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Correlation between the genetic diversity of Sitophilus Zeamais (Mots.) And the post-harvest losses of Corn in the humid agro-climatic zone of West and Central Africa (Ivory Coast, Cameroon, Ghana, Central African Republic)

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Abstract
The main origin of the huge losses of maize stocks in Africa, particularly in the Sudano-Guinean agroclimatic zone is the methods and the means of their conservation. Our study tried to explain it also by the destructive capacities of the insect, by highlighting a correlation between the adaptive predispositions, therefore destructive of the populations of the insect which are apprehensible through their high genetic diversity and these enormous losses. The results, which could be biased by protective measures for producers, appeared to be mixed. The huge losses of maize stocks observed in Ghana indeed correspond to a strong genetic diversity of the Ghanaian population of Zealese Sitophilus, comparable to a strong adaptability. But it is the opposite in other countries (Caméroun, Central African Republic, Ivory Coast) where the enormous losses are in phase with a low genetic diversity of their populations comparable to a low resilience in these countries.

Keywords: zeamais sitophilus, genetic diversity, agroclimatic zone

Introduction
Maize is an important staple food for more than 1.2 billion people in Africa and Latin America [12]. In the humid zone of West and Central Africa in particular, it is massively exploited and consumed. In Ghana, corn accounts for 50% of cereal production [12]. A survey carried out by ACDIC in 2008 in Cameroon revealed that this cereal is consumed in almost 99% of households. It is the same situation in Ivory Coast where this culture provides 15% of the energy needs of the population [14]. Unfortunately, this and other economic functions are greatly threatened by postharvest losses, caused by *Sitophilus zeamais* (Motschusky, 1855). About 15% of the maize harvested is destroyed each year by weevils in Ghana [10]. These insects are known as major devastators of cereals during conservation north of the Cameroon [2]. Many studies have been done to try to explain these huge post-harvest losses. This is the case with that of Guéye M.T and Seck D in 2011, which demonstrated that the types and storage methods of corn crops influence the vulnerability of grains to insect attack.
Our study fits into this perspective. It aims to establish a correlation between the genetic diversity of *S. zeamaïs* and the loss of corn stocks caused by this same insect in 4 countries belonging to the same humid agroclimatic zone of West and Central Africa [3]. The principle underlying our study is that the genetic diversity of a population increases its adaptive potential [7]. and that the high adaptability of an insect, comparable to its survival greatly increases its nuisance to stocks.
To reach this objective, a total of 52 insects were sampled, including 15 in Ivory Coast, 20 in the Central African Republic, 10 in Ghana and 7 in Caméroun. The sequences of the Cytochrome B gene corresponding to these insects were exploited by software for studying population genetics (Bioedit, DNAsp, Mega, Harlequin…) in relation to parameters of genetic variability (h, N, K, Pi, Hd, dn, ds, S, V, R).

1. Materials and Methods
1.1. Sampling
1.1.1. Sampling locations
Harvesting of zeamais Sitophilus individuals was carried out in five (4) countries in the humid zone [3]. These are Ivory Coast, Cameroon, Ghana, Central Africa. Table I summarizes the sampling.

Table 1: Sampling country

Countries	Sample code	Number of individuals	Geographic	coordinates
			Latitude	Longitude
Ivory Coast	SzIc	15	07°32’96’’N	05°32’49’’W
Central Africa	SzCa	20	06°36’40’’N	20°56’22’’E
Ghana	SzGh	10	07°56’47’’N	01°01’23’’W
Cameroon	SzCn	07	07°22’11’’N	12°21’17’’E

1.1.2. Harvest of individuals
In each of the above countries, 250 g to 1 kg of infested corn were collected from storage locations, through project partners. The samples have been sent to the laboratory where they are kept in jars with mesh lids for mass breeding. The insects collected at the end of this breeding were kept in alcohol at 95 ° C, then transported to the laboratory for a genetic study. Each sample is identified by a code : the first 2 letters designate the binomial name of the species (S for Sitophilus and z for Zeamais), the 2 letters which follow indicate the country of origin (example : SzIc, with S = Sitophilus, z = zeamais, Ic = Ivory coast. SzGh, with S = Sitophilus, z = zeamais, Gh = Ghana.

1.2. Molecular method of analysis
The cytochrome B gene was chosen to be amplified. The choice is explained by its particularity to keep very long

without wear and it is used regularly in the studies of insects.

1.2.1. DNA extraction

Extraction is the technique of releasing DNA from the cell. It includes the individualization of cells (digestion) and the destruction of their plasma and nuclear membranes (lysis). The digestion of the cells consisted of placing their legs and prothorax in tubes containing ATL buffer and proteinases K. After incubation, the tubes were centrifuged to separate the supernatant from the cell debris. To destroy cell membranes, cell lysis buffer (LA) was added first, then ethanol (96%) after incubation, in the tubes. Then the tubes are passed through columns with a silica membrane. Finally the centrifugation of the tubes made it possible to retain DNA on the siliceous membranes of the columns because it was negatively charged.

1.2.2. DNA purification

The DNA of the tubes was purified by adding 2 buffers AW1 and AW2 in each column. After centrifugation of the tubes and precipitation of the DNA at the bottom, the buffers and the contaminants are discarded. The columns are then replaced in other tubes in which AE buffer has been added to unhook the DNA. The DNA is thus removed and stored at -20 ° C.

1.2.3. PCR of the mitochondrial Cytochrome B gene.

The PCR of the mitochondrial Cyt.B gene was carried out by 2 primers defined by Simon *et al* (1996). For each sample (tube), the amplification was made from a total volume of 25 µl, including a mixed volume of 23 µl and a volume of 2 µl of DNA extract. The mixed volume was made up of : 18.3 µl of milli water, 2.5 µl of 10X buffer, 1 µl of additional Mgcl 2, 0.5 µl of Dntp, 0.25 µl of each primer and 0.2 µl of Taq polymerase.

Table 2: Identification of the primers used and programming of the PCR

Gene	Primer Names	Primer Sequences	PCR Program
Cyt.B	CB-J-10933(F) CB-N-11367(R)	5-TATGTACTACCATGAGGACAAATATC-3 5-ATTACACCTCCTAATTTATTAGGAAT-3	1. Initial denaturation: 94°C, 3 min; 35 denaturation cycles: 94°C, min 2. Hybrization: 47°C, 1 min 3.Elongation: 72°C, 2 min; elongation finale: 72°C, 8 min

1.2.4. Bioinformatics analyzes

The sequences were corrected and aligned by the Clustal software implemented in the Bioedit program version 7.2.5 [3].

The evaluation of the diversity of the sequences was made on the basis of certain parameters of genetic variability. These are, on the one hand, the standard indices which are among others the variable sites in parsimony and in singleton, the number of haplotypes (h), the average number of nucleotide difference (k), the percentage of transition (S) and of transversion (V), the non-synonyms (dn) and synonyms (ds) substitutions, the mutation rate (R) and on the other hand the Haplotypic (Hd) and nucleotide (Pi) diversity. These two indices have the distinction of highlighting the diversity and divergence of haplotypes. The parameters h, k, Hd, Pi were calculated by DNAsp ver software. 5.10.01 [3]. While those such as dn, ds, S, V and r were estimated by the MEGA7 ver software. 7.0.18 [2].

2. Results and discussion

2.1. Results

2.1.1. Postharvest losses of Corn in the Sudano-Guinean zone

Table 3: Postharvest losses in corn stocks in humid agroclimatic zone

Countries	Ivory Coast							Ghana				Central Africa									Cameroon
HACZ, Areas	CE	CO	C	W	N	NW	SW	West	Middle zone		North	Est	Trop. Sub-wet (Bossangoa)			Forest Guinea (Obo)			Sudanese ubangean (Sibut)			Est, South, West , Center, North
									Great season	Small season			Bag	Cob	Attic	Bag	Cob	Attic	Bag		Attic	
Losses	+++		+++	+++	++	++	++	+++	10,9%	20-30%	30-40%	20401t	13000t	3%	15%	20%	2%	14%	15%	2%	12%	15%	5 - 15%
References	RATNADASS, 1984 ; TRAN 1987 ; MOYAL et TRAN, 1990							N.F. Alhassan, P. Kumah et al (2018) ; Opit, G.P et al (2014) ; Emmanuel Bruce 92016)					FAO, PAM, 2017									Tangno et Tinkeu, 2014 L.S.T. Ngamo et Hance, 2007

CE= MidEst ; CW= Midwest ; C= Center ; O= West ; NW= North-West ; SW= South-West

+++= Very abundant ++= Abundant HACZ= Humid agroclimatic zone

The analysis of the summary table III of losses in the humid agro-climatic zone indicates that the losses during the conservation of maize vary from one country to another. But overall they are important regardless of the country. In Ivory Coast, losses are at least abundant. They vary from 10 to 40% in the middle zone of Ghana, while in the north and east of this country, they are estimated respectively at 20,401 tonnes and 13,000 tonnes. In the Central African Republic, post-harvest losses vary according to the means of storage, but they are high overall because only, 3 estimates out of 9 vary between 2 and 3%, the other measures achieved at least a loss rate of 14%. The losses are also significant in the Caméroun areas (they range between 5 to 15%).

2.1.2. Genetic variability of sequences

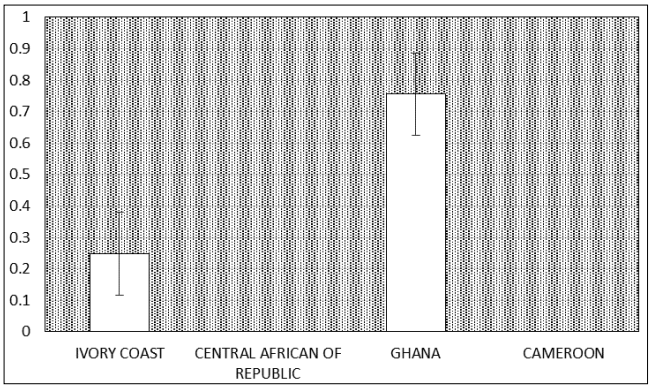
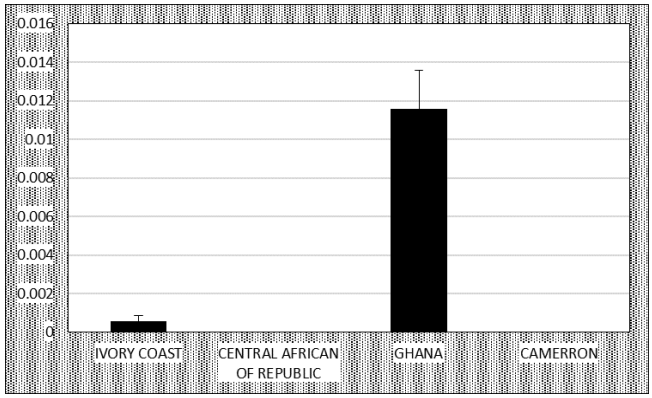


Fig 1: Haplotypic Diversity (A) and Nucleotidic Diversity (B) of *S. zeamais*.



NUCLEOTIDIC DIVERSITY (B)

Haplotypic diversity (Hd) and nucleotide diversity (Pi) are very high only in Ghana (Hd = 0.75 ± 0.130; Pi = 0.0116 ± 0.002). The ivory coast is characterized by a relatively low genetic diversity (Hd = 0.248 ± 0.131; Pi = 0.00053 ± 0.0003). The populations of other countries, namely those of Cameroon and the Central African Republic are genetically homogeneous (Hd = 0.000 ± 0.000; Pi = 0.000 ± 0.000). The values of the other parameters of genetic variability (Table IV) confirm these trends. Indeed, Ghana has the highest number of haplotypes (h = 5), variable sites (S = 11), but also the highest substitution rate (R = 1.112) and the number of means of nucleotide difference (K = 5.133). The values of these quantities are relatively low for the Ivory Coast (h = 2, K = 0.248, R = 0.820) and zero for the 2 other countries (Cameroon and Central African Republic). On the other hand, non-synonymous substitutions are more important than synonymous substitutions for all countries.

Table 4: Parameters of genetic diversity

Parameters Countries	n	h	N	K	dn	ds	Dn/ds	S	V	R	Monomorphic sites	Variables	Sites
												Singleton	Parcimony
Ivory coast	15	2	442	0,248	345,33	95,67	3,60	99,68	0,34	322,4	441	0	1
Ghana	10	5	442	5,131	345,4	95,6	3,61	33,34	66,68	1,152	431	1	10
Centralafrica	20	1	442	0,000	345,33	95,67	3,60	57,3	42,7	0,451	442	0	0
Cameroon	7	1	442	0,000	345,33	95,67	3,60	33,34	66,68	0,451	442	0	0
Sum	52	6	442	2,372	345,56	95,44	3,62	88,96	11,04	7,594	430	1	11

n=number of individuals, h= numbrer of haplotypes, N= number of sites, K= average number of nucleotide differences, dn= non-synonymous type substitution, ds= synonymous type substitution, S= transition percentage, V= transversion percentage, R= mutation rate

2.1.3. Distribution of haplotypes in the humid agroclimatic zone

The haplotype distribution map reveals a majority H1 haplotype, appearing sub-regional because it is shared between 4 countries in the wetland. The other haplotypes are specific to a given country.

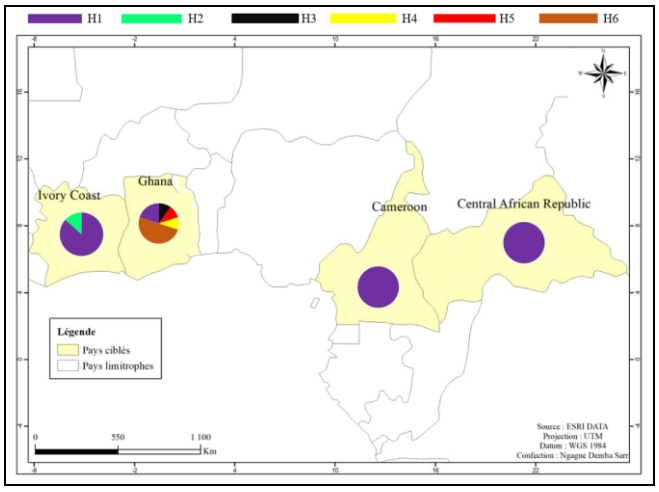


Fig 2: Geographic distribution of haplotypes in the humid zone

2.2. Discussion

It has already been demonstrated through scientific studies that the means of storing and preserving corn contributes to the increase in the loss of stocks of this cereal [14]. Would they act through the genetic diversity of insects ? To answer this question, we set ourselves the objective of highlighting a possible cause and effect relationship between the high genetic diversity of the populations of *Sitophilus zeamais* and the high vulnerability of corn stocks to this insect. The results are mixed depending on the country. The Republic of Ghana, whatever the agroecological zone considered (West, Center, East and North), is characterized by high rates of losses of corn stocks. These losses are in phase with very high values of haplotypic diversity (Hd = 0.756 ± 0.010) and nucleotide diversity (Pi = 0.0116 ± 0.002) of the Ghanaian population of S. zeamais. Similar high genetic diversity values have been found by Andolfaho *et al.* [2] in countries in the same humid agroclimatic zone (Botswana, Zimbabwe) with Drosophila melanogaster and Drosophila simulans from the X chromosome loci of these flies. Knowing that the high genetic diversity of a population gives it strong adaptive potentials [2], one could conclude that the enormous losses of maize stocks in Ghana would be the fact of the survival of the population of S. zeamais from this country. This hypothesis is however not confirmed by the evolution of the genetic diversity of the populations of the insect of other countries of the wetland. In fact, the population of S. zeamais from the Ivory Coast is characterized by relatively low genetic diversity. These values, similar to those of Thangaraj *et al.* [2] in India on Sitophilus oryzae (Pi = 0.0017 ± 0.001; 8 haplotypes on 143 individuals), are in phase with a strong loss of maize stocks from the Ivory Coast. It is the same situation in Cameroon and in the Central African Republic. These countries, which are characterized by zero genetic diversity values, have seen their maize stocks seriously deteriorated by the insect. Zero genetic diversity values for the Cytochrome B gene were obtained in Senegal (BMC and SBA) by Sarr *et al* in 2019 and by Bambou *et al.* [4] in the Central African Republic, even if the latter category of values is not confirmed with the COI gene (Hd = 1,000 ± 0.0027) ; Pi = 0.083 ± 0.005) and the 28S ribosomal gene (Hd = 0.862 ± 0.011; Pi = 0.011 ± 0.001) of the insect. In all cases, these very low values of genetic diversity had to correspond to small losses of stocks in the corresponding countries, if we stick to our null

hypothesis, because a drastic loss of the size of a population accompanied by a reduction gene exchange generally leads to an increase in the overall inbreeding of the population, often decreasing the phenotypic ability of the offspring [8]. Soulé [7] adds to this assertion in these terms : any alteration in the genetic diversity of a population jeopardizes its adaptive potential. In other words, the genetic homogeneity of a population causes its extinction.

But, it is important to underline that a lack of sampling can lead to a very weak genetic diversity of a population of insects having been coming from a country however genetically heterogeneous. Indeed, in Africa in particular, during certain periods of the year, corn samples are scarce because the priority of satisfying household food demand drastically reduces the corn stocks where insects are inserted, so much so that sampling at these times may prove to be biased, since it does not take into account all of the haplotypes actually subservient to the area [14]. Protective measures by producers, for example the use of pesticides, can also reduce the loss of corn stocks despite the existence of insects with great destructive power due to their high genetic diversity.

The genetic diversity of the populations of these wetland countries is not influenced by sharing haplotypes. Exempt Ghana and to a lesser extent the Ivory Coast, the other countries have private haplotypes. Ghana's high genetic diversity could therefore be due to the means of storing and preserving maize, as it was the case in Senegal and Guinea [19], since sharing the same climate, these countries cannot differentially undergo its genetic effects.

Conclusion

Genetic diversity determines the adaptability of a population of individuals and therefore its nuisance. Our study was to verify whether the high or low losses encountered in the countries correspond respectively to high or low genetic diversity of the corresponding populations. The results have been mixed : In Ghana, indeed, the high genetic diversity comparable to a high survival capacity of this population of S. zeamais corresponds to a high loss of corn stocks. This is not the case, however, for other countries. But the latter result can be explained through bias. Indeed, a huge development of insects may not necessarily lead to significant crop losses, because these farmers use pesticides to eliminate these insects.

Another importance of this study is to have highlighted the countries where the extinction of the insect is very plausible because of the low genetic diversity which characterizes it, it is the case of the Caméroun, of the Côte d ' ivory and from the Central African Republic, but also to have revealed the countries where the insect has a strong adaptive capacity because of the genetic heterogeneity which characterizes it (Ghana). Instead of using pesticides, the use of which can harm biodiversity to eliminate the insect, we can recommend growing corn in countries where the genetic diversity of its main pest (S. zeamais) is low. That is to say where it is reluctant to survive and recommend in countries where it is high that is to say where it can survive the exploitation of other crops.

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