

Haplotypes of Beetles *Sitophilus zeamais* and *Sitophilus oryzae*, Storage Pests of Maize in Senegal and Republic of Guinea.

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Abstract

Maize is the first worldwide production and a staple food for many African countries. These countries face problems related to adequacy and adaptation storage systems made vulnerable by disparaging product insects. And losses due to seed-weevils of corn can exceed 25% of the harvest or even 40% in six months of storage. The objective of this study is to determine the influence of agro-ecological and agro-climatic areas on the genetic and structure diversity of *Sitophilus* spp by the PCR-sequencing technique of mitochondrial gene, cytochrome B.

The study highlights the presence of two species in the samples : *Sitophilus zeamais* and

Sitophilus oryzae with 18 and 16 haplotypes respectively in seven localities in Senegal and Republic of Guinea. In Senegal, the two species have high haplotype, nucleotide diversity characterizing population stability with a large effective. However in Guinea, *S. zeamais* is characterized by a rapidly growing population from an ancestral population with low numbers and where time is not enough to find a great diversity between haplotypes. Networks and phylogenetic trees of maximum parsimony and maximum likelihood indicate that the two species would be structured according to agro-ecological and agro-climatic areas.

Keywords : *Sitophilus zeamais* ; *Sitophilus oryzae* ; Haplotypes ; Agro-ecological areas ; agro-climatic areas ; Senegal ; Republic of Guinea ; PCR-Sequencing ; Cytochrome B.

I. Introduction

Native at Central America, specifically in Mexico, maize (*Zea mays*, L.) is introduced in Africa in the sixteenth century by Portuguese

explorers [29 ; 49]. It is widely grown as a cereal for its grain rich in starch (72-73% of its weight) and represents the first cereal production in front of rice and wheat [5 ; 9 ; 28].

In Senegal, the development of its culture is linked either to the fact that maize is an ancient culture of which product is traditionally used in human and animal food or by the fact that corn can cross the lean periods [22]. Meanwhile in Republic of Guinea, corn is also one of the main cereals and ranks 3rd after rice and fonio in terms of acreage cultivated. Middle Guinea is the largest maize growing area of the country [13].

However, as all the cereals, maize is not spared by insects-pests stocks particularly the genus *Sitophilus*. Commonly called seed-weevil, the two species, *Sitophilus zeamais* and *Sitophilus oryzae*, are considered the most harmful insects stocks in tropical countries.

While ensuring food security of a country, one of key-factors is the availability of food resources through adequate and appropriate crop conservation. Previous studies have shown the extent of damage caused by these insects in stocks. Indeed, the larva of *Sitophilus oryzae*, for example, would consume 10 mg of grains during its development while an adult would consume 0.49 mg per day [21 ; 26 ; 51 in 27]. Similarly, in rural areas where conservation techniques of agricultural commodities are little developed, *Sitophilus zeamais* can cause post-harvest losses up to 90% for 5 months of storage [11 ; 35 in 33]. Therefore a better understanding of the bio-ecology and structuring of genus *Sitophilus*, primary pest of corn in Senegal and Republic of Guinea, would allow us to better fit the ecological struggle which is an alternative to the chemical process because it better preserve environment and consumer health.

Thus the aim of this study is to determine, using the PCR-Sequencing method of the mitochondrial gene cytochrome B, on one hand the structure and genetic diversity of the genus *Sitophilus* in different

agro-ecological and agro-climatic areas and on the other hand to determine the affinities between allopatric populations or not. All this in order to find a flaw in the chain of their bio-ecological evolution to intervene later through friendly methods of the nature and public health.

II. Material and Method

II.1. Sampling

Sampling is performed at 6 administrative regions of Senegal specifically at communities :

- Bambey : 14° 42' N and 16° 27' W (Diourbel) ;
- Mbassiss : 14° 04' N and 16° 25' W (Fatick) ;
- Keur Ayip : 13° 35' N and 15° 36' W (Kaolack) ;
- Missirah : 13° 31' N and 13° 30' W (Tambacounda) ;
- Salemata : 12° 37' N and 12° 49' W (Kedougou) ;
- Diaroume : 12° 59' N and 15° 37' W (Sedhiou) .

The study is extended to a locality of the natural region of Middle Guinea, Labe : 11° 19'N and 12° 16' W (Republic of Guinea).

Each of these areas is part of a defined area whether depending on a climate or on ecology.

In our sampling strategy, approximately 20 kg of maize are stored in storage locations of our partners-producers. After a sufficient time for the infestation, about 1 kg is taken from each locality either in attics, stores, kitchens or in the fields. These samples are then brought and kept for mass breeding laboratory to increase the population sampled for a second generation. Then there is the collection of insect pests (*Sitophilus* spp) in laboratory where they are preserved in alcohol 96° in view of the realization of the technical PCR-Sequencing. The following table summarizes the different parameters of the sampling.

Table 1: Summary of sampling

Localities	Agro-ecological areas	Agro-climatic areas	Number of samples	Phenotype of maize	Codes of sampling
Salemata	Upper Casamance/Eastern Senegal	Sub-guinean	13	MJ	SSaJ
Diaroume	Lower and Middle Casamance	Soudano-guinean	11	MJB	SDiM
Bambey	North of groundnut basin	Sahelo-soudanese	06	MJ	SBaJ
Missirah	Upper Casamance/Eastern Sénégal	Soudanese	12	MJB	SMiM
Keur Ayip	Southern groundnut basin		09	MJ	SKeJ
				MJB	SKeM
Mbassis				12	MO
Labe	Middle Guinea	Foutanian	10	MJ	SLaJ

II.2. Genetic study

DNA extraction, PCR and Sequencing of the cytochrome B : Abdomen, elytra and antennae of samples were kept apart to avoid contamination by fungi and nematodes and to allow for morphological observation. A partial Cyt. B gene region was amplified by PCR to characterize mitochondrial DNA of *Sitophilus zeamais* and *Sitophilus oryzae*. It is a method that allows in vitro amplification of a DNA sequence of the gene cytochrome B by a DNA-polymerase by the elongation of two primers: CB1 (5' -TAT GTA CTA CCA TGA GGA CAA ATA TC -3 ') and CB2 (ACA CTC CTC 5'ATT AAT TTA TTA GGA AT-3'). The 25ml of PCR-mixture reaction for the cytochrome b contained 18.3µl of ultrapure water, 2.5ml of enzyme buffer supplied by the kit-Qiagen, 1µl of MgCl₂, 0.5µl of dNTP, 0.25µl of each primer, 0.2 mµl of Taq polymerase and 2 µl of DNA extracted. After an initial denaturation step at 94°C for 3 min, followed by 35 cycles comprising repeated distortion at 94° C for 1 minute, annealing at 47°C for 1 minute and elongation of the complementary DNA strand at 72°C for 1 minute ; and a final extension at 72°C for 10 minutes completes the PCR. This step is followed by sequencing samples by MacroGen, an american society in South Korea.

II.3. Genetic analysis

The sequences alignment is done manually checked and corrected using the BioEdit software that uses the Clustal W. algorithm [46] 2005 Version 7.0.5.3 Hall (1999). Standard indices of genetic variations (distance genetic inner and between haplotypes, number of polymorphic sites, number of informative sites, nature and position of mutations...) are explained with the software MEGA5 (Molecular Evolutionary Genetics Analysis 5) version 5.05. The number of haplotypes Nhp ; haplotype diversity (H) and nucleotide diversity (Pi) ; genetic differentiation index F_{ST} [48 ; 50] and genetic distances are used to describe the distribution of genetic variability between populations. These parameters are estimated using the software version 5.10.01 DNAsp Rozas *and al.*, (2010). The Mantel's test was performed by

XLSTAT 2012.6.03 version to find out if the matrices (genetic and geographic distances) are correlated : isolation by distance. Networks of haplotypes are drawn by the publisher Network Version 4.6.1.1. The phylogenetic affinities of the two species (*S. zeamais* and *S. oryzae*) were estimated by three methods: Neighbour-Joining method is based on the matrix of genetic distances ecotypes (distance Kimura 2-parameter) taken two by two to model the evolutionary process ; the method of Maximum Parsimony believes that a tree is optimal when its total length (number of steps needed to explain the game of analyzed data) is minimal then a consensus of all trees selected is then performed ; the method of Maximum Likelihood is used to test all the stories that may have led the current data set analyzed. Reconstructions were rooted by a sequence of *Caryedon serratus*. The robustness of the nodes was evaluated for 1000 bootstrap replicates. A bootstrap is considered significant if its value is greater than 70%.

III Results

III.1. Analysis of the genetic variation of the used DNA sequences

Analysis of the sequences by the blast showed that samples are composed of two species (*Sitophilus zeamais* and *Sitophilus oryzae*) whose only interspecific relationships have been yet to be determined. They will therefore be studied separately. For this case :

- ***Sitophilus zeamais*** : 49 sequences were identified, each is constituted of 410 bp of which 380 conserved sites, 30 polymorphic sites, 28 parsimony informative sites and two singletons sites. The mutations frequency is 53.35% of transition type and 46.64% of transversion type ; with adenine to 39.37%, 29.95% of thymine/uracil, 19.45% to cytosine and 11.23% of guanine. Transitions between purine bases (54.17%) outnumber those between pyrimidine bases (45.82%). There are 4 populations for agro-climatic and/or agro-ecological areas. In fact that these areas overlap completely the localities sampled (Table 2) :

- Northern Groundnut Basin area or Sahelo-sudanian population (Bambey) is composed of 6 samples, 15 variable sites and 3 haplotypes ;
 - Middle Guinea area or Foutanian population (Labe) is formed of 10 samples, 10 variable sites and four haplotypes ;
 - Southern Groundnut Basin area or Sudanese population (Keur Ayip and Mbassiss) that shows 21 samples, 20 variable sites and 10 haplotypes ;
 - Upper Casamance/Eastern Senegal area or Sudanese population (Missirah) is constituted of 12 samples, 23 variable sites and 6 haplotypes.
- The two latter populations defined agro-ecologically match one agro-climatic population. That is to say the Sudanese population composed of 33 samples, 28 variable sites and 14 haplotypes.
- In case *Sitophilus oryzae* : 24 sequences were studied , each is constituted of 410 bp including 386 conserved sites, 24 variable sites, 19 parsimony informative sites and 5 singletons sites. The mutations frequency is 54.17% transition type and 45.82% of transversion type and most mutations have not led to a change of amino acid in polypeptide chain ; with 39.76% of adenine,

thymine/uracil to 28.90%, 19.81% to cytosine and 11.53% of guanine.

Transitions between purine bases (49.83%) outnumber those between pyrimidine bases (3.52%).

There are 2 populations (Table 3) :

- Lower and Middle Casamance area or Sudano-guinean population (Diaroume) which has 11 samples, 19 polymorphic sites and 7 haplotypes ;

- Upper Casamance/Eastern Senegal area or Sub-guinean population (Salemata) with 13 samples, 22 polymorphic sites and 10 haplotypes.

The ratio (R) transition/transversion in 2 species is 1.224, this shows a non-saturation sites because greater than 1. The sequences are therefore informative.

The sequences composition in A-T is greater than in C-G unlikely situation where the composition would be reversed ; indeed there are two hydrogen bonds for A-T against 3 for C-G.

And there are no deletions in sequences.

Table 2 : Number of samples, of haplotypes and polymorphic sites in each area.

Parameters Populations	Number of samples	Number of polymorphic sites	Number of haplotypes
North Groundnut Basin (NGA) or Sahelo-soudanese	06	15	03
Middle Guinea (MG) or Foutanian	10	10	04
Lower and Middle Casamance (LMC) or Soudano-guinean	11	19	07
Upper Casamance/Eastern Senegal /Salemata (UCES/S) or Sub-guinean	13	22	10
South Groundnut Basin (SGA) or Soudanese	21	20	10
Upper Casamance/Eastern Senegal/Missirah (UCES/M) or Soudanese	12	23	06
Soudanese = SGA + UCES/M	33	28	14

III.2. Diversity and structure genetic of two species

III.2.1. Haplotypes : Haplotypes designate individuals which have identical sequences at any point and haplotype groups or lineages individuals which share mutations that are specific to these individuals.

There are 18 haplotypes (Table 3) for *S. zeamais*. The majority haplotype (H2) is composed of 22 individuals (44.90%) from localities Bambey (4), Labe (3) Mbassiss (7) Missirah (7) and Keur Ayip (1). H2 could be a

sub-regional haplotype because it is present in the whole agro-ecological areas up to Republic of Guinea. The number of individual haplotypes is 11 (22.45%). There are 4 private haplotypes with 2 belong to the community of Keur Ayip (H3 and H13 with respectively 2 and 3 individuals), one which come from Labe (H7 : 5 individuals) and another from Mbassiss (H17 : 2 individuals).

There are so two other haplotypes each composed of two individuals.

In *S. oryzae*, there are 16 haplotypes. The majority haplotype is made with 8 individuals (33.33%)

which are from Diaroume (5) and Salemata (3). There are 14 individual haplotypes (58.33%) and one private haplotype H5 belonging to the locality Salemata.

III.2.2. Structuring : The haplotype and nucleotide diversity are very strong in both species (Figure 1a and 1b). Thus, haplotype diversity in each individual is over 50%.

There is a difference in average every 63.24 bp for the specie *S. oryzae* ($P_i = 0.0163310^{-4}$) while it is 77.82 for *S. zeamais* ($P_i = 0.0128510^{-4}$).

Sequences analysis by the blast shows a zonation of species of the genus *Sitophilus* in Senegal. Indeed all individuals *Sitophilus oryzae* are located south of the Gambia River (Diaroume and Salemata) while those of *Sitophilus zeamais* met in the North of the River Gambia (Mbassis, Bambey, Keur Ayip and Missirah).

III.2.3. Phylogenetic trees : There are across phylogenetic trees (Neighbour-Joining,

Maximum of Parsimony and Maximum of likelihood) two main clades (C1, C2) highlighting a separate structure depending on the species and the agro-ecological, agro-climatic areas. One clade is constituted of individuals of *Sitophilus zeamais* and then the other of *Sitophilus oryzae*. C1 clade is composed of individuals of *S. zeamais*. It is composed of 6 haplotype groups (HG). The HG1 and HG5 have the particularity to contain at least one individual from each of the sampled localities except Keur Ayip and Bambey respectively. The HG2 (100%) and HG4 (74%) are made up of individuals from Keur Ayip.

H3 (100%) is formed of individuals from Labe.

Finally HG6 (100%) is constituted of 3 individuals from Bambey Mbassis and Missirah.

There are individuals of *S. oryzae* which form clade C2 that is made 2 haplotype groups (H7 and H8). The HG7 is formed of 3 individual haplotypes.

Table 3: Summary of different haplotypes in each specie.

Parameters Species	Haplotypes
<i>Sitophilus oryzae</i>	H₁ : 1 [SSaJ2] ; H₂ : 8 [SDiM7 SDiM8 SDiM9 SDiM10 SDiM11 SSaJ3 SSaJ5 SSaJ10] ; H₃ :1[SSaJ11] ; H₄ : 1[SSaJ9] ; H₅ : 2 [SSaJ13 SSaJ7] ; H₆ : 1 [SDiM1] ; H₇ : 1 [SSaJ1] ; H₈ : 1 [SSaJ8]; H₉ : 1[SDiM3] ; H₁₀ : 1 [SDiM6] ; H₁₁ : 1 [SDiM4] ; H₁₂ : 1 [SDiM2] ; H₁₃ : 1 [SDiM5] ; H₁₄ : 1 [SSaJ12] ; H₁₅ : 1 [SSaJ6] ; H₁₆ : 1 [SSaJ4]
<i>Sitophilus zeamais</i>	H₁ : 1 [SBaJ1] ; H₂ : 22 [SBaJ2 SBaJ4 SBaJ5 SBaJ6 SKeM1 SMbJ7 SMbJ8 SMbJ10 SMbJ11 SMbJ13 SMbJ14 SMiM5 SMiM8 SMiM9 SMiM10 SMiM13 SMiM11 SMiM14 SLaJ7 SLaJ8 SLaJ9 SMbJ1] ; H₃ : 3 [SKeJ1 SKeJ3 SKeJ4]; H₄ : 2 [SBaJ3 SMiM12] ; H₅ : 1 [SMbJ12] ; H₆ : 1 [SKeM3]; H₇ : 5 [SLaJ3 SLaJ4 SLaJ10 SLaJ5 SLaJ2] ; H₈ : 1 [SKeM4] ; H₉ : 1[SLaJ1] ; H₁₀ : 1 [SMiM3] ; H₁₁ : 1 [SMiM1] ; H₁₂ : 1 [SMiM4] ; H₁₃ : 2 [SKeJ5 SKeJ2]; H₁₄ : 2 [SMiM2 SMbJ3] ; H₁₅ : 1 [SKeM2]; H₁₆ : 1 [SLaJ6] ; H₁₇ : 2 [SMbJ2 SMbJ5] ; H₁₈ : 1 [SMBJ4]

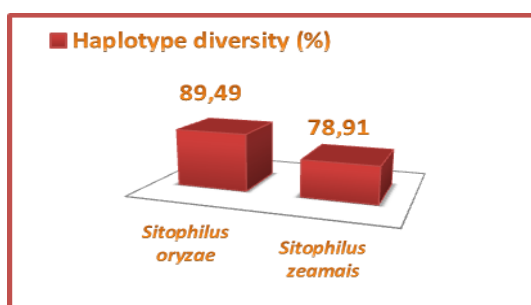


Figure 1a : Diversity haplotype

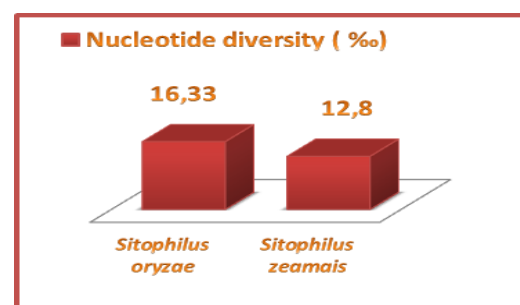


Figure 1b : Nucleotide Diversity

A mismatch distribution is a tabulation of the number of pairwise differences between all DNA sequences in a sample [25].

Mismatch distribution analysis for both species shows that they are multimodal (Figure 2.a and 2.b). We note that *S. oryzae* is characterized by more stability than *S. zeamais*.

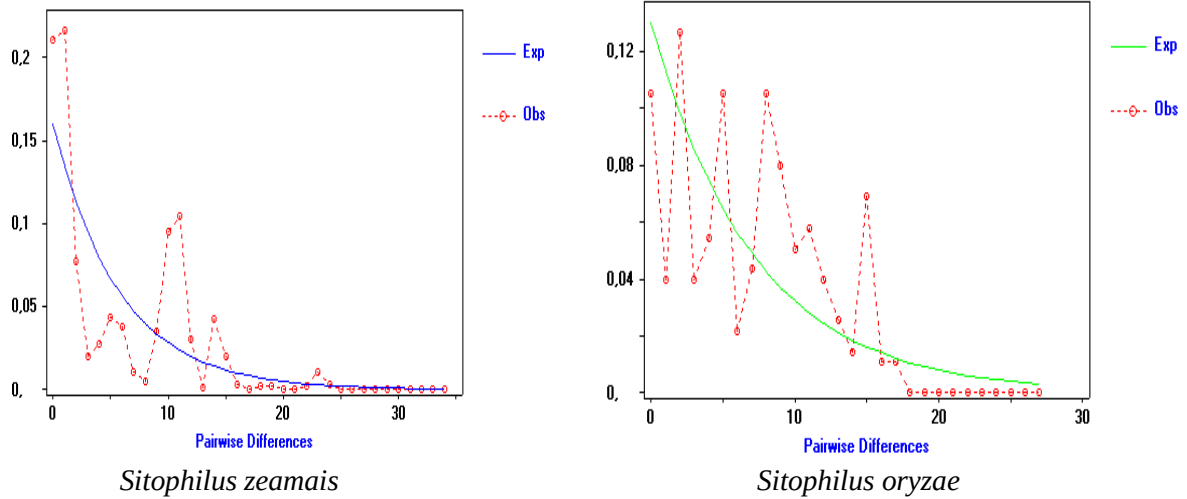


Figure 2.a : Mismatch distribution with assumption of constant population size

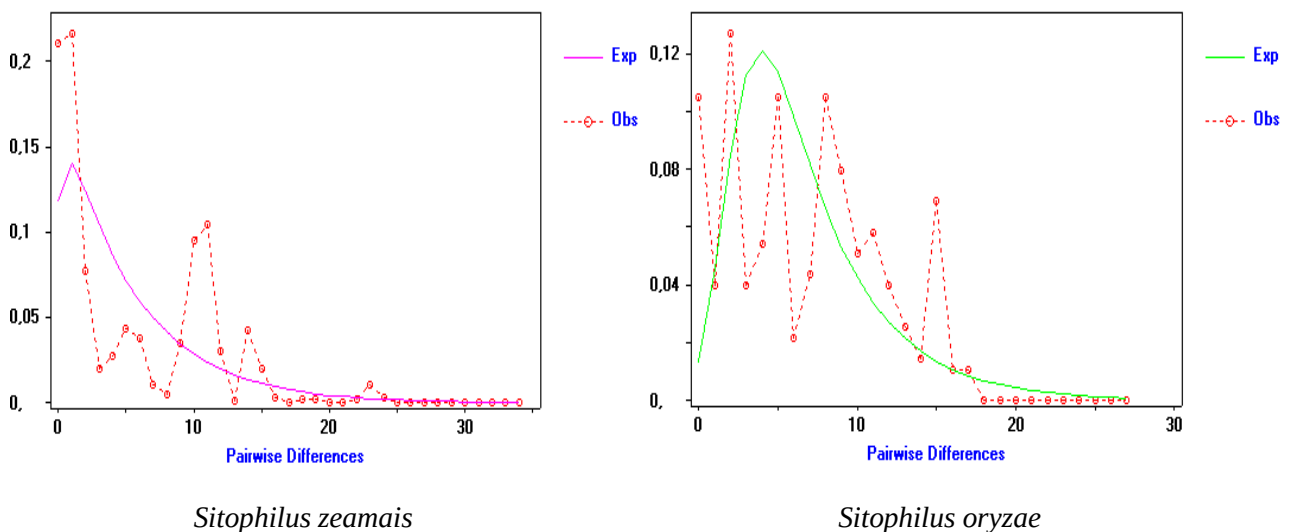


Figure 2.b : Mismatch distribution with assumption of population growth/decline

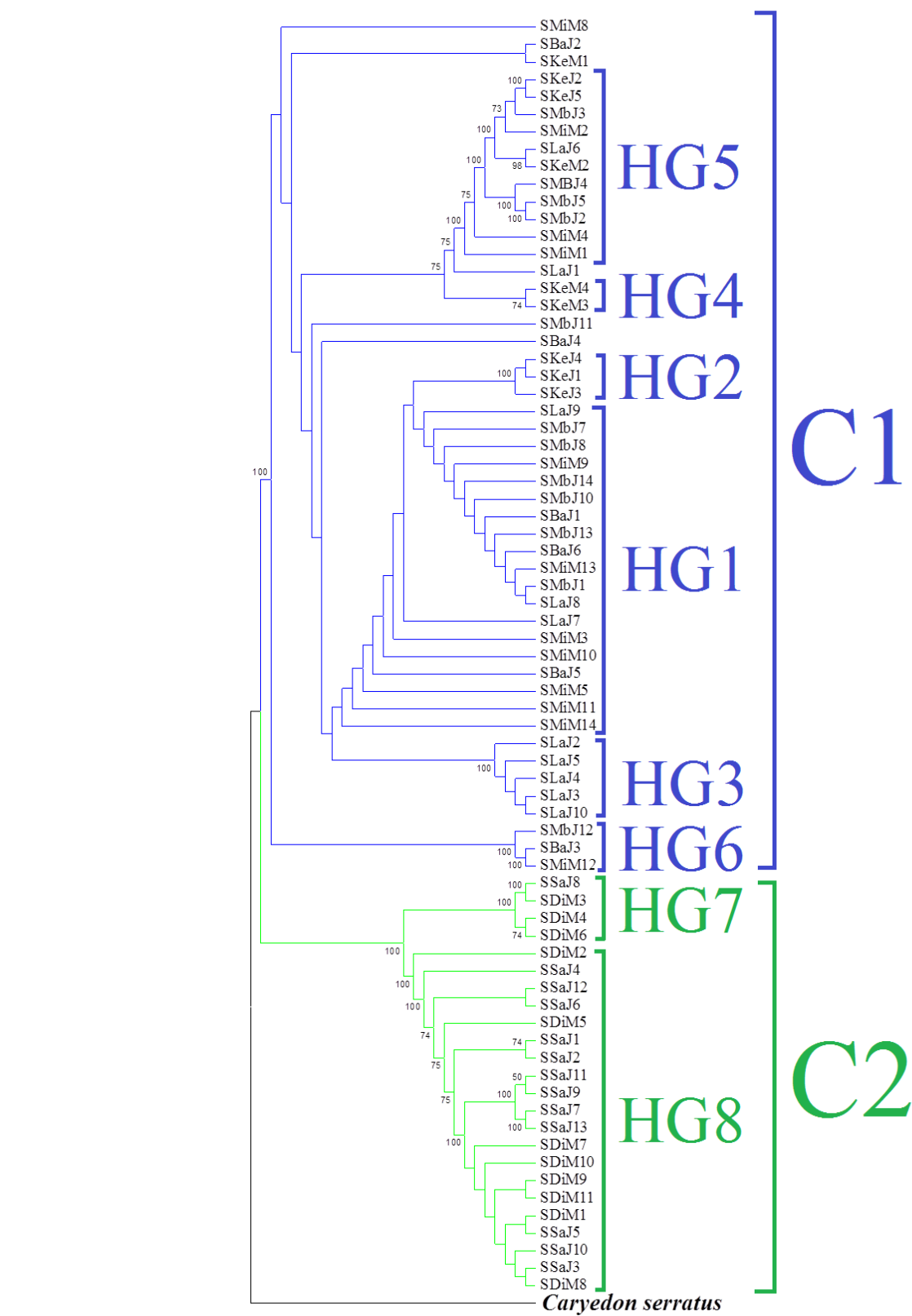


Figure 3 : Phylogenetic tree of both species ; MP (BT = 1000) , Kimura 2-parameter .

III.3. Genetic diversity and populations structure

The populations considered throughout this study are defined either in terms of agro-ecological or agro-climatic areas, or administrative areas (for calculating the Mantel's test). Within populations in both species, haplotype diversity (>50%) and nucleotide diversity (There is not reference, comparison is made between populations) are very strong with a predominance of populations constituted by *S. oryzae* ; with the exception of the very low nucleotide diversity from the population of Middle Guinea constituted of individuals of *S. zeamais* (Figure 4).

For studying of the genetic variability of populations of *S. zeamais*, the coefficient of gene differentiation (G_{ST}) is 0.098. In *S. oryzae*, genetic diversity within populations (H_s) is equal to 0.017 and genetic diversity between populations (D_{ST}) is nil (0.00).

Genetic distances of inner areas (Figure 5.a) are low. However, the genetic distance the population foutanien Middle Guinea is lower (0.0065), there is hardly divergence.

Genetic distances of populations between areas of *S. zeamais* (Figure 5.b) the weakest are those between populations of Senegal and Middle Guinea. And the highest was obtained between the population sahelio-sudanian of North Groundnut Basin and Sudanese population of South groundnut Basin (0.016). It should be noted that the most important genetic distances are obtained within or between populations of different variety. Genetic distances between haplotypes of *S. oryzae* are important in many cases; whereas these genetic distances are less in *S. zeamais*. However it should be noted in this latter case the haplotype H4 (SBaJ3 ; SMiM12) which shows a significant genetic distance relative to the other haplotype variant of 0.025 to 0.061 with H17 (SMbJ2 ; SMbJ5) that is a private haplotype locality Mbassis in the Sudanian area of Southern Groundnut Basin .

In *S. oryzae*, genetic distance of populations of the Lower and Middle Casamance (Diaroume) and Upper Casamance/Eastern Senegal (Salemata) is the highest (0.017).

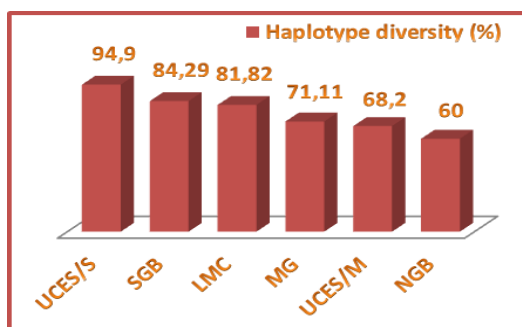


Figure 4.a : Haplotype diversity

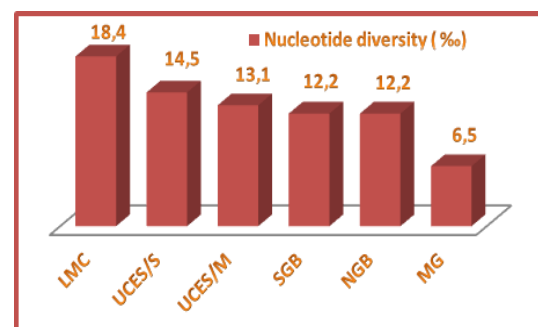


Figure 4.b : Nucleotide Diversity

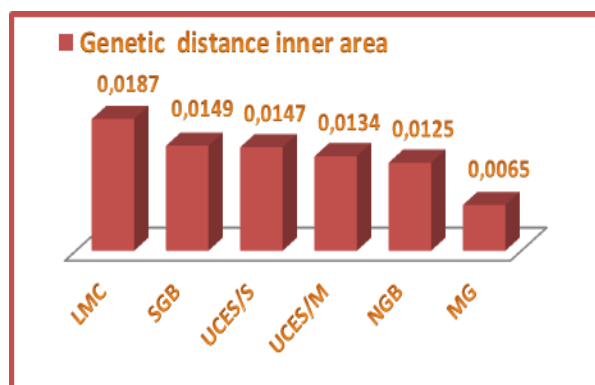


Figure 5.a : Genetic distances inner areas

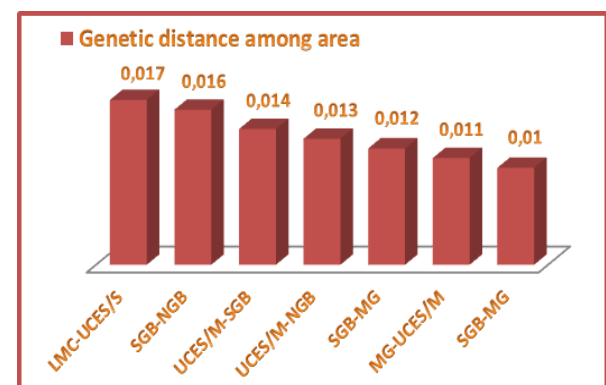
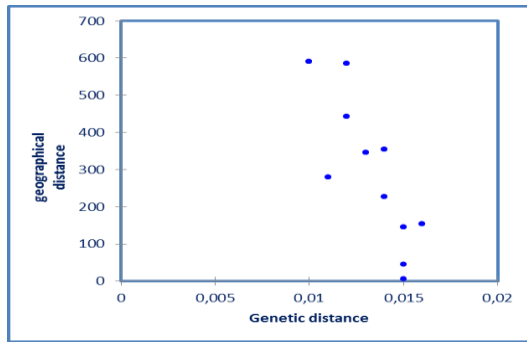


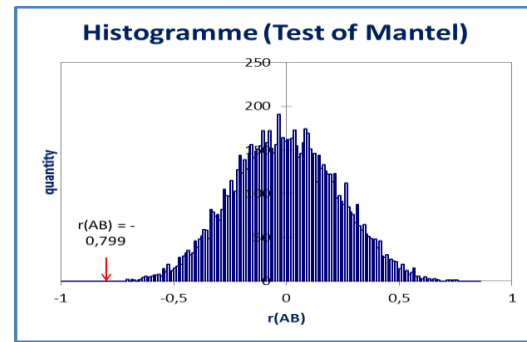
Figure 5.b : Genetic distances between areas

Mantel's test (Figure 6) is designed only for *S. zeamais*. To do this test, we need at least 3 populations, or *S. oryzae* is composed only 2 populations.

Thus from Figure 6.a, genetic distance is correlated to the geographical distance for *S. zeamais*. This is corroborated by the Pearson correlation coefficient which measures how much two variables are correlated seeking linear correlations.



6.a



6.b

Figure 6 : Mantel's test (geographical distances based on genetic distances (6.a)) with Pearsons' correlation (6.b) for *S. zeamais*.

N_m is the effective number of migrants per generation [50] and is calculated from the formula $F_{ST} = 1 / (4N_m + 1)$ with the assumption that the populations that we observe are arranged in island and we are at migration-drift equilibrium. F_{ST} quantifies the differentiation between samples and N_m gene flow between populations.

The degree of differentiation (Table 4) is very low ($F_{ST} = 0.0067$) between populations of *S. oryzae* ; there is a significant gene flow between these two populations. They are, in reality, a single population. This is corroborated by

genetic distance because the genetic distance inner area is greater than that between areas.

Genetic differentiation between populations resulted 0.67% of the total variability (Table 4). Both populations are panmictic with a substantial flow of migration ($N_m = 37.25$).

In *S. zeamais*, F_{ST} is variable depending on the population considered. It is strong between population inside the Groundnut Basin in general and between the populations of Groundnut Basin and Middle Guinea. While it is nil between the Groundnut Basin, in general, and Upper Casamance/Eastern Senegal (Table 4).

Table 4: Changes in genetic differentiation and gene flow between populations

Population 1	Population 2	F_{ST}	N_m
BMC	HCSO/S	0,0067	37,25
NBA	SBA	0,1294	1,68
NBA	MG	0,0863	2.65
NBA	HCSO/M	00	00
SBA	MG	0,1248	1,75
SBA	HCSO/M	00	00
MG	HCSO/M	0,056	4,21

III.4. Networks of haplotypes

It allows to see genetic differences between haplotypes. It highlights the degree of relatedness between different samples.

According to the haplotype network (Figure 7), there is a distribution function of the localities, that is to say, agro-ecological and agro-climatic. The mutation rate is one (1) transfer all or 20180 years 4.9510^{-5} mutation per year.

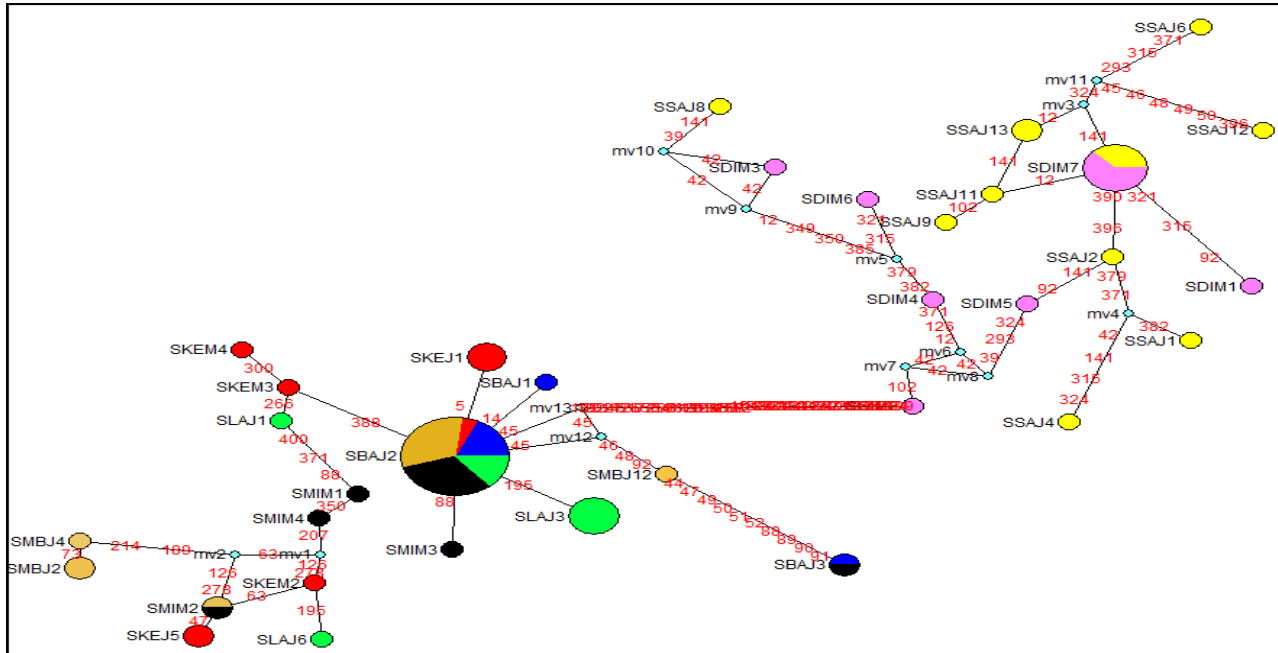


Figure 7: Networks of haplotypes *S. zeamais* and *S. oryzae*, NETW4610.

Caption: haplotypes represented in proportion of localities grouped in a circle (in color).



IV. Discussion

The aim of this study is to determine, on one hand, genetic diversity and structure of *Sitophilus* spp and on the other hand, genetic impact of Senegalese and Guinean agro-ecological, agro-climatic areas on *Sitophilus* spp.

Nucleotide substitutions show, on all the sequences, the presence of 18 haplotypes for *S. zeamais* and 16 for *S. oryzae*. The haplotype majority of *S. zeamais* could be a sub-regional because this haplotype presents in all localities Senegal up to Republic of Guinea. However, we must highlight the imbalance of sampling in favour to the Sudanian areas (33 of 49 individuals). This has an effect in the estimation of haplotype diversity so that nucleotide diversity.

Populational structure in both species is, overall, that of a stable population with large

effective size or the admixture signal from populations that have been isolated from each other by the values of nucleotide diversity and haplotype. Demographic stability is confirmed by the mismatches distributions : the populations of *Sitophilus* spp are in balance, there is no demographic expansion.

Zonation of species in Senegal could be explained by the fact that both species have a preference for the grain they depreciate (selection principle of guest of Hopkins: a female from a given host, prefer to lay its eggs on the same plant). Indeed, *S. oryzae* has a preference for small grains such as rice unlike *S. zeamais* prefers large grains such as corn, according to Seck (2009). This preference for the choice of

spawning is reported in *Caryedon serratus* (Coleoptera) from Sembene and Delobel (2008) which show a significant positive correlation between the number of eggs on a seed and the weight of this seed. There is also the fact that the Lower and Middle Casamance is an area of traditional rice-growing with *Oryza glaberrima* [18]. Some practices also make southern Senegal is a favorite area for *S. oryzae*. Indeed there is for rice (which is the main cereal consumed in Senegal), a continuous availability yet available of corn is short (~6 months for the Upper Casamance/East Senegal, 1~2 months for the Lower and Middle Casamance).

The availability of rice is accentuated by the importance of spending on the purchase of rice in Lower and Middle Casamance (46%) and Upper Casamance/Eastern Senegal (22%), a record compared to other parts of the country (ESASU, 2008). This situation favors the persistence of habitat and development support, critical factors in the formation and maintenance of host races in phytophagous insects [43].

Differentiation and genetic structuring of the species *Sitophilus* spp would be determined on agro-ecological and agro-climatic areas according the phylogenetic trees constituted of two clades that are separately monophyletic and paraphyletic each another. And ecology and climate have an impact on both species. However it should be noted that the survival of the insect is not exclusively dependent on the environment because endocytobiontes (intracellular symbiont) are involved in the adaptation of the host (*Sitophilus* spp) changes in environment [14]. In *S. oryzae* for example, the elimination of endocytobionte causes an increase in development time, decreased fertility and loss of flight ability in adults [23 ; 31 in 7]. Therefore anthropogenic activities could also be related to the zonation of species of *Sitophilus* spp.

So according to Sézonlin (2006), when the molecular analyzes indicate that we are in front of differentiated populations of a species of phytophagous, we will need to adapt the control methods based on regions or countries. Our case suggests us an adapted from north to south in the case of Senegal struggle.

In clade constituted of individuals of *S. zeamais*, there exists a genetic specificity of some individuals at 2 haplotype groups (HG4 and HG2) and (H3) belonging to Keur Ayip and Labe respectively as according the same variety of yellow corn. In this case, it may issue two assumptions:

➤ Keur Ayip is a locality on the border between Senegal and Gambia which trade with countries such as Sierra Leone, Nigeria according Diome and al (2011) as well as Labe which is characterized by its commercial dynamism where the high probability of introduction of haplotypes from other localities;

➤ The sampling location in Keur Ayip being a warehouse for storing several seeds, there may be individuals sampled have had different diets where differentiation in their genetic structure as demonstrated Sembene and al (2012) that diet has a structuring, differential effect at the genetic level in *Caryedon serratus* which is a granivore beetle like the genus *Sitophilus*.

There is also the haplotype groups (HG1 and HG5) gathering individuals from Missirah, Mbassis, Keur Ayip and Labe. In this case, the structure is not based on the variety of corn because they include several varieties. So the variety is not critical in the structuring of *S. zeamais*. The constitution of these haplotype groups may be related to dispersion due to human activities through trade or a structuring effect related to control methods (example pesticides). In these haplotype groups, there is a distribution share and other individuals from the same agro-ecological area. This shows a significant genetic variability within the same area. Lack of individuals from Keur Ayip and Bambey respectively on HG1 and HG5 also show high genetic diversity between these two areas particularly ; and within the Groundnut Basin in general as indicated by the genetic distance between areas (0.016) which is the highest.

For the presence of *S. zeamais* in the Republic of Guinea, in Labe exactly, one could hypothesize an infestation of Labe, which is the second city of Guinea and capital of Middle Guinea, from population of Senegal by genetic drift (generally erosive action) through trade. Because on the one hand, genetic distance decreases as geographic

distance increases starting from the Groundnut Basin to Labe ; and on the other low divergence between the sequences of Labe.

This may be due to genetic drift by founder effect resulting in the loss of genetic diversity observed in Labe. On the other hand, Southern Senegal is the favorite area of *S. oryzae* and that has virtually the same climate in Northern Guinea (Middle Guinea), so an infestation would come from North to South. In exception of the population of the Middle Guinea is characterized by a rapidly growing population from an ancestral population with low numbers and where time is not enough to find a great diversity between haplotypes ; all other populations are characterized by a stable population with large effective.

The study of genetic differentiation of populations shows that there is weak genetic diversity between populations of *S. oryzae*. Indeed, the study of genetic diversity in populations of *S. oryzae* shows that the contribution of genetic diversity within populations ($H_S=0.017$) in the total genetic diversity ($H_T=0.017$) is greater than the contribution of diversity genetic between population ($D_{ST}=0.00$). The two populations of *S. oryzae* are reality one population. In addition to the genetic distance inner area of LMC (0.0187) is greater than that between areas UCES/S and LMC (0.0170). Then these two populations are panmictic. However for *S. zeamais*, there is a greater or lesser genetic diversity between populations, then the contribution of gene diversity between populations (G_{ST}) to the total gene diversity is on average 9.8%.

The largest genetic distance of populations between areas of *S. zeamais* was obtained between the North and South of Groundnut Basin area (0.016) ; this confirms a high variability within Groundnut Basin. The smallest is between North of Groundnut Basin area and Middle Guinea (0.010), areas that have had the same yellow variety and the largest geographic distance.

In inner area level, genetic distance is highest in the South of Groundnut Basin area (0.0149), an area which includes 3 varieties sampled. The variety can be distinctive in genetic divergence. Geographical distance would be critical in the genetic structure of *S. zeamais* as confirmed by

the Mantel's test. This is not corroborated by the work of Sembene and al. (2012) in the case of the groundnut seed-beetle *Caryedon serratus* Ol. (Coleoptera, Chrysomelidae) where geographical distance between communities does not seem to influence the genetic structure of different populations.

The highest value of genetic differentiation ($F_{ST}\sim 12\%$) was observed between the South of groundnut Basin area and North of Groundnut Basin area on one hand, and the South of Groundnut Basin area and Middle Guinea area on the other hand ; indicating a more or less significant reproductive isolation between these populations. F_{ST} values found between populations of *S. zeamais* of Groundnut Basin in general and the Upper Casamance/Eastern Senegal (Missirah) on the one hand and those of *S. oryzae* (Diaroume and Salemata) on the other hand show that there is no genetic differentiation between these zones, thus there is a strong gene flow that has in generally a homogenizer effect.

V. Conclusion

In Senegal, two species of *Sitophilus* spp are present in maize stocks ; namely, *Sitophilus zeamais* and *Sitophilus oryzae* present in Senegal and Republic of Guinea with respectively 18 and 16 haplotypes. The haplotype majority of *S. zeamais* could represent a sub-regional haplotype.

In Senegal, two species are characterized by high haplotype and nucleotide diversity. Thus, these species show demographic dynamics of a stable population with a large effective. Demographic dynamics in two populations of species highlights a high genetic variability which can cause the emergence of strains resistant to control methods. While *S. zeamais* of Guinea is determined by a rapidly growing population from an ancestral population with low numbers and where time is not enough to find a great diversity between haplotypes. The presence of *S. zeamais* may be due to infestation by individuals from Senegal.

Zonation of species is found during sequence analysis by the blast. All individuals from South of the River Gambia are *S. oryzae*, while those from the northern belong to *S. zeamais*.

These two species would be structured according to the agro-ecological and agro-climatic areas. These areas could well influence the structure of species of *Sitophilus* but also cultural practices and eating habits would play a significant role. This zonation puts an emphasis on the need to adapt the struggle according to the agro-ecological and particularly the North and South areas of Senegal.

However, a better understanding of the characteristics and relationships between ecotypes of these species require :

- To diversify localities sampled especially in Republic of Guinea, trying to have a balance in

the sampling locations to obtain objective results on the genetic diversity for example ;

- Extend tests to study microsatellites to see, for example, if genetic diversity is under the influence of the type of marker (enzyme and microsatellite) ;
- To make a phylogenetic reconstruction based on the combination of data from the nuclear gene (28 S, for example) and a mitochondrial gene (cytochrome b, for example) ;
- To determine the mechanism of host adaptation by endocytobionte to find a flaw in the chain of biological reactions implemented.

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