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Assessment of the Genetic Diversity of Sitophilus Zeamais in Countries of the Sahelo-Sudanian Zone (Senegal, Mali, Niger, Burkina Faso, Guinea Conakry)

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Abstract

Maize is exploited substantially in the countries of the semi-arid zone of West Africa [5] where it plays essential economic and social functions. But these functions are seriously threatened by heavy losses, caused mainly by the corn weevil (*Sitophilus zeamais*) (Motschusky, 1855). This article aims to assess the genetic diversity of the insect in 5 countries in the semi-arid zone. This evaluation will highlight the country (ies) where the susceptibility of *S. zeamais* to survive or disappear is high, because the genetic diversity of a population is positively linked to its adaptive potential [12]. Exploitation of 60 sequences of the cytochrome b gene from insects from countries in the area (Senegal, Mali, Niger, Burkina Faso, Guinea Conakry) has led to the conclusion that genetic diversity is high in Senegal, Guinea Conakry but especially in Burkina Faso and Niger. These countries would therefore favor the adaptability of the insect. However, it is very low in Mali. Thus this country would be unfavorable to the survival of *S. zeamais*.

Keywords: Sitophilus Zeamais ; maize ; Semi-arid zone ; Cytochrome B.

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1. Introduction

In Africa, maize occupies a fundamental place in the food of the households and the cattle. Of the 111 million tonnes of maize consumed worldwide, Africa accounts for 25% [16]. Very resilient, this plant adapts to various climates. Thus maize is exploited particularly in the Sudano-Guinean zone where it is however strongly deteriorated by the corn weevil, a beetle of the curculionidae, known under the scientific name of *Sitophilus zeamais*. The huge losses it causes have induced research. But most of them revolve mainly around the identification of vegetarian biopesticides that are restrictive on the development of the insect. The few rare genetic studies carried out in the sub-region characterize the pest and they are local : Ndiaye and his colleagues [17]. The purpose of this article is to assess the genetic diversity of *S. zeamais* in the semi-arid zone of West Africa, covering among other 5 countries (Senegal, Niger, Burkina Faso, Mali, Guinea Conakry) and look for its origin. The usefulness of this study is to identify among these countries the most likely to favor the survival or the extinction of the insect by the genetic diversity which they create. Indeed, the alteration of the genetic diversity of a population jeopardizes its adaptive potentials [13]. To reach this objective, 60 insects were harvested across West Africa, including 20 in Senegal, 10 in Mali, 10 in Niger, 10 in Burkina Faso and 10 in Guinea Conakry. Cyt gene sequences. B corresponding to these insects were exploited by software for studying population genetics (Bioedit, DNAsp, Mega, etc.), in relation to parameters of genetic variability (h, N, K, Pi, Hd, dn, ds, S, V).

2. Materials and Methods

2.1. Sampling

2.1.1. Sampling locations

Harvesting of *zeamais Sitophilus* individuals was carried out in five (5) countries in the semi-arid zone. (White, 1983). These are Senegal, Mali, Burkina Faso, Guinea Conakry and Niger. Table I summarizes the sampling.

Table 1: Sampling country

Countries	Sample Code	Number of individuals	Geographic	coordinates
			Latitude	Longitude
Senegal	SzSn	20	14°29’51’’N	14°27’09’’W
Mali	SzMl	10	17°34’14’’N	03°59’46″ W
Burkina Faso	SzBf	10	12°14’99’’N	01°33’42’’W
Guinea Conakry	SzG	10	09°56’44’’N	09°41’48’’W
Niger	SzNg	10	17°36’28’’N	08°04’54’’W

2.1.2. 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 : SzSn, with S = Sitophilus , z = zeamais, Sn = Senegal. SzBf, with S = Sitophilus, z = zeamais, Bf = Burkina Faso.

2.2. 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 [7].

2.2.1. 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.

2.2.2. 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.

2.2.3. PCR of the mitochondrial Cytochrome B gene

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)	5-TATGTACTACCATGAGGACAAATATC-3	1. Initial denaturation : 94°C, 3 min ; 35 denaturation cycles : 94°C, min 2. Hybrization : 47°C, 1 min
	CB-N-11367(R)	5-ATTACACCTCCTAATTTATTAGGAAT-3	3.Elongation : 72°C, 2 min ; elongation finale : 72°C, 8 min

The PCR of the mitochondrial Cyt.B gene was carried out by 2 primers defined by Simon and his colleagues (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.

2.2.4. Bioinformatics analyzes

The sequences were corrected and aligned by the Clustal software implemented in the Bioedit program version 7.2.5 [17]. 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 [17]. While those such as dn, ds, S, V and r were estimated by the MEGA7 ver software. 7.0.18 [17].

3. Results and discussion

3.1. Results

3.1.1. Cultivation practices in semi-arid areas

3.1.2. Genetic variability of sequences

Table IV summarizes some parameters of genetic variability of *Zeamais Sitophilus* populations in five (5) countries from West Africa. The analysis of Figure I shows that the haplotypic diversity (Hd) is very high in Burkina Faso (0.867 ± 0.007) and in Niger (0.844 ± 0.103). It is relatively high in Senegal (0.784 ± 0.083) in Guinea (0.711 ± 0.117). On the other hand, the Hd is very low in Mali. The nucleotide diversity (Pi) and the average number of nucleotide differences (K) show roughly the same evolution. Indeed, these quantities are symbolized by high values in Burkina Faso (Pi = 0.013 ± 0.003; K = 5.911) and in Niger (Pi = 0.011 ± 0.002) ; K = 7.089). In Senegal, they are relatively high (Pi = 0.011 ± 0.002; K = 4.932). On the other hand, in Guinea and Mali, the Pi and the K are respectively weak and zero (Guinea : Pi = 0.003 ± 0.001; K = 1.68, Mali : Pi = 0.000 ± 0.000; K = 0.000). Non-synonymous substitutions are more important than synonymous substitutions in all countries (Dn / ds greater than 3). Transition type mutations are very important in Guinea (R = 1.83). On the other hand, they are low in Mali (R = 0.451) ; in Niger (R = 0.299) and in Burkina Faso (R = 0.97). The number of variable sites is high in Senegal (S = 22), Niger (S = 21) and Burkina Faso (S = 15). On the other hand, it is relatively low in Guinea (S = 6) and zero in Mali (S = 0).

Table 4

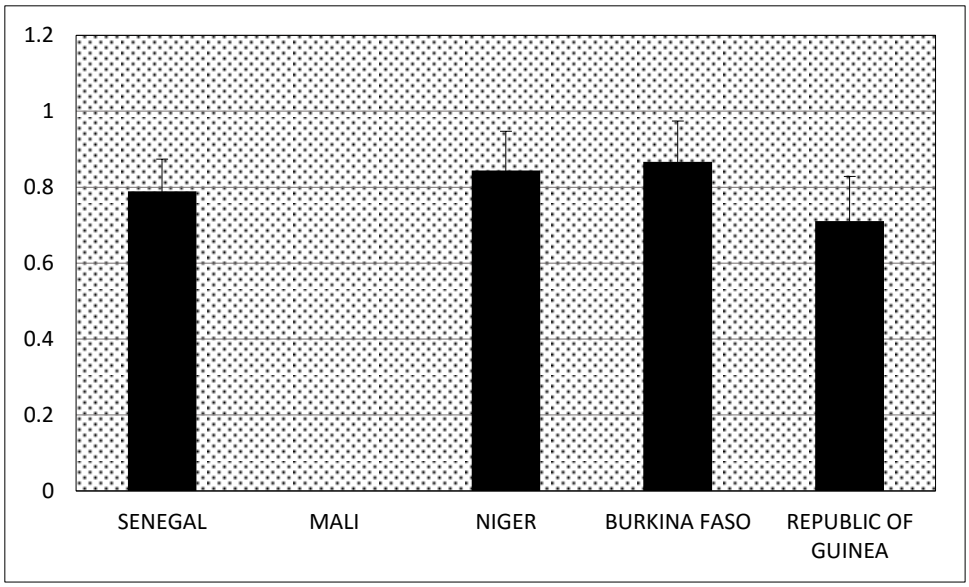
Countries	Farming Methods	
	Farming Practices	Conservation methods and means
Senegal Source : Guéye M.T., Seck D. (2012)	- Stock processing : fonides, k-othrine, plants+cooking salt -Cropping system : fallow, fertilization (smoke, minerals),	-Means of storage : Bag, attic (in nguer), barrel, store, rack+bag, room habitat, above the roofs of the huts -Modes de stockage : en épis, en grains
Mali Sanogo O. and his colleagues 1992	- Stock processing : mineral fertilizers, organic manure -Farming system : drying, winnowing, crop rotation	-Stockage means : traditionnel (clay ganaries, hangars, barrels, jar), modern (store, silos) -Stockage mode : in grain
Burkina Faso Sanou (1991) F. Sankara and his colleagues 2017	- Treatment of stocks : use of repellents plants (Hyptis spicigera), use of inert material (ash+limestones), chemical products (aluminium phosphide) -Cropping system : cropping on ridges, row sowing, crop rotation (maize-coton), weeeding, hilling	- Means of storage : attic made of banco, stores, houses. -Storage methods : in ears (attics), in grains (bag, stores)
Niger Source : Diagnostic, 2010	- stocks treatment : mineral (NPK), organic smoke -Cropping system : sowing with a pocket sometimes without plowing, weeding, hoeing, de-stemmeing.	- Stockage means : bags, barrels - Storage methods : in dry ears.
Guinea Conakry Sékouna Camara and his colleagues IRAG	- Stock processing : mineral, organic -Cropping System : crop rotations (maize-cowpea, Maize-groundnut), irrigated farming, in the shallows	- Stockage means : granaries of plant origin - Storage methods : cement stores, bags.

Table 3: Parameters of genetic diversity

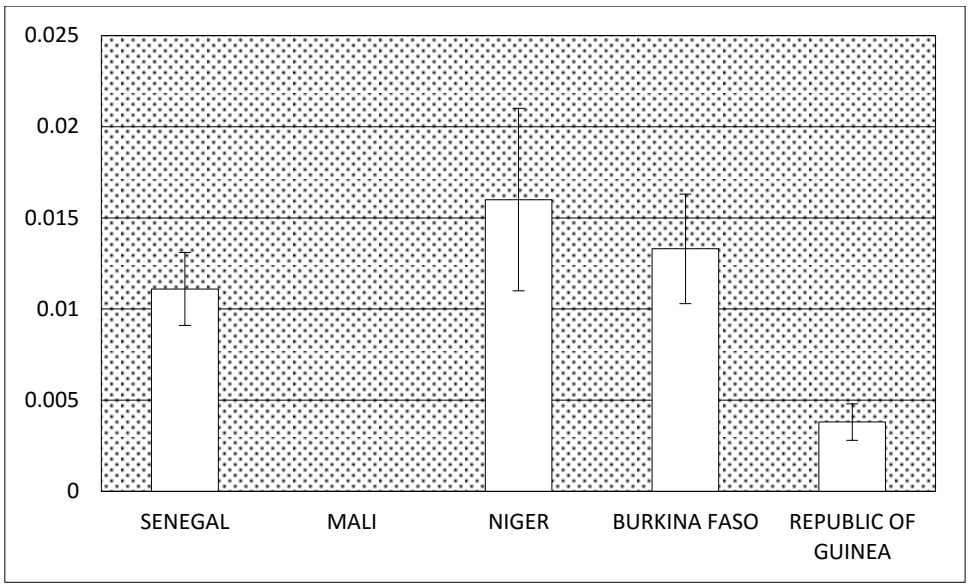
Parameters / Countries	n	h	N	K	dn	ds	Dn/ds	S	V	R	Monomorphic sites	Variable sites	
												Singleton	Parcimony
SENEGAL	20	8	442	4,932	345,85	95,15	3,63	66,54	33,46	1,83	420	12	10
MALI	10	1	442	0,000	345,33	95,67	3,60	33,33	66,66	0,451	442	0	0
NIGER	10	6	442	7,089	345,57	95,47	3,62	31,88	68,12	0,299	420	9	13
BURKINA FASO	10	7	442	5,911	345,20	95,80	3,60	58,71	41,3	0,977	427	5	10
GUINE CONAKRY	10	4	442	1,689	345,53	95,47	3,61	76,97	23,02	3,262	436	4	2
SUM	60	22	442	4,99	345,56	95,44	3,62	61,31	38,68	1,353	391	23	28

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



HAPLOTYPIC DIVERSITY(A)



NUCLEOTIDIC DIVERSITY (B)

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

3.1.3. Distribution of haplotypes in the semi-arid zone

The haplotype distribution map shows that haplotype number 10 is in the majority and is present in all countries

except Burkina Faso. The predominantly secondary haplotype H1 is shared between Burkina Faso and Niger. Countries are therefore mainly made up of private haplotypes.

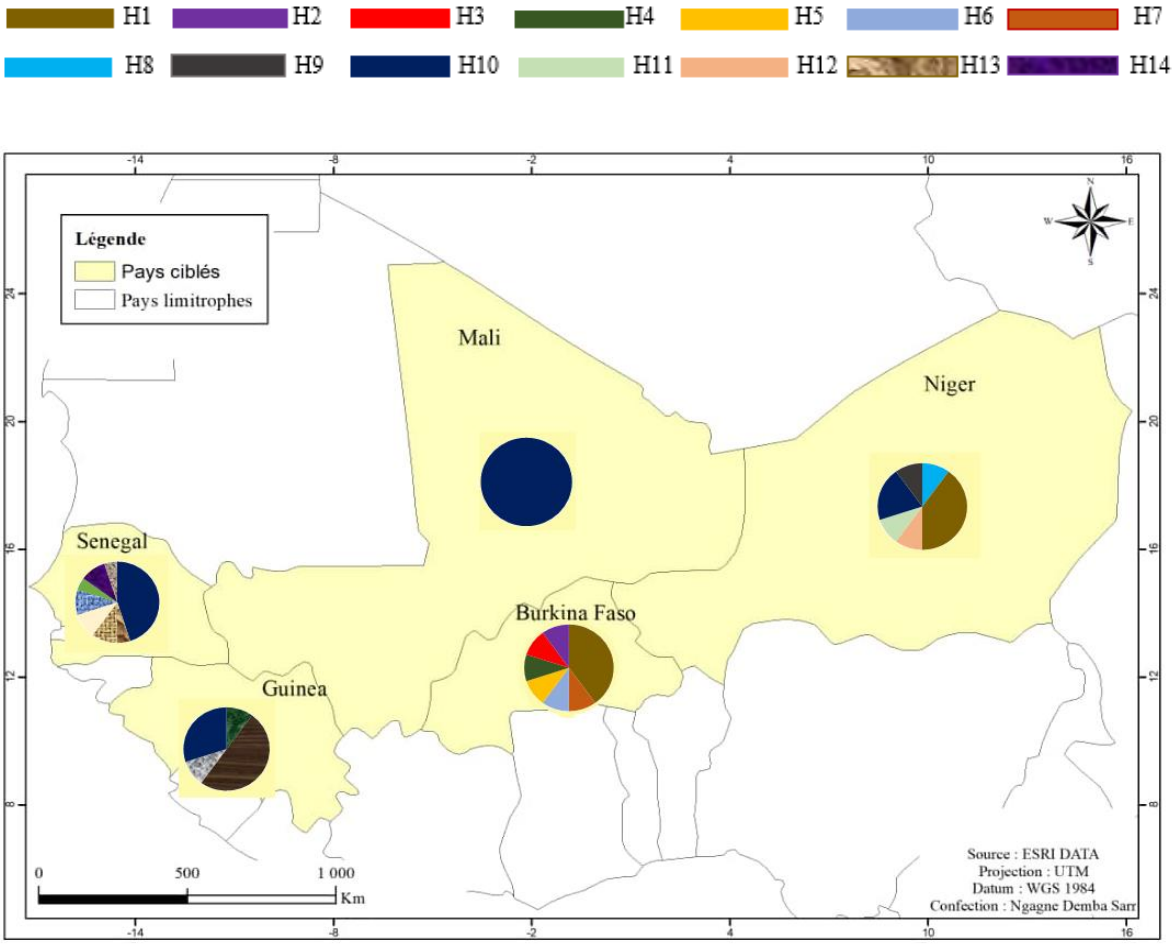


Figure 2: Geographic distribution of haplotypes in the Sahelo-Sudanian zone

3.2. Discussion

The objectives of the article were to highlight a possible differential in genetic diversity of populations of *S. zeamais* in 5 countries of West Africa, sharing the same climatic conditions (Semi-arid zone) [5] to explain it on the basis of possible exchanges of haplotypes between countries or on the basis of the evaluation of cultural practices and techniques of producers (especially conservation method) which influence the genome of populations, as Ndong and his colleagues have demonstrated in a study highlighting clearly the genetic effects of storage means on populations of *S. zeamais* who infested maize in Senegal and in Guinea. Finally, the aim of this study was to predict the impact of genetic diversity on the adaptive potential of the insect. Because the level of intra-population genetic diversity is a key factor for the long-term survival of species [13]. Genetic diversity is high in Senegal, Guinea and especially in Niger and Burkina Faso. A similar genetic diversity was highlighted by Sezonlin and his colleagues [18] in a neighboring country (Benin), on a maize stalk pest, Busseola Fusca (Lepidoptera, noctuidae). However, genetic diversity is very low in Mali. We tried to find out whether these strong values of genetic diversity of populations are the result of farming practices which vary from country to

country or the fact of the transfer of haplotypes. The results have been mixed for some countries and clear for others. Exploitation of the haplotype distribution map in the semi-arid zone indicates that Niger and Burkina Faso which are characterized by large values of parameters of genetic variability (Hd, Pi, h, K, S, dn…) shares a single haplotype, covering 4 individuals out of 24. No other sharing of individuals between each of these 2 countries and the others is observed. A transfer of insects in a commercial setting could be at the origin of this weak sharing. Indeed, the exchange of grains from one zone to another can be accompanied by a transfer of larvae, cocoons, oviposits or even adults [19] since the exchange of haplotypes is weak enough to explain the high genetic diversity in these countries sharing the same climate, certain cultural practices in Niger : no-till sowing, sowing with a pocket and storage of corn in barrels ... [18] and in Burkina Faso : storage of maize in banco granaries… [21] is believed to be its origin. Similar studies on the same insect have shown that storage means are capable of differently affecting the genetic diversity of its populations [1] strong genetic diversity has also been observed in Senegal and Guinea. The presence of a high number of haplotypes in these 2 countries would not also be due to the transfer of variants between these countries and the others in the Sahelo-Sudanian zone, Indeed, Guinea has no individual in common with each of the other countries. Senegal shares a single haplotype with Niger and Mali. Thus the specific nature of the type of conservation of maize in Senegal and Guinea could be at the origin of the high genetic diversity of the populations of these countries. Because unlike some countries in the area, corn is kept in Guinea and in Senegal mainly in bags, in stores, in granaries built in seedlings, in barrels, above the roofs of houses [19] unlike the other countries of the semi-arid zone, Mali is characterized by a very low value of genetic diversity. Similar genetic diversity values were found by Sarr and his colleagues in 2009 in the Low Medium Casamance and the South Peanut Basin (Senegal). The genetic homogeneity of the Malian population of *S. zeamais* can be fatal to the insect because any alteration in the genetic diversity of a population can jeopardize its adaptive potential [12] but these values could also be the signal for a severe and prolonged bottleneck [13] since the countries in question here share approximately the same climate [5] and there has not been a substantial exchange of haplotypes between them, the high genetic diversity of Niger, Burkina Faso and to a lesser extent Senegal and Guinea, is the consequence of specific cultural practices in these countries. Such genetic heterogeneity provides important adaptive skills to the insect. Thus the republics of Niger, Burkina Faso, Senegal and Guinea would individually favor the survival of *S. zeamais*, unlike that of Mali. But this interpretation could be biased by a sampling defect which can lead to a low census of haplotypes compared to the reality in a country, for example due to a sporadic nature of the harvest.

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