Correspondence analysis



of the total inertia, resulting in an ideal explanation of the genetic diversity within the population. After the assignment of individuals, three distinct genetic clusters appeared: group 1 (gray), solely composed of hybrids, was largely dissociated from the other groups by the first axis; group 2 (yellow) and group 3 (blue) were discriminated by the second axis but appeared genetically nearby. Group 2 included all Large-White pigs and part of the local pigs whereas the remainder of the local pigs constituted group 3.

Genetic structure

The analysis of the genetic structure and the Evanno test revealed the number K of subpopulation existing in the pig's population. Delta K values varied between 0.47 for $K=4$ and 0.97 for $K=3$, with the highest Delta K corresponding to $K=3$ (Supplementary Figure and Supplementary Table 2). Thus, three (3) subpopulations were identified in the population, as shown in Fig. 5.

Figure 5 illustrates the genetic structure of the studied population, which comprises three (3) distinct subpopulations. All individuals of subpopulation 1 (red), constituting 3% of the population, were hybrids. Subpopulation 2 (green) represented 59% of the individuals and consisted of the local pigs and the Large-White pigs. The local pigs included in this group underwent an important introgression from exotic genes, in particular from the Large-White pigs. The local pigs of this subpopulation originated mainly from

the departments of Ouémé, Littoral, and Couffo. Subpopulation 3 (blue) comprised 38% of the total population and consisted of local pigs, indicating a strong predominance of the local genes, and origins from the departments of Zou and Plateau. In this structure, pigs from the departments of Atlantic and Mono were distributed between subpopulations 2 and 3.

Genetic variability within subpopulations

In total, 138 alleles were found in subpopulation 2, 96 in subpopulation 3, and 33 in subpopulation 1 (Supplementary Table 3). Alleles common to the three subpopulations were the following: 115 (So026), 136 (SW122), 142 (SW951), 168 (SW857), 225 (So002), 246 (So227), and 248 (So005). The number of private alleles (Table 4) was high within subpopulation 2 (50 private alleles) and weak within subpopulation 1 (2 private alleles), while subpopulation 3 exhibited an intermediate number (11 private alleles). The symmetrical allelic distance between subpopulation 1 (hybrid) and subpopulation 2, and that between subpopulation 1 and subpopulation 3 were 0.787 and 0.713, respectively. The lowest allelic distance (0.441) was observed between subpopulations 2 and 3.

Overall, the microsatellite markers were polymorphic in subpopulations 2 and 3, whereas in subpopulation 1, five markers were monomorphic (Table 2). The highest average number of alleles ($N_a = 8.12$ alleles) was observed in

Fig. 5 Genetic structure of pig's population in southern Benin

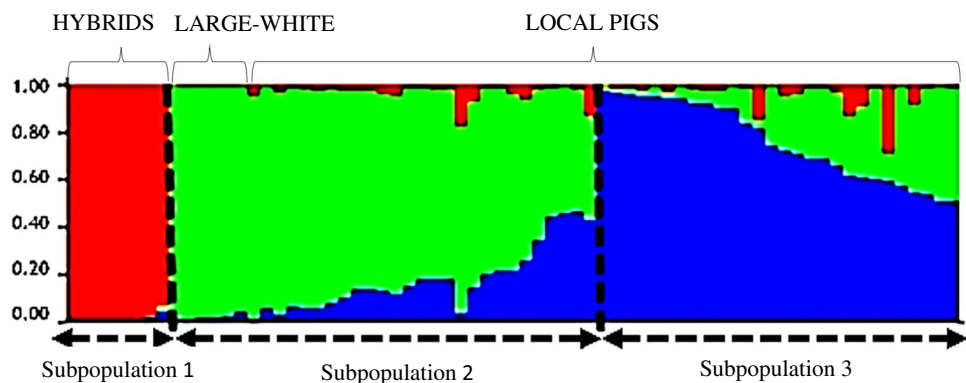


Table 2 Genetic variability within each subpopulation

Subpopulations	P_1	N_a	R	H_e	H_o	F_{IS}	PIC	H_s	HWE test (χ^2)
1	12/17	1.94 ± 0.55	1.80 ± 0.52	0.26 ± 0.19	0.27 ± 0.25	-0.09 ± 0.41	0.22 ± 0.16	0.28 ± 0.20	33.34 ns
2	17/17	8.12 ± 2.26	3.87 ± 0.78	0.67 ± 0.13	0.59 ± 0.15	0.14 ± 0.13	0.66 ± 0.14	0.68 ± 0.14	Infinity***
3	17/17	5.71 ± 1.69	3.26 ± 0.69	0.59 ± 0.16	0.48 ± 0.17	0.14 ± 0.15	0.55 ± 0.15	0.59 ± 0.16	Infinity***

P_1 : number of polymorphic loci, N_a : number of alleles, R : allelic richness, H_e : expected heterozygosity, H_o : observed heterozygosity, F_{IS} : inbreeding coefficient, PIC: polymorphism information content, H_s : genetic diversity, HWE: Hardy-Weinberg equilibrium, ns: no significant deviation from HWE

***: Significant deviation from HWE at threshold of 0.1%

subpopulation 2, while the lowest was found in subpopulation 1. Subpopulations 2 and 3 showed close allelic richness (3.87 and 3.26, respectively). The lowest allelic richness was obtained in subpopulation 1, which showed expected and observed heterozygosity values largely lower than those obtained in subpopulations 2 and 3. Subpopulation 2 showed an 8% heterozygote deficiency while subpopulation 3 showed an 11% heterozygote deficiency.

The inbreeding coefficients (F_{IS}) were similar in subpopulations 2 and 3. The highest PIC values were recorded in subpopulations 2 and 3, whereas subpopulation 1 revealed the lowest PIC value. The genetic diversity was higher in subpopulations 2 and 3 than that in subpopulation 1.

Analysis of molecular variance

According to the three hierarchical levels of diversity, the AMOVA results (Table 3) showed that 77% of the genetic variation resided in the whole population while 9% of the variation was observed among subpopulations, with variance components of 4.41 and 0.53, respectively. In addition, 14% of the genetic diversity was explained by differences among individuals within subpopulations. The indices of fixation F_{IS} , F_{ST} , and F_{IT} were estimated at 0.15, 0.09, and 0.23, respectively ($p < 0.001$).

Genetic differentiation and gene flow

The pairwise genetic differentiation between subpopulations (Supplementary Table 4) was computed according to three

methodological approaches, namely, F_{ST} (Weir & Cockerham 1984), $-\ln(1 - F_{ST})$ (Reynolds et al. 1983), and $F_{ST}/(1 - F_{ST})$ (Slatkin 1995). All three methods produced similar values. These results showed a weak differentiation (3 to 5%) between subpopulations 2 and 3. In contrast, subpopulation 1 showed a strong differentiation from the two other subpopulations (18 to 29%).

The genetic distances between (Supplementary Table 5) subpopulation 1 and the two others were higher (0.17 and 0.15, respectively) than those between subpopulation 2 and subpopulation 3 (0.06). The estimated gene flow between subpopulations 2 and 3 was 10.82 (Supplementary Table 5), while that between subpopulation 1 and subpopulations 2 and 3 was more limited (2.22 and 1.72, respectively).

Bottleneck analysis

A sign test showed that the number of expected loci with heterozygosity excess was estimated at 09.91, under an infinite allele model (IAM), 10.07 under a two-phase model (TPM), and 10.04 under a stepwise mutation model (SMM). It further showed that the number of loci observed with heterozygosity excess was 8 under IAM, 4 under TPM, and 2 under SMM (Table 4). The difference between the observed and the expected loci with heterozygosity excess, as determined by the sign test, was significant under SMM and TPM, but not under IAM. Similarly, the Wilcoxon test was significant under TPM and SMM, but not under IAM. A qualitative mode-shift analysis showed a normal L-shaped curve (Fig. 6).

Table 3 AMOVA results partitioning the genetic variability within and among populations at three levels

Source of variation	df	Sum of the squares	Variance components	Proportion of variation (%)	Fixation index
Among subpopulations	2	52.59	0.53	9.27	$F_{IS} = 0.15^{***}$
Within subpopulations	66	360.54	0.77	13.56	$F_{ST} = 0.09^{***}$
Within population	69	293	4.41	77.17	$F_{IT} = 0.23^{***}$
Total	137	706.13	5.72	100	

***Significant difference at the threshold of 0.1%

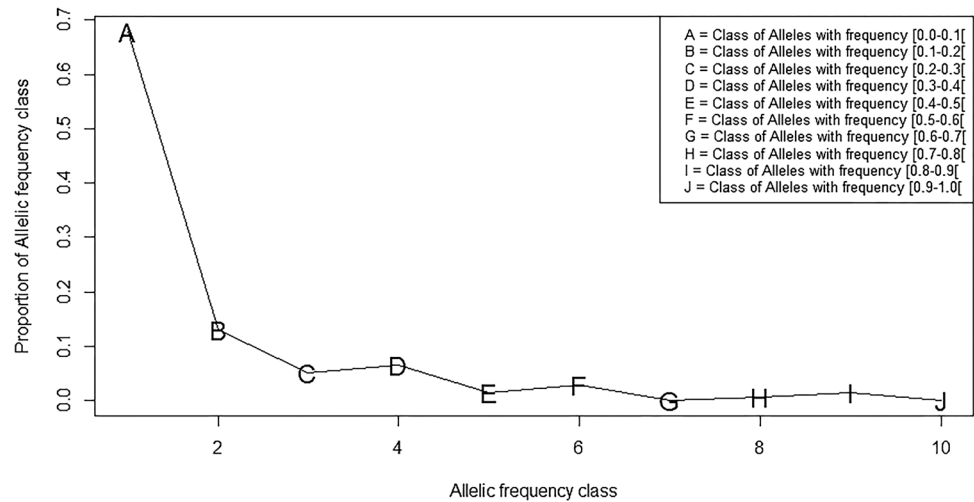
Table 4 Bottleneck test in local pig's population in southern Benin

Test		IAM	TPM	SMM
Sign test	Expected number of loci with heterozygosity excess	9.91	10.07	10.04
	Observed number of loci with heterozygosity excess	8	4	2
	<i>p</i> -value	0.24 ^{ns}	0.00**	0.00***
Wilcoxon signed-rank test	<i>p</i> -value (on tail for He excess)	0.78 ^{ns}	0.02*	0.00***

IAM infinite allele model, TPM two phase model, SMM stepwise mutation model, ns not significant

*Significant ($p \leq 5\%$); **significant ($p \leq 1\%$); ***significant ($p \leq 0.1\%$)

Fig. 6 Mode-shift analysis resulting in a normal L-shaped curve



Discussion

This is the first study with a detailed evaluation of genetic diversity and phylogenetic relationships among pig populations in southern Benin using molecular markers. A limitation of this study was the small sample size, which may have reduced the power of our results. We used a limited number of sampled pigs as it was difficult to sample more animals. Pig farmers are often reluctant to offer their animals for sampling as fresh pig blood is of high economic (used in various food specialties) and spiritual value, and very useful in traditional medicine in Benin (Djimènou 2019).

Large-White pigs were used in this study as the exotic breed. These pigs have been widely used for decades in pure breeding as well as in crossbreeding with the local pigs in Benin (Youssao et al. 2009; Houndonougbo et al. 2012; Djimènou et al. 2017a).

Hybrid pigs resulting from crosses of the local pigs with the Bush-pigs were used in this study as a reference group. These hybrid individuals are not only viable but also fertile and can reproduce for generations. Domestication and breeding of the Bush-pigs may be used for the repopulation of national parks (the W Park and the Pendjari Park in northern Benin). We used hybrids in this study also as a means to improve our knowledge about the Bush-pigs.

Fourteen (14) of the 17 investigated loci showed a PIC value over 0.5, indicating that the microsatellite loci used had very high informative capacity on diversity analysis within the population (Botstein et al. 1980).

The average PIC value (0.61) observed in this study was higher than the one (0.51) reported by Zaman et al. (2015) and Meléndez et al. (2014), in the Indian and the Colombian pigs, respectively. The total number of alleles observed (152 alleles) was higher than that (134 alleles) reported by Meléndez et al. (2014) in 62 local pigs using 20 microsatellite loci in Colombia. Moreover, the average number of alleles in

this study (8.94 ± 2.64) was higher than that (5.58 alleles) observed by Ayizanga (2016) in the local pigs of Ghana, that (6.7 alleles) obtained by Meléndez et al. (2014), that (5.72 alleles) observed by Montenegro et al. (2015) in pigs of Rocha Pampa in Uruguay, and that (7.07 alleles) reported by Canul et al. (2005) in pigs of Mexico. Furthermore, the average number of alleles found in this study was also higher than those (4.35 to 6.11) observed by Fan et al. (2003) and Li et al. (2002) in the breeds of Chinese pigs as well as those (7 to 7.74 alleles) observed by Behl et al. (2002) in the Indian pig breeds, those (5.36 alleles) found in the local and commercial pigs of Portugal (Vicente et al. 2008), and those (5.14 alleles) observed in the indigenous pig of Iberia and Island by Gama et al. (2013). In contrast, the observed number of alleles (8.94) was similar to that (8.2) found by Martinez et al. (2005) in the Cuban Creole pigs. All these results show that there is a high diversity in the swine population of southern Benin.

In this study, the observed average heterozygosity (0.46) was lower than those reported (0.634, 0.692, and 0.531) by Swart et al. (2010) in the South African, the Mozambican, and the Namibian local pigs, respectively, and by Meléndez et al. (2014) in the Columbian pigs (0.55). Our result is, however, close to that reported by Ayizanga (2016) (0.48) in the 86 genotyped Ghanaian local pigs using 12 microsatellites. The observed deficit in heterozygotes ($H_o = 0.46$ and $H_e = 0.51$) was confirmed by the average inbreeding coefficient ($F_{IS} = 0.13 \pm 0.13$). These results suggest a likely consanguinity in the pig populations of southern Benin causing a loss of genetic variability. They are in agreement with those reported in the Ghanaian pigs that showed a significant deficit in heterozygotes (Ayizanga 2016). The value of F_{IS} is, however, twice as low as that (0.26) obtained in the Ghanaian pigs (Ayizanga 2016). The deficit in heterozygotes can be explained by the presence of null alleles (San-Cristobal et al. 2003; Katiravan et al. 2008; De Meeüs 2012). Ollivier

(2009) also referred to this phenomenon of heterozygote deficiency as a result of the presence of null alleles, which can cause an excess of false homozygotes, a well-known limitation of using microsatellite markers. De Meeûs (2012) recommends testing for null alleles in all genetic diversity studies using microsatellite markers. He specified that, when all loci are rejected by the test, which was not reported in the present study, the deficit in heterozygotes would be ascribed to biological reasons. Despite the elimination of loci representing null alleles, the heterozygote deficit persisted, excluding the probability that the observed deficit could be entirely caused by the presence of null alleles. Therefore, inbreeding and null alleles can be considered contributing factors to the heterozygote deficiency observed in the current analyses.

This study also shows that the deficit in heterozygotes is associated with a high inbreeding coefficient (F_{IS}) and an increase in genetic diversity (H_S) along with an important deviation of the population from the Hardy–Weinberg equilibrium. These results show that the sampled animals did not come from a single pool of randomly fecundated gametes. The results can also be explained by the presence of the Wahlund effect. The three clusters or subpopulations detected in this study by a factorial correspondence analysis, as well as by a genetic structure analysis, confirmed this assumption. Hybrids resulting from crossings between the local pigs and the Bush-pigs showed a homogeneous genetic constitution, which was evidently distinct from that of the other two subpopulations. Subpopulation 2 has been characterized by a high degree of introgression of the Large-White pig genes into the local pigs that are assigned in this subpopulation. This genetic erosion is observed mainly in the local pigs originating from the Departments of Ouémé, Littoral, and Couffo. These molecular results confirm the results reported from recent studies performed by Djimènou et al. (2017a), who reported that Ouémé and Littoral departments were the zones where the Large-White pigs were mostly bred in southern Benin. The current study extends this zone to the Department of Couffo. Local pigs that showed a very weak introgression of exotic genes in subpopulation 3 come from the Departments of Zou and Plateau, whereas those coming from the Departments of Atlantic and Mono were distributed between subpopulations 2 and 3. Djimènou et al. (2018) reported that the local pigs in southern Benin were morphologically structured into three morphotypes according to their body size. The degree of gene introgression observed in the current study can explain the observed phenotypic diversity in local pigs in southern Benin, which has been mentioned in Djimènou et al. (2018). We can conclude that the genetic specificity of the local pigs in southern Benin is under threat. The threats on the local pigs are probably related to the changes on the breeding systems that are only focused on immediate outcomes to satisfy a strong market

demand for swine meat. These threats can also be explained by the lower production performances of the local pigs than the exotic breed's production (Youssao et al. 2009).

The phylogenetic relations between pairs of animals within subpopulations showed a genetic homogeneity of the hybrid and the Large-White pigs. In contrast, the local pigs displayed a subdivision into four subgroups, indicating a large genetic diversity in the local African pig populations in southern Benin. The observed interconnection between these genetic subgroups means they have shared at least one common ancestor. The only isolated Large-White pig found among the local pigs showed that this individual, although showing a Large-White phenotype, had a high proportion of genes from local pigs.

A high genetic variability was observed within subpopulations 2 and 3, in contrast to the hybrid subpopulation. Indeed, the expected heterozygosity obtained ($H_e=0.26$) for subpopulation 1 (hybrids) was two times lower than that of subpopulations 2 and 3, estimated at 0.67 and 0.59, respectively. Besides, the values of observed heterozygosity of 0.59 and 0.48 for subpopulations 2 and 3, respectively, followed by the high values of the estimated genetic diversity parameters, confirmed a high heterogeneity within each of these two subpopulations. The low genetic diversity detected in subpopulation 1 ($H_S=0.28$, $PIC < 0.25$, less than 2 alleles per locus) could be a consequence of many factors: a strong selection pressure; a lack of breeding programs or technical problems, like loci with null alleles; or problems related to the specificity of the microsatellite markers applied to the Bush-pig genome. Jarne and Lagoda (1996) showed that the percentage of loci that successfully cross-amplify between species may decrease with increasing genetic distance. An interesting study will be the use of specific microsatellite markers for Bush-pigs to confirm or invalidate this hypothesis. The symmetrical allelic and genetic distances estimated through various models showed that the hybrids were largely distinct from local and exotic pigs and more genetically close to their parent, the Bush-pig. The fecundity of hybrid animals resulting from crosses between the local pigs and the Bush-pigs suggests that these two species may have the same number of chromosomes (38 chromosomes). A cytogenetic characterization of the Bush-pig may confirm this assumption (Albayrak and Inci 2007).

The weak genetic differentiation observed between subpopulations 2 and 3 confirmed gene flow between these two subpopulations. These findings are clearly in line with the traditional management of pig genetic resources characterized by animal exchanges and trading between farmers within and among departments and regions in southern Benin (Djimènou et al. 2017a,b; 2018).

Based on the results from the different bottleneck tests, it can be stated that the local pig's population has not undergone a recent bottleneck, although this population

suffered a drastic and brutal reduction in its numbers following the 1997 African swine fever episode in Benin. The different tests used to identify the existence of a recent bottleneck in the local pig population in southern Benin were not significant under the infinite allele model (IAM). However, under the Wilcoxon test, it has been shown that if a bottleneck has really occurred in a population, it will be detected very strongly with the IAM hypothesis, moderately with the TPM, and weakly with the SMM (De Meeûs 2012). The high degree of introgression of Large-White pig genes into local pigs is a consequence of uncontrolled crossbreeding aimed at increasing productivity. This erosion of the local pig genome is responsible for the drastic loss of advantages related to the adaptability to tropical climatic conditions, disease resistance, and meat quality of the local pig (Youssao et al. 2004a, b; Djimenou et al. 2021). Even if crossbreeding is a shortcut to achieve a high level of animal protein production in Benin, it is essential to conserve the genes for adaptation and disease resistance, hence the importance of safeguarding our local pig resources. The data from this study are not yet sufficient for the establishment of a conservation program for local pigs in Benin. Indeed, the study is only based in southern Benin and does not allow to draw a conclusion at the national level. Also, the genetic distances observed between the identified genetic groups are a great asset to maximize genetic progress by selection within the local pigs. Unfortunately, these pigs are no longer true local pigs because of the erosion degree of their genome by foreign genes. The extension of the study will allow better mapping of the distribution and genetic status of local pigs for sustainable management in Benin.

Conclusion

The current study showed a high level of genetic diversity in pig populations in southern Benin. The observed diversity was mainly due to a within-breed genetic variation. Local pigs in southern Benin are affected by a great introgression by the Large-White genes. The study revealed a strong differentiation of hybrids, compared to the local pigs and the Large-White pigs, which were genetically close. In addition, the local pig's populations in southern Benin have not undergone a recent bottleneck.

This study demonstrates that pure breeds of the local pigs are scarce in southern Benin. Due to the limited sample size used in the current study, however, future studies will be required to investigate other regions of Benin using a larger number of local pigs to identify the distribution zone of local pigs pure breeds for their conservation and sustainable management in Benin.

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Author contribution DD, KOD, and ASH designed the proposal; DD and CCAAM collected all the data; DD and DGK performed the statistical analyses; DD, KOD, and ASH wrote the manuscript; and DGK, HAS, and AC reviewed the manuscript.

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Declarations

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