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Genetic diversity and population structure of cavy (*Cavia porcellus L*) in three agro ecological zones of Côte d'Ivoire

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Abstract

To investigate the genetic diversity and population structure of cavies (*Cavia porcellus* (Linnaeus, 1758) from three agro ecological zones (North, central, and South) of Ivory Coast, 14 microsatellites markers were used. A total of 131 cavies were genotyped. The measure of population diversity for the three populations revealed a mean allele frequency of 6.0, 5.5 and 6.429 (P<0,05) for the north, central and south populations respectively. The observed heterozygosity (Ho) was 0.511 ± 0.66, 0.505 ± 0.55 and 0.567 ± 0.064 (p < 0,05) for the north, central and south populations and in all cases lower than the expected heterozygosity (He) (0.577 ± 0.059 , 0.634 ± 0.051, 0.645 ± 0.052) respectively. This indicates low heterozygosity across the three populations in the whole population., The population specific inbreeding coefficients (F_{St}) were 0.1695, 0.2768 and 0.2245 (P < 0,01) for the three separate populations and a mean of 0.2257.There were no clear differences in the population structure with only 2.59 % variation among the three populations and 21.99 % variation among individuals within a population. There were high rates of inbreeding in all the three populations (mean 0.2257 (p < 0,01). Therefore the tree population would mix. It is difficult to select non-related animals and thus control inbreeding in the target populations or selection for particular traits of interest.

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Introduction

Cavies (*Cavia porcellus*, *Rodentia*, *Caviidae* L.) were domesticated in Andean region 2500 to 3600 years ago (Chauca, 1997). Nowadays, cavies play an important role in the economy by providing food security and livelihoods for Andean peasant families (Lammers *et al.,* 2009).

Food security is a major challenge for most African nations including Côte d'Ivoire. All over Africa, general food supply remains very low compared to an increasing demand due to growing populations, rising urbanization coupled with rural-urban migration as well as, partly, increasing wealth (Neumann *et al*., 2007). Most households however do not get a regular supply of animal protein from large livestock due to its cost and cultural issues. Large livestock such as cows and goats are a source of wealth and prestige. It however has been shown that a regular supply of small quantities of animal protein is important for physical and cognitive development of children (Grillenberger *et al*., 2006). Small livestock such as cavies can be used to address this nutrition gap both quantitatively and qualitatively.

Domestic cavy or 'guinea pig' (*Cavia porcellus* L.) occur more widely in Africa than is generally known because it is usually not included in livestock statistics. Despite their reported distribution over a belt from West Africa to East Africa (Ngou-Ngoupayou *et al*., 1995), domestic cavies have consistently been ignored in research and development for better production. This deficiency is also observed in most African countries where cavies are kept.

Cavies have great potential in contributing to addressing food security challenges in developing countries (Lammers *et al.* 2009). This is has been demonstrated albeit under unfortunate circumstances in Côte d'Ivoire and in the Kivu provinces of DRC which suffered civil strife and armed conflict (Rossi *et al*., 2006). Cavies have helped rural people to not completely lose their livestock populations in pillage as they were easier to transport and regenerate due to their small size and short breeding times. Trade in cavies has served as a source of income for paying school fees alongside its role as a protein source (Metre, 2005).

There is now increasing awareness of the commercial viability of cavies. In Cote d'Ivoire it has also been noted that small-scale family farms for fattening or breeding cavies are in existence. These farmers rear cavies for commercial purposes only and in so doing make a living for themselves and contribute to satisfying the market's need for meat protein.

Therefore, it is important to develop proper and informed technical and breeding support programmes for cavy farmers. An understanding of the cavy population is critical to this. To cover this gap in our scientific and technical knowledge we undertook a study to assess the genetic diversity of cavies in Cote d'Ivoire. The aim of this study was to investigate the diversity, inbreeding levels and population structure of cavies in Côte d'Ivoire so as to better inform the breeding strategies to be used in improving small-scale cavy farming. This paper reports the genetic diversity indices, inbreeding levels and population structure of cavies from three zones of Côte d'Ivoire.

Materials and methods

Sample collection

131 blood samples were collected from cavies kept in 7 regions of Cote d'Ivoire. 2 regions in the north, 2 regions in the central and 3 regions in south were sampled from May to October 2011 (fig.1).

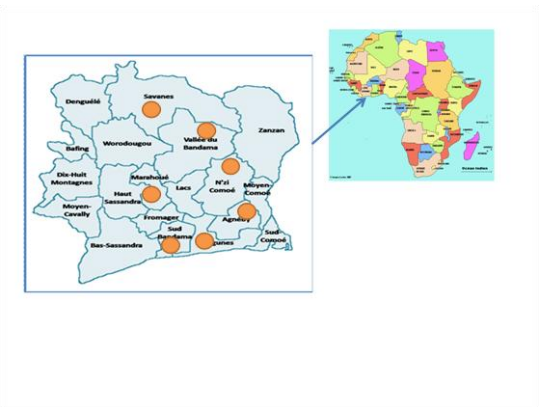

Fig. 1. Map of sampling area (Côte d'Ivoire).

Blood collection and DNA extraction

Blood samples were collected from 131 cavies aged three months old and weighing over 250g. The procedure involved taking120 µl of blood from the ear region and putting it on Whatman ® FTA cards at room temperature. The FTA cards were airdried and then stored and transported to Biosciences Eastern and Central Africa (BecA), International Livestock Research Institute (ILRI) hub laboratories for processing. DNA extraction was carried out using Invitrogen kit Pure link® genomic DNA extraction kit using the manufacturer's instructions. The quality of the extracted DNA was confirmed by OD reading using a Nanodrop ® ND-8000 spectrophotometer and by electrophoresis on a 0.8% gel red stained agarose gel.

Genotyping was performed using 14 SSR microsatellite markers following a modified version of the protocol published by Kanitz *et al.* (2009). Polymerase Chain Reaction (PCR) amplification was carried out in a 10 µl total volume reaction mixture, containing 0.2µL of each primer, 0.2 mM dNTP mix, 6.32 µl Milli Q water, 1 x buffer (Fermentas), 5U/ µL Taq polymerase (Fermentas) and of 10 ng / µL template DNA. PCR amplification was performed in a GenAmp ® 9700 PCR system (Applied Biosystems) with the following conditions: 95°C for 3min, followed by 35 cycles of 30 s at 94 °C, 30 s of hybridization and 60 s of extension at 72 °C, and final extension step at 72 °C for 20 min (Table). All the 14 SSR markers amplified well and the PCR fragments were resolved on the ABI 3730 genetic analyzer. The data was captured using the GenScan ® collection software (Applied Biosystems) and the allelic data analysed using the GeneMapper ® software version 4.1 (Applied biosystems). A total 118 data points were achieved out of the expected 131 data point giving an overall success rate of 90 % .The data was compiled into a spreadsheet as a standard GeneMapper output file and used in subsequent analysis.

General Statistical Analysis

Genotypes were assigned for each animal based on allele size data. Polymorphism Information Content proportion (PIC) was estimated and allele richness was calculeted with Power Maker software (Bolstein *et al*., 1980). Allele frequency, and the number of different alleles and fixation index of each locus were statistically analysed using GenAlex software.

Genetic diversity within and among Agroecological region

Expected (He) and observed (Ho) heterozygosity , number of allele (Na) were calculated for each region using Arlequin software for deviations from Hardy-Weinberg equilibrum **(**Guo and Thompson, 1992). The inbreeding coefficient `(Fis), population variation and population structure (Fst) for populations were estimated using Weir and Cockerham (1984**)** method.

Genetic distances between guinea pigs of each region were estimated using GenAlex version 6.4 (Nei *et al*., 1983) The genetic and geographical distances between population pairs were correlated, in order to establish whether genetic separtion could be linked to the isolation of populations. Bootstrap values were obtained using 10000 replications

Admixture were investigated. For grouping individuals into a K-th number of population, Bayesian probabilistic group assignment was done using STRUCTURE software (Pritchard *et al*., 2000). K values analyzed ranged from to 1-7 and each one was simulated fives times. Correllated allele frequency with mixing model was used for runs with 100,000 iteration following a 10,000 burn-in period. The DeltaK method described by Evanno *et al.*(2005) was applied for inferring optimal k-values. DISTRUCT software was used to graphically visualize the clustering pattern of the animals.

Results

Micosattelite markers and F-statistics

All the 14 microsatellite markers used in this study were amplified succesfully in all the populations. All loci were found to be polymorhic with alleles ranging from 4 (cavy 2 and cavy 6) to 14 (cavy 14). The average number of observed alleles for all three

populations per locus ranged from a low of 2.67 (cavy 2 and cavy 16) to 9 (cavy 14) and a global mean of 5.98 ± 0.37 for the average number of alleles and 3.175 ± 0.19 for the effective number of alleles as shown on Table 1.

Table 1. Microsattelite markers, TNa = Total Number of alleles, MNa = Mean number of alleles, Ne = No. of Effective Alleles, Polymorphic information content per locus, Ho = Observed Heterozygosity = No. of Hets / N He = Expected Heterozygosity, F statistics (Fis, Fit, Fst) per locus.

Marker	TNa	MNa	Ne	PIC	Ho	He	Fis	Fit	Fst
cavy2	4	2.67	1.11	0.175	0.02	0.10	0.794	0.795	0.004
cavy3	5	4.00	1.69	0.428	0.22	0.39	0.429	0.455	0.045
cavy5	8	5.33	3.47	0.774	0.64	0.70	0.089	0.153	0.071
cavy 6	11	8.67	4.43	0.802	0.58	0.77	0.246	0.269	0.030
cavy7	9	6.00	3.54	0.775	0.53	0.71	0.258	0.307	0.066
cavy8	12	8.33	2.66	0.629	0.51	0.62	0.179	0.196	0.021
cavy9	5	3.33	2.51	0.568	0.50	0.59	0.144	0.169	0.029
cavy10	6	4.67	2.86	0.641	0.43	0.64	0.335	0.362	0.041
cavy11	10	8.00	4.71	0.783	0.69	0.79	0.122	0.133	0.013
cavy 12	14	8.33	3.62	0.752	0.91	0.72	-0.273	-0.250	0.019
cavy13	11	7.67	4.40	0.724	0.43	0.77	0.441	0.450	0.016
cavy14	11	9.00	4.81	0.7994	0.78	0.79	0.020	0.027	0.007
cavy15	8	5.00	2.50	0.5962	0.63	0.60	-0.049	-0.044	0.004
cavy16	4	2.67	2.16	0.255	0.52	0.48	-0.085	0.030	0.106
Mean		5.98±0.37	3.175±0.19	0.622	0.528±0.04	0.619±0.03	0.189±0.07	0.218±0.07	0.034±0.008

The mean He across loci was 0.619 ± 0.03 with estimates per locus ranging from 0.10 (cavy 2) to 0.79 (cavy 11). For Ho, the mean for all loci was 0.528 ± 0.04 and the range was 0.02 (cavy 2) to 0.91 (cavy 12) and was lower than the He in eleven of the loci studied with cavy 12, 15 and 16 being the exceptions.

The mean estimated Fst for the 14 loci was 0.034 ± 0.008 and the range was between 0.004 (cavy 2 and cavy 15) and 0.106 (cavy 16). The mean within breed deficit of heterozygosity (Fis) pooled across the 14 loci was 0.189 ± 0.07. Nevertheless, there were differences among loci for this deficit with values ranging from -0.273 (cavy 12) to 0.794 (cavy 2). The mean estimated Fit across the 14 loci was 0.218 ± 0.07 with values ranging from -0.250 (cavy 12) to 0.795 (cavy 2). It is also notable that the polymorphism information index for the 14 microsatelites used varied from 0.175 (cavy 2) to 0.802 (cavy 6).

Population Diversity and relationships

The mean average values for number of alleles for individual populations, effective alleles, observed and expected heterozygosoties and average loci with private alleles is shown on Table 2. The south showed the highest average for both the average number of alleles (6.426 ± 0.693) and loci with private alleles (0.714 ± 0.194). In all three populations the expected heterozygosity was higher than that of the observed heterozygosity with the lowest being from 0.505 ± 0.052 (Central) against an expected heterozygosity of 0.634 ± 0.052 , the global mean for all three populations was estimated at 0.528 ± 0.036 against an expected hetorozygosity of 0.619 ± 0.031 (Table 3).

For evaluate loci, there is no heterezygote deficite with loci lcavy 14 in north population and south population, and no hetezygote deficite with locus 12 in north population. Then the north population and south have the highest number of loci in Hardy-Weinberg equilibrum (HWE) (Table 3).

Population variations

Analysis of molecular variation among populations was small (2.59 %) while within populations and individual was medium (21.99 %) and large (75.42 %)

respectivly (table 4). The F statistics or fixation indices for the pairwise comparisons were calculated for all loci and ranged from very significant to highly significant.

Table 2. Estimation of intrapopulation diversity indices and population inbreeding coeficient.

Population		Na	Ne	Ho	He
North	Mean	6.000	2.882	0.511	0.577
	± SE	0.703	0.307	0.066	0.059
Central	Mean	5.500	3.250	0.505	0.634
	± SE	0.532	0.336	0.059	0.051
South	Mean	6.429	3.393	0.567	0.645
	± SE	0.693	0.332	0.064	0.052
Total	Mean	5.976	3.175	0.528	0.619
	± SE	0.369	0.186	0.036	0.031

Na represents mean number of allele per population, Ne= mean number of effective alleles, Ho= observed heterozygosity, He= expected heterozygozity, (GenAlex data).

Table 3. Intrapopulation genetic diversity measures for each agroecological region of cavies from Cote d'ivoire.

North	Locus	Na	Ho	He	UHe	HWE	Central	Locus	Na	Ho	He	UHe	HWE
	cavy2	3	0.059	0.112	0.114	***		cavy2	3	0.000	0.057	0.058	***
	cavy3	4	0.118	0.263	0.267	***		cavy3	4	0.303	0.528	0.537	**
	cavy5	5	0.710	0.644	0.654	ns		cavy5	5	0.533	0.706	0.718	ns
	cavy 6	8	0.606	0.761	0.773	ns		cavy 6	8	0.529	0.759	0.770	*
	cavy7	6	0.548	0.683	0.694	ns		cavy7	5	0.419	0.685	0.696	ns
	cavy8	8	0.529	0.640	0.649	**		cavy8	8	0.472	0.580	0.588	*
	cavy9	3	0.514	0.548	0.556	ns		cavy9	3	0.486	0.529	0.537	ns
	cavy10	4	0.455	0.581	0.590	*		cavy10	5	0.371	0.717	0.728	***
	cavy11	9	0.697	0.770	0.781	***		cavy11	7	0.647	0.815	0.827	***
	cavy 12	10	0.840	0.727	0.742	***		cavy 12	7	1.000	0.659	0.675	**
	cavy13	8	0.333	0.746	0.764	***		cavy13	6	0.545	0.794	0.813	**
	cavy14	9	0.818	0.778	0.790	***		cavy14	8	0.710	0.803	0.816	*
	cavy15	5	0.700	0.623	0.638	ns		cavy15	6	0.556	0.614	0.632	***
	cavy16	2	0.222	0.198	0.209	ns		cavy16	3	0.500	0.625	0.714	ns

South	Locus	Na	Ho	He	UHe	HWE
	cavy2	3	0.000	0.117	0.118	***
	cavy3	4	0.240	0.366	0.370	***
	cavy5	6	0.681	0.762	0.770	ns
	cavy 6	10	0.612	0.798	0.807	***
	cavy7	7	0.617	0.767	0.775	ns
	cavy8	9	0.529	0.645	0.651	***
	cavy9	4	0.510	0.687	0.693	ns
	cavy10	5	0.451	0.623	0.629	ns
	cavy11	8	0.725	0.771	0.779	***
	cavy 12	8	0.701	0.766	0.776	***
	cavy13	9	0.414	0.772	0.786	**
	cavy14	10	0.800	0.793	0.801	***
	cavy15	4	0.525	0.556	0.568	ns
	cavy16	3	0.433	0.611	0.667	ns

Na = mean number of allele per population, Ne = mean number of effective alleles, Ho = observed heterozygosity, He = expected heterozygozity, UHe = Unbiased heterozygosity index, HWE = Hardy –Weinberg Equilibrum *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), $^{ns}(p > 0.05)$.

Table 4. Population variation with AMOVA (Distance method- Pairwise differences) and average F statistics for the 3 populations.

Source of variation	df	sum of squares	variance components	Percentage variation
Among populations	2	10.03	0.03963	2.59
Among individuals within populations	120	219.33	0.33665	21.99
Within individuals	123	142	1.15447	75.42
Total	245	371.37	1.53075	

	Fixation Indices	P values	Significance
F_{IS}	0.22577	0	***
F_{ST}	0.02589	0.001	**
F_{IT}	0.24581	0	***

*** higth significant

The Fis value = 0.225 indicates a deficit of heterozygotes important at the population level taken isolation in the overall population (Fit = 0. 245) probably due to the Wahlund effect. The value of Fit indicates an overall deficit of heterozygotes 24.5 % considering the three populations studied. Concerning to Fst, genetic differentiation between populations is = 0.02589, which can be considered a moderate overall value, indicating the origin of the total genetic variation in the species.

The inbreeding coefficients for the three populations are shown on table 5. All regions show inbreeding with the highest index in the Central region (0.276), the south (0.224) and the North (0.169).

The genetic distances were calculated for these populations. The closest distance was obeserved beteween south population and population from central (D = 0.099) and north (D = 0.063). The largest was beteween north central and north (D = 0.101) (table 7).

Table 5. Population specific inbreeding coeficients (10100 permutations).

Agro ecological zone	F_{IS}	P-value
North	0.16959	0.000792
Central	0.27684	0.00
South	0.22455	0.00

Table 6. Genetic distance (below the diagonal) and Genetic identity (above the diagonal) (GenAlex V 6.41) among the population of cavy of Cote d'ivoire.

	North	Central	South
North		0.904	0.939
Central	0.101		0.905
South	0.063	0.099	

(P < 0.01).

Population Structure

Ancestral populations

The number of ancestral population underlying the observed genetic diversity was assessed with the Bayesian approach implements by STRUCTURE. The likelihood of the observed data given the number of inferred ancestral populationd [Ln Pr (X|K)] is shown in fig. 2 for numbers of infered populations ranging from K = 1 to K = 7 with the average for 6 repliations for all values of K. The mean value of Ln Pr (X|K) increased up to K= 5 and dropped afterwards with a large increase in its variance. It was therefore assumed that K = 5 is the most likely number of ancestral populations that contribute to the observed genetic variability in the three populations studied.

Admixture

The contributions of the assumed ancestral popultions are graphically represented in fig. 3 for values of K ranging between K = 1 to K = 7. From the visual output from DISTRUCT the three regions have a lot of genetic admixture. It is however interesting to note that a section of the North and another from the

south seem to share a near similar genetic profile (see circled areas) the rest seem to share a similar admixture profile.

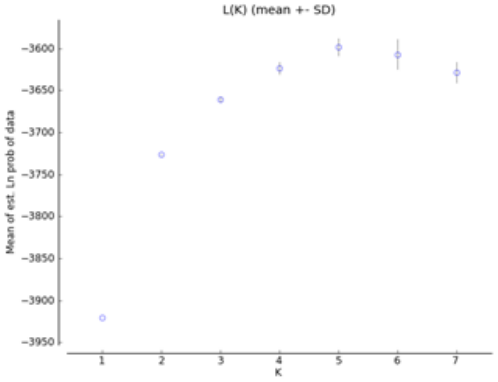

Fig. 2. Plot estimating probabilities of the data [Ln Pr (X/K)] for different number of infered clusters (K = 1 to K = 7) for the mean of 5 runs at each K.

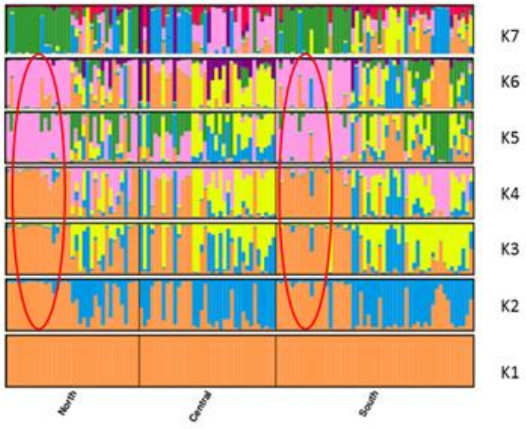

Fig. 3. Graphical representation of the estimated membership fractions of individuals in the 3 populations analysed in each of the K inferred clusters for K = 1- 7.

Discussion

The study of genetic variation plays an important role in developing breeding strategies for economical animal species (Maudet *et al.*, 2002). The advantage of using microsatellite markers for estimating genetic variation has been used to investigate in farm animals and cavy populations from Columbia and Brasilia.

In the present study, fourteen microsatellites were used to evaluate the genetic diversity within and between three populations of cavies in Cote d'Ivoire. All microsatellite were found to be in polymorphic state in all populations studied contrary to the report findings of William *et al.* (2011) when evaluating the same microsatellite loci in 3 populations of cavies in Columbia. According to Botstein *et al.* (1980), the used markers indicated relatively high Polymorphic Information Content (PIC= 0, 175 to 0.802), allowing the detection of significant differences in genetic structure of cavies' lines. However, it is necessary to look for additional microsatellite loci to increase the number of available evaluated alleles. Only cavy 2 and cavy16 were very low PIC< 0.26 (table 2), this was accordance with Burgos *et al.* (2007) and Solarte *et al.* (2007), who stated that cavy population is affected by forces that modify allele frequency, such as selection, genetic drift and other, bottlenecks. As cavy production systems are influenced by market behaviours, especially during the season of high demand and school year, producers sell most of their animals, keeping only the necessary few to breed a fresh population. Also, most cavy farmers move with their domestic animals when they exude to the central or south area vice-versa.

Considering average expected heterozygosity, overall loci were found to be high within the tree population. It was found that there was heterozygote deficit in all the three populations. Solarte *et al.* (2007) also noted the same with studies in cavies' population from Columbia. This has been also reported by Tapio *et al.* (2003) and Santucci *et al.* (2007) in animal domestic studies.

The Inbreeding coefficient (Fis = 0.225) indicates that there is a deficiency of heterozygotes in the sub populations. There is inbreeding. The genetic differentiation between the total populations is founded to be very small. There is non-random mating (inbreeding) across the general total population. The Fst value (0.0258) show that genetic differentiation between the populations is small.The low levels of population structure and high rate of inbreeding has been reported by William *et al.* (2011) in Columbia cavies population and also registered in various animal production systems. This has been attributed to non-

random mating production system, selection by features of economic importance, and the intensive use of reproductive technologies (Wu *et al*., 2009). Cavies have also rapid growth, high reproductive and this can contribute a highly susceptible inbreeding issue in traditional production systems.

According to genetic distance and genetic identity data, there is a little separation between north and central, south population, the probability of encountering a common allele in any two populations was presumed to be greater in north, south population and central. This could be attributed to the production system where breeders (pupils) exchange or buy cavies without take care of origin. Also, the rural population of north moves to south for growing cocoa and pupil during the school holydays move from their place to another area with their cavies because it could be easy to carry it.

Concerning the K value, it has been placed at K = 5 from the visual output from DISTRUCT the three regions have five ecotype breeds and a lot of genetic admixture. But STRUCTURE show in graphical representation (fig.3) that individual of population of cavies from the North and another from the south seem to share a near similar genetic profile. The rest seem to share a similar admixture profile .So we can define this like two types or variety of cavy. According to Lawson *et al.* (2007) in domestics breeds can be categorize into type or variety if the population is genetically similar. Though the data seems to show as many as five possible breeding ecotypes it is doubtful is there is any phenotypic evidence to correlate this and if there the phenotypes have any traits of interest and advantage to the farmer.

So we can conclude, the genotyping data suggests that there is little genetic diversity across three populations of cavies studied in Cote d'Ivoire and define two types.

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