

Diversity and Genetic Structure of *Sitophilus Spp.* Haplotypes, Primary Pest of Stored Maize in Senegal and Guinea: Genetic Impact of Storage Infrastructure and Agro-Climatic Zones

¹**Assane Ndong**

Faculty of sciences and Technology
Department of Animal Biology
BIOPASS Laboratory UMR 022 IRD-CBGP
University Cheikh Anta DIOP
Dakar, Senegal
assanendong85@yahoo.fr

²**Cheikh Thiaw**

Senegalese Institute of Agricultural Research
Regional Center for the Study on
Improvement of Adaptation to Drought
(ISRA/CERAAS), Thies Escale, Senegal.
thiacheikh@hotmail.com

³**Toffene Diome**

Faculty of sciences and Technology
Department of Animal Biology
BIOPASS Laboratory UMR 022 IRD-CBGP
University Cheikh Anta DIOP
Dakar, Senegal
eltof@yahoo.fr

⁴**Mama Raky Ndiaye**

Faculty of sciences and Technology
Department of Animal Biology
BIOPASS Laboratory UMR 022 IRD-CBGP
University Cheikh Anta DIOP
Dakar, Senegal
kiiraa10@hotmail.fr

⁵**Mamadou Kane**

BIOPASS Laboratory
UMR 022 IRD-CBGP
Bel-Air, Dakar, Senegal
Mamadou.kane@ird.fr

⁶**Mbacke Sembene**

Faculty of Sciences and Technology
Department of Animal Biology
BIOPASS Laboratory UMR 022 IRD-CBGP
University Cheikh Anta DIOP,
Dakar, Senegal, mbacke.sembene@ird.fr

Abstract: *In Senegal, as in most neighbouring Sahelian states, corn is one of the major food crops and its promotion is one of the basic objectives of food security defined in most countries. While food security means producing enough by promoting new seed varieties, it also involves storage of this production. The post-harvest component was then wrongly little considered in Senegal. Thus Sitophilus spp. causes losses ranging from 25 to 40% in farmers' stocks. However, their biology and physiology are poorly understood because of their endogenous development and the lack of specific gene molecular manipulation. The aim of this study is to identify both the different species in the genus Sitophilus infesting stored maize in Senegal and Republic of Guinea and the number, the polymorphic diversity and genetic structure of haplotypes that exist there, through the analysis of cytochrome B gene. On the other hand, it is to determine the genetic impact of storage infrastructure and agro-climatic zones in the affinities between allopatric populations and population dynamics. In Senegal the genus Sitophilus is composed of Sitophilus zeamais and Sitophilus oryzae found specifically in areas that are their own and without overlapping sympatric. They are characterized respectively by an average genetic differentiation of 5.26% based on 18 haplotypes and 6.68% with 16 haplotypes. In addition to the genetic structure of this genus based on agro-climatic zones, genetic distances between populations of S. zeamais are negatively correlated with geographic distance. There is a decreasing gradient of genetic diversity based on storage infrastructure starting from stores of traders (market) to the kitchen passing respectively through the producer stores, attics, fields and huts. The high genetic diversity observed in stores and markets is the result of the effect of food by cross infection between cereals.*

Keywords: *Sitophilus zeamais, Sitophilus oryzae, Sitophilus spp, storage infrastructure, agro-climatic zone, Cytochrome B, Genetic diversity, Maize.*

1. INTRODUCTION

Cereals are the world's basic staple food and they provide an energy and protein source for many populations, particularly in the developing world. Maize is widely cultivated as well as cereal grains rich in starch (72-73% by weight) and its suitability for the production of ethanol. It is the first cereal production before rice and wheat [1, 2, 3]. In Senegal, the development of culture is linked either to the fact that maize is an ancient culture that the product is traditionally in food and feed, or by the fact that corn can cross the lean periods [4]. Meanwhile in the Republic of Guinea, corn is one of the main cereals and ranks third after rice and fonio in terms of acreage. Middle Guinea is the largest maize growing area of the country [5].

In most neighbouring Sahelian states, corn is one of the major food crops and its promotion is one of the basic objectives of food security defined in most countries. While food security means producing enough by promoting new seed varieties, it also involves storage and good conservation of this production for consumption according to the needs. It also requires the transformation of the raw materials to increase the value and generate incomes [6, 7]. This is all the more justified by the fact that agricultural production is seasonal in Senegal and Guinea Conakry whereas the needs of consumers are felt all around the year. Besides, this need of storing the harvests is reinforced by the importation of cereals (maize) whose local production is not enough. Thus, the setting of an adequate phytosanitary policy to protect populations from the risk of food shortages during the agricultural inter-season is a necessity that the developing countries must achieve. In Sahelian countries where the dry season is longer, the storage of harvest is a question of survival.

Unfortunately, cereal grain losses during storage can reach up to 50% of the total harvest, which represents a worldwide loss equivalent to thousands of millions of Euros [8] because the post-harvest component was then wrongly little considered in these countries, particularly the management of staple food storage and that of the seeds. In Senegal, we notice the existence of pretty marginal types of storage. According to [9], some producers spread the grain of maize on sieve at first and then, store them in sacks after selecting (sieve + sack, equivalent of 8, 1%). Others conserve the grain of maize in bamboo fences (2, 7%), grains of maize are stored in living rooms (2, 1%), in stores, in attics and above houses' tops (2, 7%). The use of baskets, cans, installed sieves above the roofs of houses, kitchens and huts. The competition of insects infecting the storages of stored foodstuffs is then a high risk which hinders the achievement of food security goals. The maize is becoming more and more difficult to conserve, its enemies in Senegal and Guinea, the weevil, *Sitophilus spp.* and newly *Carpophilus hemipterus* L. (comm. Pers), causing "genocides". According to the farmers, the most important losses are inflicted by different primary pest species that constitute the *Sitophilus* genus. The latter is world widely known as one of the greatest stored cereal devastators, not only because of its own consumption but also, because it opens the door to a series of garbage eater, the most common of which is the red *Tribolium* of corn starch that finishes the damage. It also permits the development of mould, *Aspergillus flavus*. Ratnadass and Sauphanor [10] attribute to *Sitophilus spp.* the main losses of stored maize with a decisive role of dampness in the level of insect populations. The maize grain losses due to the weevil can out pass 25% of the harvest or even reach 40% in six months of storage [11]. The *Sitophilus oryzae* larva, for instance would consume 10 mg of grains during its development while the adult would consume 0, 49 mg per day [12]. As the proverb goes, "misery loves company": the practiced chemical struggle is expensive and complex because of its ecologic and health drawbacks. The populations' traditional methods of struggle, in accordance with their standards of living; haven't proved their efficiency when applied on massive storages.

The *Sitophilus spp.* weevils are among the most cosmopolite stored cereals devastators. However, their biology and physiology are mainly misunderstood because their stages of development take place within the grains of cereals and also due to the absence of specific genes molecular manipulation [13]. Hence, the aim of this study based on the mitochondrial gene, Cytochrome B is to characterize genetically; the different primary pest species of the *Sitophilus* genus, found in the stocks of maize in Senegal and Guinea and also their zoning according to the agro-climatic areas on the one hand. On the other hand, it also aims at determining the number, the polymorphic diversity and the genetic structure of the different haplotypes existing in the different storing infrastructures and population dynamics.

2. METHODS

2.1. Sampling

2.1.1. Studied Sites

The targeting of the studied zones has to do with the importance of the production, the infestation, the infrastructures of maize storage, the geographical position (that is to say the belonging to the localities of agro-climatic zones) and the presence of physical or natural barriers (borders, insulate zones ,parks). The sampling has been experienced around 6 regions of Senegal, precisely in the following localities “**Fig1a**” : Bambey (Diourbel region) situated at 14°42’ N / 16°27’W ; Mbassis, (14°04’ N / 16°25’ W) insulate zone ; Keur Ayip (13°35’ N / 15°36’ W) situated at the Gambian border ; Missirah (13°31’ N / 13°30’ W) ; Salémata (12°37’ N / 12°49’ W) and the Diaroumé crossroads (12°59’ N / 15°37’ W) which is the turntable of the country’s southern trade exchange. Moreover, the study is extended to a locality belonging to the natural region of Middle Guinea which is the greatest maize area of the Republic of Guinea: Labé (11° 19’ N and 12° 16’ W). Each of these localities is part and partial of a zone, defined in accordance with the climate, agro-climatic zone “**Fig1b**”. From North to South, we successively find the Sahelo-Sudanese zone (SAS), the Sudanese zone, (SU), the Sudano-Guinean zone (SUG), the Sub-Guinean zone (SG) and the Fouta Tropical zone (FTR).

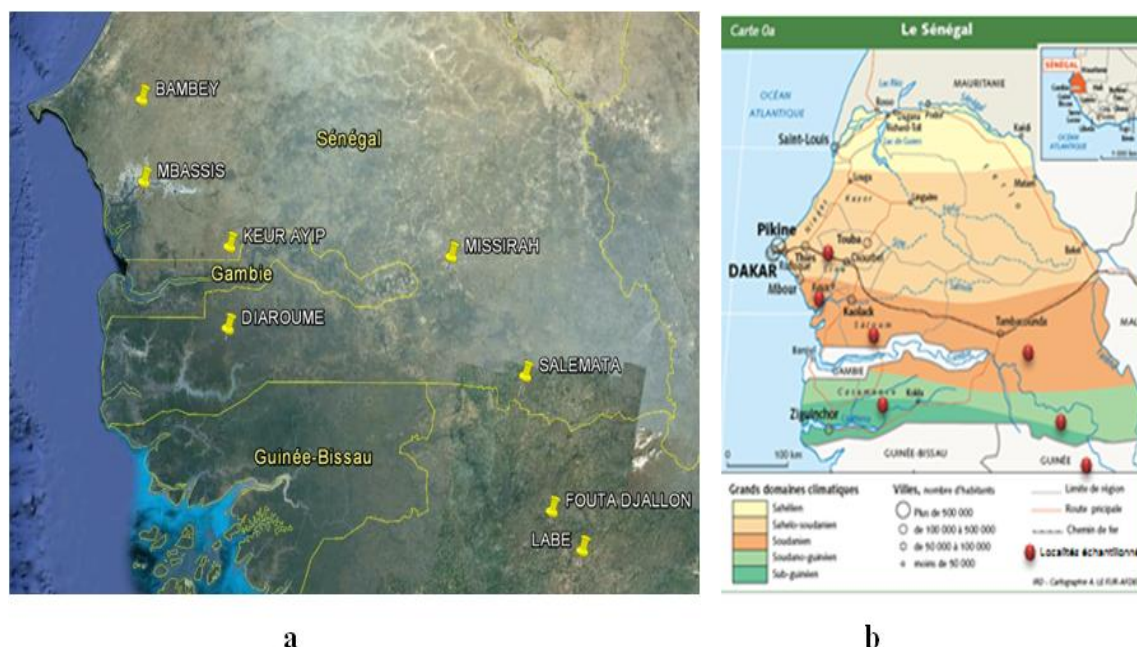


Fig1. Geographical position of localities where the sampling has been experienced (a) and dispatching of the areas according to the agro-climatic zones (b)

2.1.2. *Sitophilus spp.* Samples

Collections of maize samples around these localities will permit to take a census of insects living in the constituted stocks. The sampling starts in fields, immediately after the harvest; as the ears mature and it will go on until the drying and storage in stores or in market places. Consequently, 1 kilogram of maize cultivated in the country by local producers; is either taken from attics, kitchens, huts, rooms, fields or stores (**Table 1**). Then these samples are taken to the insectariums via plastic bags, insects are collected there and conserved in 96% alcohol. So the maize which has been got rid of adult insects also contains eggs and other kinds of larva. This allows making of it a massive breeding within glass jars of (16 cm high and 9 cm in diameter) with a wire meshed cover so as to increase the sampled population by a second generation.

Each individual of a sample is coded by using the first letter of the genus name in capital, followed by the two first letters of the locality where it stems from (the first letter in capital and the second lower-case letter) plus an order number. For example the code SMb1 represents an individual of *Sitophilus* stemming from Mbassis locality and with an order number 1.

Table1. Recapitulation of collected samples' characteristics: country, administrative region, locality, GPS data, storing Infrastructure and agro-climatic zone.

Country	Region	Localities	GPS Data	Storing Infrastructures	Agroclimatics Zones
Sénégal	Diourbel	Bambey	14°42' N / 16°27' W	Huts	Sahelo-Sudanese (SAS)
	Fatick	Mbassis	14°04' N / 16°25' W	Fields	Sudanes (SOU)
	Kaolack	Keur Ayip	13°35' N / 15°36' W	Stores	
	Tambacounda	Missirah	13°31' N / 13°30' W	Attics	
	Kédougou	Salémata	12°37' N / 12°49' W	Stores	Sudano-Guinean (SOG)
	Sédhiou	Diaroumé	12°59' N / 15°37' W	Rooms	Sub-Guinean (SUG)
G. Conakry	F. Djalón	Labé	11°19' N / 12°16' W	Kitchens	Tropical (FTR)

2.2. Mitochondrial Nucleotidic Polymorphism Study

2.2.1. Genomic DNA Extraction

It is done thanks to the Qiagen Kit, with some modifications around the steps 1, 2, 8, and 9. The extraction begins with a dissection of the insect, which consist in a meticulous removing of the abdomen, the elytra and the small wings to avoid contamination through nematodes, fungus, acarions and to permit ultimately identifying observations of species. The digestion of the complex head-thorax-legs is followed by a succession of cell and tissue lyses steps, of purification separating the DNA from its cell impurity and elution that consists in retrieving the DNA in 2 tubes (the first with a volume of 40 µl and the second with a volume of 20µL).

2.2.2. PCR of the Mitochondrial Gene Cytochrome B

The PCR is realized on mitochondrial gene fragments coding: the Cytochrome B. This gene has been amplified by primers CB1 (5' TATGTACTACCATGAGGACAAATATC-3') and CB2 (5' ATTACACCTCCTAATTTATTAGGAAT-3'). The amplification has been realized for a sample within a 25 µl volume of reaction that contains a mixture of 21 µl, composed of 16.3 µl milli-Q water; 2.5 µl of buffer 10x; 1 µl of additional MgCl₂; 0.5 µl dNTP; 0.25 µl of each primers (forward et reverse) ; 0.2 µl of polymerase Taq and 4 µl of DNA extract. It is done in the in following amplification conditions: initial denaturing at 94°C for three minutes, followed by 35 cycles of denaturing at 94°C for 1 minute, hybridation at 47°C for 1 minute and a pulled blade of complementary DNA at 72°C for a minute and a final pulling at 72°C for 10 minutes puts an end to the PCR. The sequencing has been carried out by South Korean company: Macrogen.

2.3. Statistics and Genetic Analysis

The alignment of obtained sequences is carried out with the 7.080 version of BioEdit software by checking and correcting meticulously and manually their nucleotidic successions referring to the electrophoregram. After the observation of a weak alignment rate between two groups of samples, the construction of a haplotype network is compulsory. It is a tree under the form of a star which displays the joining (intra/inter-specific composition) made up of individuals that share common nucleotide successions. The 4.6.1.1 Network software is used to reconstruct this network via the Maximum of Parsimony method. Not only does it allow seeing the median vectors, but also the number and the position of changing steps between this joining. The Median Joining option or MJ network is used because it takes into account the multi-states of data (for example: at the nucleotide position, it may be A, C, G, T, and the ambiguity such as N).

Here, a haplotype means a nucleotide sequence that can be common to several individuals but it differs from other haplotypes by one or several substitutions of nucleotides. A private haplotype is a haplotype which is only found in a unique population. A main haplotype or majority haplotype is the haplotype that has the greatest number of individuals: this is the most representative haplotype. An individualistic haplotype is only composed of a unique individual. A haplogroup or descendants or phylum corresponds to individuals sharing the same genetic pool with their specific mutations. The DNA polymorphism analysis such as haplotypical and nucleotidic diversities, standard indications of genetic variations (polymorphic sites' number, informative sites' number, the nature and the position of the mutations, rate of transition and Transversion) and the number of haplotypes for each specific joining have been done thanks to the DNAsp version 5.10.01 of [14].

The genetic distance that indicates the genetic difference between the populations can be related to the locality or even to host plant. The MEGA 5 software helped us to do these analyses in intra and inter populations: intra/inter locality; intra/inter agro-climatic zones and intra/inter phylum (descendants). A phylogenetic reconstruction through the Maximum of Likelihood (ML) method has also been carried out thanks to this software. The robustness of the knots is evaluated upon repetitions of 1000 bootstrap and the HYK + G, model, which is more applicable to our sequences, is used too. The phylogenetic affinities are completed by the Bayesian method so as to compare and innovate. As for a prospective resolution of the tree with our data, a probability value of 3, 000,000 of generations is scored in the evaluation of hot and cold chains. A *Caryedon serratus* Ol. Sequence is used as an out-group. The test of Mantel is solely carried out for one of the sequences' joining by XLSTAT 2012.6.03 version in order to find out whether the matrixes (genetic and geographic distances) are correlated. Because to do such a test, we need at least 3 populations, while we have only 2 populations for the other joining whatever the used criterion may be. The null hypothesis (0H) indicates that the matrixes aren't correlated; the alternative hypothesis (AH) indicates that the matrixes are correlated. If the value of r (AB) is positive the matrixes are correlated in the same direction, if the latter is negative then, the matrixes are correlated in the opposite direction. The Arlequin 3.1 version software [15] has permitted to perform the test of D Tajima, Fs de Fu and of Ewens-Watterson between the localities on the one hand, and the test of molecular variance analysis (AMOVA) in intra/inter agro-climatic zones, On the other hand.

Besides, a combination of interesting indications to test the demographic expansions as the Fs de Fu uses the information from the distribution of haplotypes so as to test the demographic growths. The effects of a selection in "background noise" can be distinguished from a demographic increase phenomenon or from an expansion of area, by comparing the values of Fs, F* and D*: if Fs is significant and F* and D* are not, so a demographic expansion or an area expansion will be indicated while the opposite result will suggest a phenomenon of selection. These indications are also calculated by the program of DNAsp 5.10.01 version [14].

3. RESULTS AND DISCUSSION

3.1. Analyses of *Sitophilus spp* Genetic Variability

The fasta file, from the alignment of our data set counts 73 sequences of 410 basic pair (bp). Yet, it's necessary to point out that these aligned sequences globally present a weak alignment rate (21, 46% of difference). Consequently, a much accentuated polymorphism that divides into two groups our data set. The first group is composed of 49 sequences which represent much more likelihood, thus constituting a first species. A second group of 24 sequences presenting a genetic gap compared to the other group but the intra group polymorphism is less accentuated. Among the 49 individuals, 6 individuals stem from Bambey, 9 from Keur Ayip, 12 from Mbassis, 12 from Missirah and 10 from Labé. Among the other 24 individuals, 13 originate from Salémata and 11 from Diaroumé.

The 49 individuals of the first group of sequences are found scatted in the localities that are situated in the middle-west, in the centre, in eastern Senegal and in the north of the Republic of Guinea. The other 24 are solely observed in the localities of southern Senegal and not elsewhere. It's about Salémata and Diaroumé localities which are separated from the other localities by the Gambian border, the Gambian, the Senegalese and even the Guinea river tributaries and among other highest peaks of the relief (400 m) and the Niokolokoba Parc in the south-east of the country.

The cytochrome B gene being amplified for the first time in this genre, the recognition of the two species is obtained after a comparison via bar coding in Bold and Genbank since the Cytochrome Oxydase I gene (COI) has been sequenced for these same individuals. Hence, in Senegal the *Sitophilus spp* genus is composed of two species: *Sitophilus zeamais* and *Sitophilus oryzae*.

So, these two species are separately studied without forgetting that some genetic parameters will be evaluated so as to evaluate their genetic difference. As for the *Sitophilus zeamais* specie: 49 sequences of 410 bp of length have been identified including 380 monomorph sites, 30 polymorph sites, 28 informative sites in parsimony, 2 single sites and an average number of

difference (5.26%). The frequency of mutation is estimated to 54.17% of transitional type and 45.83 of transversional type and that of the bases is estimated to 39.37 for adenine, thymine to 29.95%, cytosine 19.45% and the guanine to 11.23%.

As for *Sitophilus oryzae* specie: 24 sequences of 410 bp studied have shown 386 reserved variable sites, 19 informative sites in parsimony, 5 single sites and an average number of differences of 6.68%. The frequency of mutations is about 53.35 of transitional types and about 46.65% of transversional type in accordance with *Sitophilus zeamais* specie. Most of the mutations haven't entailed a change of amine acid in the polypeptide chain. The distribution of bases follows this rate: Adenine 39.76 %, Thymine 28.90%, Cytosine 19.81% and Guanine 11.53%.

3.2. Number and Composition of *Sitophilus zeamais* and *Sitophilus oryzae* Haplotypes

The comparison of genetic diversity parameters of *Sitophilus spp* between the two species shows (Table 2) that *Sitophilus zeamais*, presents 18 haplotypes (from H1 to H18) out of 49 individuals while the *Sitophilus oryzae* gets 16 haplotypes (from H19 to H34) out of 24 individuals (table 4). within the *S. zeamais*, the majority haplotype (H2) is composed of 22 individuals (44.90) originating from Bambey localities (4), Labé (3), Mbassis (7) and Keur Ayip (1). The H2 is also a sub-regional haplotype because it's found in all the sample localities up to Guinea. The number of private haplotype is 15 (83.33%) including 5 stemming from Keur Ayip (H3, H6, H9, H13, and H15), 1 from Bambey (H1), 3 from Mbassis (H5, H17 and H18), 3 from Missirah (H10, H11, and H12). Finally, 3 are from Labé (H7, H8, and H16). At *S. Oryzae*'s the most haplotype is constituted of 8 individuals (33.33%) from Diaroumé and Salémata areas. There are 15 Private haplotypes (93.75%) including 6 ones which belong to Diaroumé area and 9 for Salémata area. The proportion of complete haplotype/private haplotype is summed up in "Fig2" which indicates that the number of haplotypes follows a decreasing gradient of genetic diversity for the two species when we take into consideration respectively the following the storage infrastructures: stores, attics, fields, huts and kitchens. The storage infrastructure for each area is different from those found in the other areas. As a result, the results obtained in each area are directly correlated to the storage infrastructure. However the samples from Diaroumé are taken in a living room; but according to the farmer, this maize has been bought in the market. This fact is remarkable as regard to the haplotypal richness of this area. The smallest number of haplotype is found in the kitchen stocks and the biggest one in the trade stocks (stores) where many speculations are noticed.

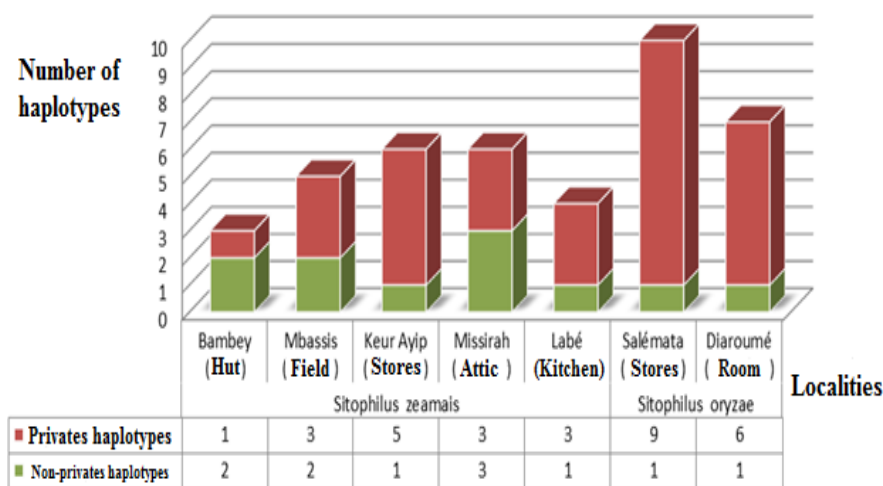


Fig2. Proportion of the private haplotypes as regard to the total number of haplotypes in each area or storage infrastructure

Another comparison of the genetic diversity parameters between the areas for this time, shows that Salémata area (*S. Oryzae*) has in full 10 haplotypes; and Diaroumé area (*S. Oryzae*) has 7 ones. As for *S. zeamais*, Bambey area has got in full 3 haplotypes; there are 5 ones in Mbassis area, 6 in both Missirah and Keur Ayib areas and finally 4 haplotypes in Labé. According to individual composition (composed of one individual or more) and the dispatching of the haplotypes (proper to one or many areas), we have noticed the presence of an important number

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of private and/or detached haplotypes. It is then fundamental to precise that all the detached haplotypes are private haplotypes, but they are not reciprocal.

In fact the explanation given by the haplotypes network “Fig3” is an evidence for the existence and the phylogenetic dispatching of both *Sitophilus spp.* species in Senegal and Guinea Republic as regard to their haplotypes.

Table2. Summary of different haplotypes of each species: Number of haplotypes (NH), the number of individuals per haplotype and haplotype composition.

Parameter Species	NH	Number of individuals per haplotype and haplotype composition.
<i>Sitophilus zeamais</i>	18	H ₁ : 1 [SBa1] ↔ H ₂ : 22 [SBa2, SBa4, SBa5, SBa6, SKe6, SMb7, SMb8, SMb10, SMb11, SMb13, SMb14, SMi5, SMi8, SMi9, SMi10, SMi13, SMi11, SMi14, SLa7, SLa8, SLa9, SMb1] ↔ H ₃ : 3 [SKe1, SKe3, SKe4] ↔ H ₄ : 2 [SBa3, SMi12] ↔ H ₅ : 1 [SMb12] ↔ H ₆ : 1 [SKe8] ↔ H ₇ : 5 [SLa2, SLa3, SLa4, SLa5, SLa10] ↔ H ₈ : 1 [SLa1] ↔ H ₉ : 1 [SKe9] ↔ H ₁₀ : 1 [SMi1] ↔ H ₁₁ : 1 [SMi3] ↔ H ₁₂ : 1 [SMi4] ↔ H ₁₃ : 2 [SKe2, SKe5] ↔ H ₁₄ : 2 [SMi2, SMb3] ↔ H ₁₅ : 1 [SKe7] ↔ H ₁₆ : 1 [SLa6] ↔ H ₁₇ : 2 [SMb2, SMb5] ↔ H ₁₈ : 1 [SMb4]
<i>Sitophilus oryzae</i>	16	H ₁₉ : 1 [SSa2] ↔ H ₂₀ : 8 [SDi7, SDi8, SDi9, SDi10, SDi11, SSa3, SSa5, SSa10] ↔ H ₂₁ : 1 [SSaJ11] ↔ H ₂₂ : 1 [SSa9] ↔ H ₂₃ : 2 [SSa13, SSa7] ↔ H ₂₄ : 1 [SDi1] ↔ H ₂₅ : 1 [SSa1] ↔ H ₂₆ : 1 [SSa8] ↔ H ₂₇ : 1 [SDi3] ↔ H ₂₈ : 1 [SDi6] ↔ H ₂₉ : 1 [SDi4] ↔ H ₃₀ : 1 [SDi2] ↔ H ₃₁ : 1 [SDi5] ↔ H ₃₂ : 1 [SSa12] ↔ H ₃₃ : 1 [SSa6] ↔ H ₃₄ : 1 [SSa4]

3.3. The Haplotypes Network

A network is made up of knots and links which relate the knots. In phylogenetic, the knots of the network are life sequences or switched off with specific mutations. Therefore they appear to be either sequences of the whole data (haplotypes) or medians vectors. The links are the differences of features. A vector is a hypothetical median (often hereditary); sequence (mv) which is necessary for the connection of the existing sequences in the network with the maximum parsimony. Links and medians vectors constitute respectively the number of changing steps and the barrier of genetic flow which separate the various knots. The number of 133 mutations helped to build a parsimonious network where the most polymorph site is situated at position 42. The latter is the highest peak of the genetic divergence in the *Sitophilus* genus considering the median vectors mv6, mv7, mv8, mv9 and mv10. The two groups played by *Sitophilus zeamais* and *Sitophilus oryzae* are separated by a number of high level changing steps (51 steps) and 5 median vectors (mv6, mv7, mv8, mv12 and mv13) which indicate a genetic barrier between these two groups “Fig3”.

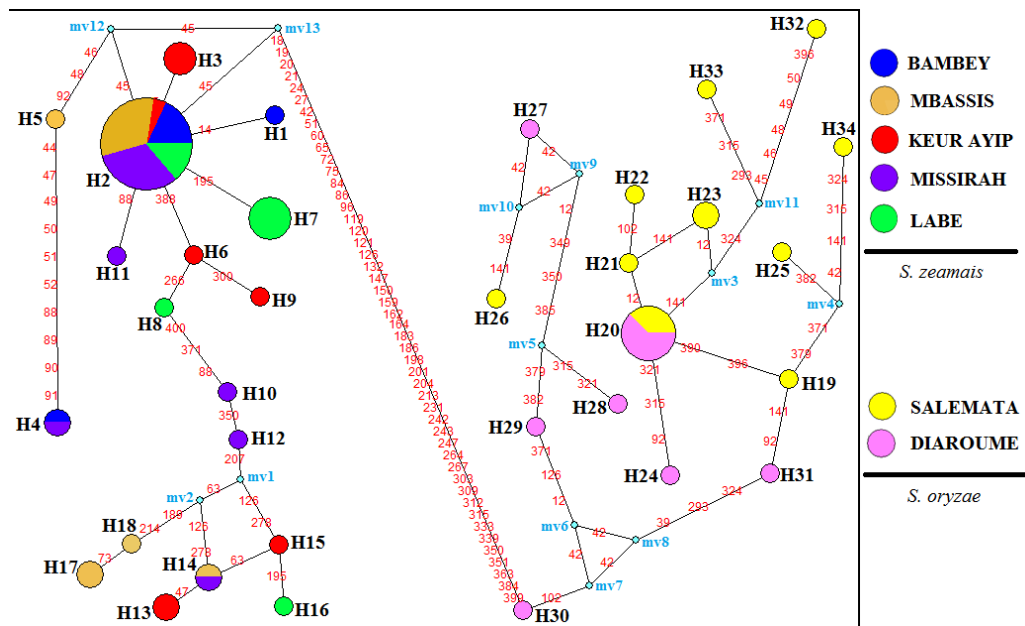


Fig3. Network of the haplotypes showing the genetic difference and haplotypal structure within the *Sitophilus zeamais* (at left) and *Sitophilus oryzae* (at right) species. Each disk indicates a haplotype and their dimension is proportional to the number of individuals corresponding to the haplotype.

Each group is characterized by a majority haplotype from where the other ones are derived. The genetically nearest *Sitophilus oryzae* haplotypes of *Sitophilus zeamais* are to be found in Diaroumé area. Both Mbassis and Missirah areas have the greatest part of the majority haplotype of *Sitophilus zeamais* and the rest is occupied by the other areas. Bambey and Labé areas occupy a great deal in the majority haplotype. The Missirah individual presents more affinity with those from Bambey (H4) and Mbassis (H14) than those from Keur Ayip. At *Sitophilus Oryzae*'s, the main haplotype which is mostly composed of individuals from the area of Diaroumé is emerging other haplotypes with a more important number than at *Sitophilus zeamais*.

Therefore, in Senegal the *Sitophilus* genus is composed of two species found specifically in their own zones without any sympatric cross-check: so there exist two domains for *Sitophilus zeamais* and *Sitophilus Oryzae* species.

3.4. Diversity, Distance and Genetic Structuring of *Sitophilus* spp

3.4.1. According to the Areas and Storage Infrastructures.

The areas of Keur Ayip (0.889 ± 0.091) located at the border with Gambia and Labé (0.711 ± 0.117) situated in the Guinea Republic where the samples are taken respectively from stock in stores and kitchen show the strongest haplotypal diversities (HD). However the haplotypal diversities noticed, is high and is estimated to 0.600 ± 0.215 in Bambey, 0.667 ± 0.141 in Mbassis and 0.682 ± 0.148 in Missirah (“**Fig4**”). These values mentioned, are obtained on the *Sitophilus zeamais* species.

In an attempt of comparison, the haplotypal diversity (Hd) of *Sitophilus oryzae* has been estimated in figure 5 at table 5 for both Diaroumé and Salémata areas. It is elevated in these two areas but more important in Salémata (0.949 ± 0.051) up to Diaroumé (0.818 ± 0.119).

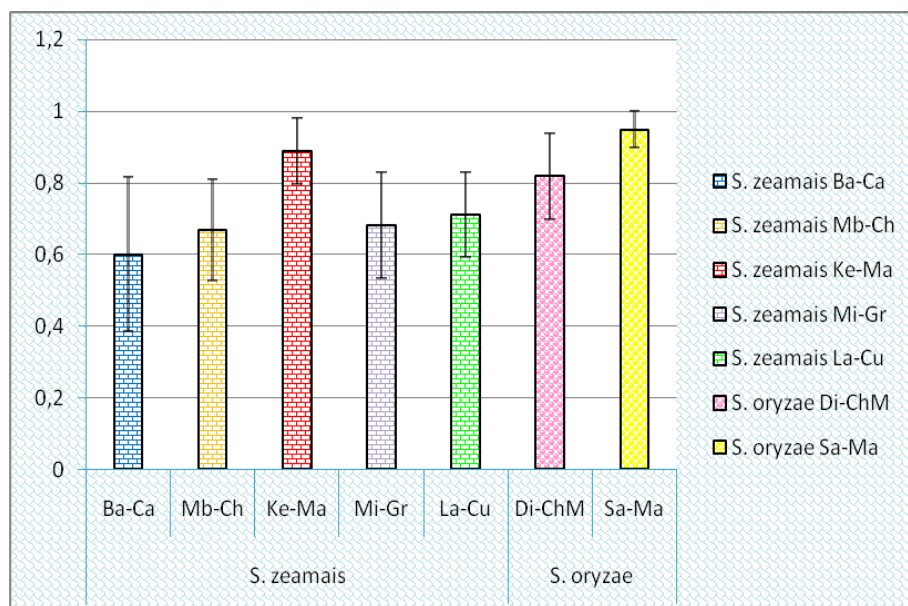


Fig4. Haplotype diversity (hd) of *Sitophilus* genus according to the areas and storages infrastructures

The nucleotide diversity (Pi) estimated in per thousand at *S. zeamais*, is elevated in all the areas and is about 0.01220 ± 0.00727 in Bambey, 0.01463 ± 0.00309 in Mbassis, 0.01477 ± 0.00299 in Keur Ayip and 0.01308 ± 0.00485 in Missirah. However it is worth noticing that the area of Labé is an exception because its nucleotide diversity is slight and is estimated to 0.00645 ± 0.00317 (**Fig5**). It is elevated in all the areas of *S. oryzae*. It is estimated to 0.01836 ± 0.00356 in Diaroumé and 0.01451 ± 0.00309 in Salémata (**Fig5**).

The genetic diversity (haplotypal and nucleotide) is more important in the *S. oryzae* species than in the *S. zeamais*.

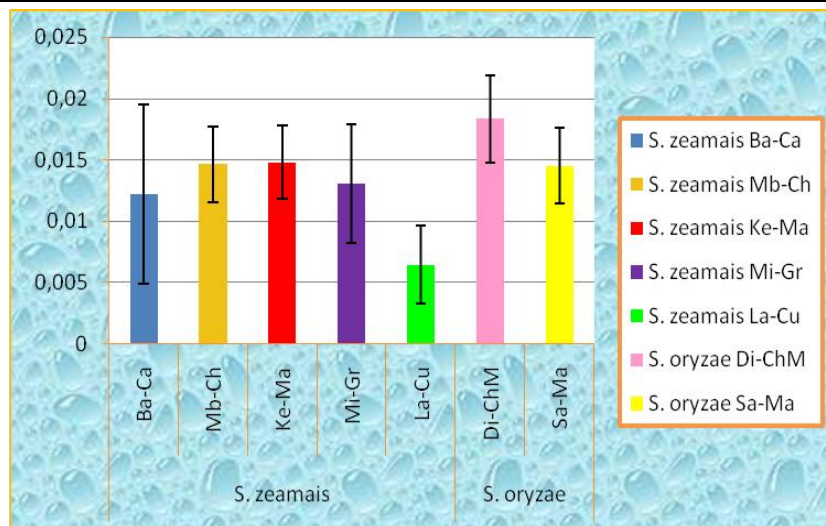


Fig5. Nucleotide diversity (P_i) of *Sitophilus* genre according to the areas and the storage infrastructures

The intra-locality genetic distance (intra storage infrastructure) which is shorter (0.007) at *S. zeamais* is obtained in Labé area (kitchen) and the longer one (0.015) in Mbassis area (field) and Keur Ayip area (store). The inter locality distance which is shorter (0.010) is noticed between the samples of Bambey (hut) and those of Labé (kitchen) and the longer one (0.016) between Bambey (field) and Keur Ayip (store). The intra specific distance of *S. oryzae* inside the areas is estimated to 0.015 in Salémata, 0.019 in Diaroumé whereas the inter locality distance is estimated to 0.017 (Table 3). The genetic distances assessed between the two species are estimated from 0.155 to 0.164 evidencing the real existence of two species from *Sitophilus* genus in the stocks in Senegal. The average distance within *S. zeamais* is about 0.013 and 0.028 for *S. oryzae*. The average divergence between the two species is estimated to 0.154 and that between the sub-clades formed by the haplotypes according to their genetic affinities is from 0 to 0.049. It is also important to mention that the genetic diversity (haplotypic, nucleotide and distance) is more important in the markets and the stores to develop progressively following a decreasing gradient of the field, attic, hut and kitchen.

The test of Mantel (Fig6a) for *S. zeamais* shows that the genetic distance is correlated to the geographical distance. This is corroborated by the correlation coefficient of Pearson (Fig6b) which measures at which point two variables are correlated when looking for the linear correlations. It varies between -1 and 1: 1= perfect positive correlation, 0= no correlation, -1= perfect negative correlation when one of the variables is increasing, the other is decreasing). In accordance with the numbers of P-value (bilateral), H_a (alternative Hypothesis) < 0.0001, Alpha (significance level) $H_0 = 0.05$ and $r(AB) = -0.799$ the null hypothesis H_0 must be rejected and keep the alternative hypothesis H_a .

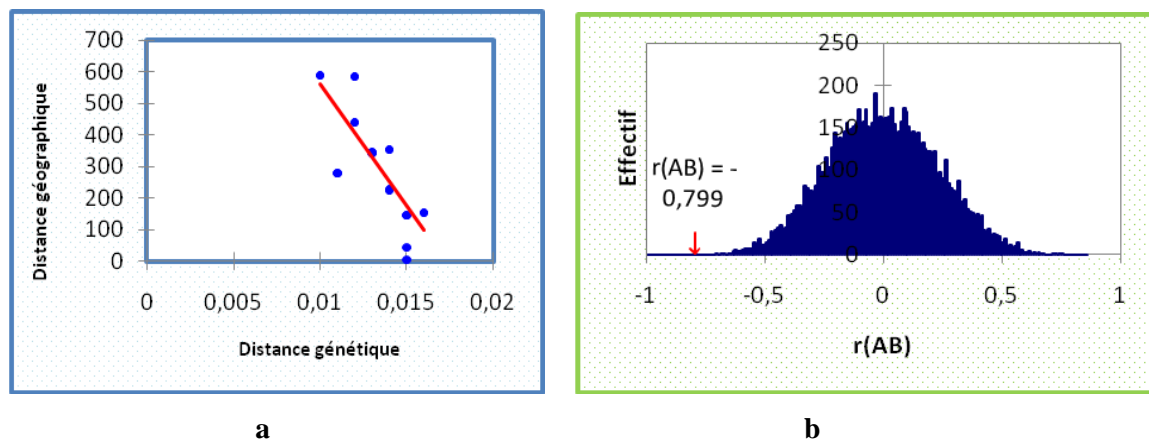


Fig6. Test of Mantel: Correlation of the geographical distance (position of the localities) in accordance with the genetic distance (a) with a corroboration of Pearson's coefficient (b) for *S. zeamais* species.

H0: the matrixes are not correlated.

Ah: the matrixes are correlated.

The risk of rejecting the null hypothesis H0 although it is true is less than 0.01%. Indeed the genetic distances are negatively correlated to the geographical distance that is to say if the latter is increasing the genetic distance is decreasing.

3.4.2. Accordance the agro-climate zones

The *S. zeamais* species is found in the agro-climate sudanian (SOU), Sahelo-Sudanian (SAS) and tropical futanian (FTR) whereas the *S. oryzae* species is mainly found in the Sudano-Guinean (SOG) and sub-Guinean (SUG) zones. The SOU zone has the biggest haplotypal (0.790 ± 0.072) and nucleotide (0.01406 ± 0.00244) diversity. The SAS zone has got the weakest haplotypal diversity (0.600 ± 0.215) and FTR, the smallest nucleotide diversity (0.00645 ± 0.00317). The haplotypal and nucleotide diversity of the both SOG and SUG zones are respectively equivalent to those obtained in Salémata and Diaroumé (Fig7; Fig8).

The smallest genetic distance is noticed inside the FTR zone (0.007) and the biggest one is inside the SOU (0.014). The most important inter-zone divergence is calculated between SAS and SOU (0.015).

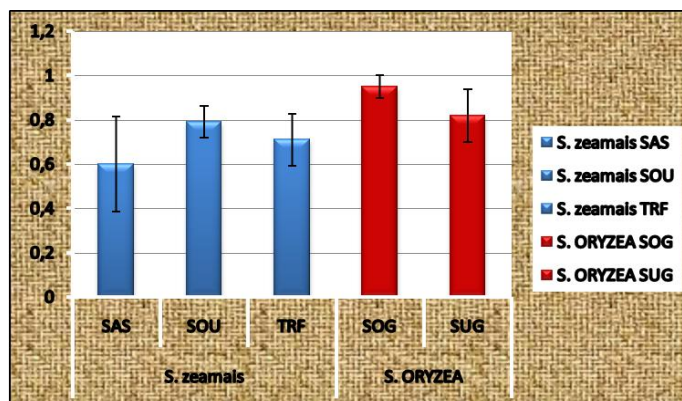


Fig7. Haplotypal diversity within the *Sitophilus* genus between the agro-climate zones

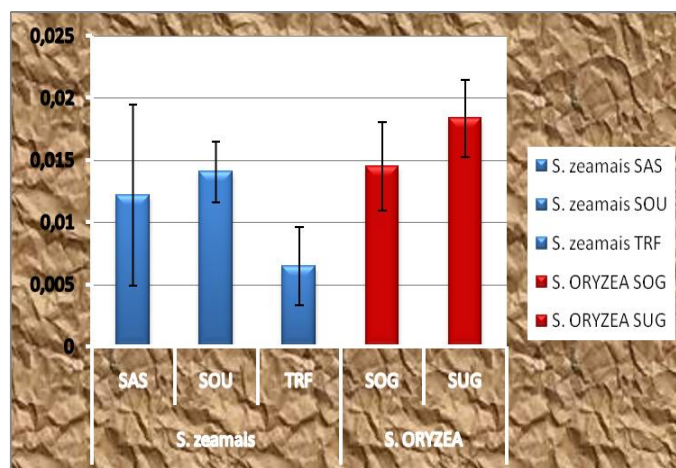


Fig8. Nucleotide diversity within the *Sitophilus* genus between the agro-climate zones

Table3. Genetic distances of *Sitophilus zeamais* and *Sitophilus oryzae* in each area/storage infrastructure: localities and storage infrastructures (LIS) intra localities distance (IAL) and inter localities (IRL).

LIS	GENETICS DISTANCES							
	IAL	Inter localities (IRL)						
		Ba	Mb	Ke	Mi	La	Di	Sa
Ba-Hu	0.012	—						
Mb-Fi	0.015	0.015	—					
Ke-St	0.015	0.016	0.015	—				
Mi-At	0.013	0.013	0.014	0.014	—			

Diversity and Genetic Structure of *Sitophilus Spp.* Haplotypes, Primary Pest of Stored Maize in Senegal and Guinea: Genetic Impact of Storage Infrastructure and Agro-Climatic Zones

La-Ki	0.007	0.010	0.012	0.012	0.011	—		
Di-Ro	0.019	0.157	0.159	0.159	0.157	0.155	—	
Sa-Ma	0.015	0.161	0.164	0.164	0.162	0.160	0.017	—

Table4. Genetic distances of *Sitophilus zeamais* and *Sitophilus oryzae* in each agro-climate zone: Agro-climate Intra zones (IAZA) and agro-climate inter zones (IRZA).

Parameters	GENETICS DISTANCES					
	IAZA	Inter agroclimatic zones (IRZA)				
		SAS	SOU	TRF	SUG	SOG
SAS	0.012	—				
SOU	0.014	0.015	—			
TRF	0.007	0.010	0.012	—		
SUG	0.019	0.157	0.159	0.155	—	
SOG	0.015	0.161	0.163	0.160	0.017	—

3.5. Mismatch Distribution, AMOVA Test and Neutrality Test

The mismatch distribution or disparities' distribution shows as well as for the two kinds of multimodal polygons (**Fig9a/9b**). It's about establishing the distribution of the differences' number (genetic distance) between the individuals of the same population taken in pairs. The P-value of mismatch distribution for a demographic expansion (Arlequin) of defined population according to the localities isn't important in any storing infrastructure. Demographic indications SSD (Sum of Squared Deviation) and RI (Raggedness index de Harpending) have been calculated between the observed and expected distributions in order to test the validity of the expansion modal. But the SSD indication presents, on the one hand weak values (In Mbassiss at 0.07 next to an expansion in the fields ;in Labé 0.15 in Kitchen ;in Bambey and Diaroumé at 0.2 respectively in hut and room).On the other hand, the SSD indication presents high values in (Missirah and Salémata at 0.4 respectively in attics and stores; Keur Ayip at 0.34 in stores).

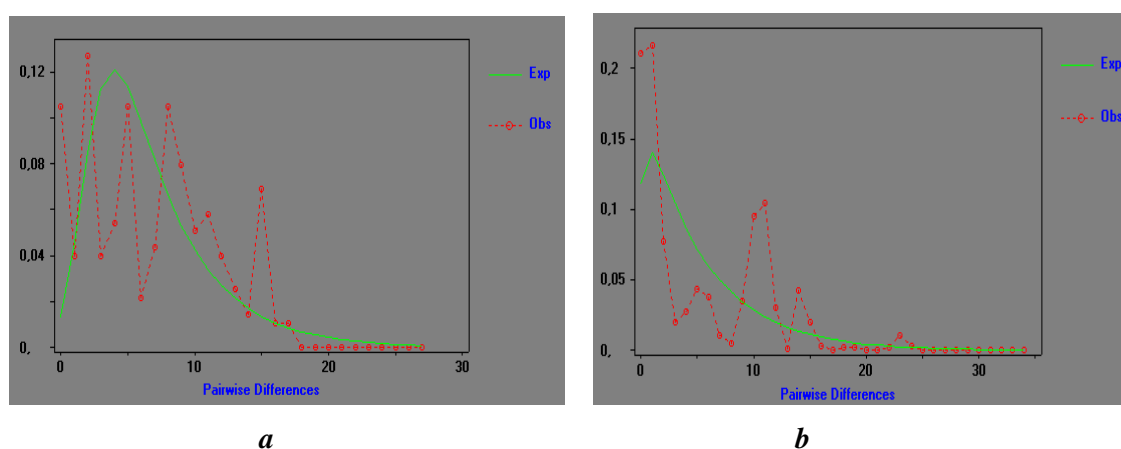


Fig9. Mismatch Distribution comparing the observed disparity to the expected one from *S. zeamais* (a) and *S. oryzae* (b)

The AMOVA test (**Table 5**) shows a genetic structure of *Sitophilus* genus in Senegal and the Republic of Guinea according to the agro-climatic zones with a significant and negative fixation indication (-0.00251). The variation rate found in this direction equals to 85.22%. The intra agro-climatic fixation indication and that of intra locality (population) are almost equaled (respectively 0.85180 and 0.85217). But the composition of the variance indicates a negative value (-0.00706) in intra agro-climatic zone with -0.04%. The percentage of variation between populations is not neglectful and is estimated to 14.82%.

The test of Ewens-Watterson (9), estimates the observed diversity and the expected one at the same time with an average number of differences of polymorphic sites between individuals taken in pairs (Watterson, 1975). And the average number of haplotypal differences between individuals taken in pairs (Ewens, 1972). It shows that no value of P-value is important in the populations of localities. Yet, it is weak in Labé (0.681) compared to the maximal values obtained in Bambey (1), Missirah (1), Diaroumé (1) and which are less important in Keur Ayip (0.847), in Mbassiss (0.948).

Table5. Molecular analysis test of variance intralocality and intra / inter agroclimatic zones of the genus *Sitophilus* in stocks in Senegal and the Republic of Guinea. The meaningful indicators ($P < 0.05$) are marked with an asterisk. D.f(degrees of freedom).

Source of variation	Fixation Indices	D.f	Sum of squares	Components of Variance	Percentage of variation
Between agro-climatic zones	-0.00251*	4	858.807	16.22697 Va	85.22%
Intra agro-climatic zones	0.85180	2	5.490	-0.00706 Vb	-0.04%
Intra locality (infrastructure)	0.85217	66	186.251	2.82198 Vc	14.82%

The test of D Tajima being significant in Bambey ($0.014 < 0.05$) is characterized by a negative value (-1.47247). In the other localities, it is not significant but it remains negative in Missirah (-1.31116), Labé (-1.27498) and Salémata (-0.69292) and it's positive in Mbassis (0.28669), Keur Ayip (1.26914) and Diaroumé (0.72407).

Le F_s de F_u isn't significant (P -value > 0.02) for a population dispatching according to the localities. It is negative in Salémata (-2.40338) and positive and twice superior in Bambey (2.95986), in Mbassis (2.77726); it is comprised between 1 and 2 in Missirah (1.20764), in Labé (1.58557); inférieur to 1 in Keur Ayip (0.42091), in Diaroumé (0.66148).

The effects of selection phenomena or that of demographic expansion is tested between agro-climatic zones (**Table 6**) by comparing the values of F_s , de D de Tajima, F^* , D^* , FL-D and FL-F. The tests of D de Tajima and that of F_s de F_u are not significant in any zone.

However, the SAS zone admits a negative D and a positive F_s added to the D^* , FL-D and FL-F, that are all negative and significant. This shows that we have a phenomenon of selection (sweeping or purifying, balanced) entailing an appearing and a rapid spreading within the population and a maintaining of ancestral and advantageous alleles ($F_s > 0$). The SOU and SOG zones have negative D and negative F_s that symbolize a population undergoing a selection for each species or a demographic expansion or an area expansion. The TRF zone having a negative D and ($F_s > 0$). a positive F_s evidences a population undergoing a selection which entails a bottleneck. The SUG zone marked by positive values makes appear a population with a maintaining of ancestral alleles or a bottleneck.

Table6. Test of neutrality within agro-climatic zones from a combination of methods based on the indication of F de F_u and D^* and F^* . The presence of sign + indicates a significant P -value.

	Tajima's D	F_s de f_u	F^*	D^*	FL-D	FL-F
SAS	-1.47247	2.960	-1.63141	-1.51001 ⁺	-2.4777 ⁺⁺	-2.6956 ⁺⁺
SOU	-0.78285	-1.410	-0.91815	-0.77496	-0.9622	-1.0904
TRF	-1.11650	1.237	-1.27406	-1.13824	-1.5984	-1.7674
SOG	-0.69292	-2.403	-0.94909	-0.88039	-1.2742	-1.3393
SUG	0.46374	0.661	0.74646	0.72551	0.7570	0.8182

The values of genetic differentiation by pair of populations (F_{st}) evaluated between agro-climatic zone indicate positive values everywhere (**Table 7**). These values are low between the SAS, SOU and TRF zones on the one hand, the SOG and SUG zones on the other hand. They are very high between the SOG and SUG zone in relation to the three other above mentioned zones (SAS, SOU and TRF). So, the F_{st} shows two great phylum: a clade embodied by a weak genetic differentiation within its favorite zones (0.08141 à 0.10267), is especially found in the SAS, SOU and TRF zones. Another clade that is different from the first one by their genetic pool is subjected in the SOG and SUG zones, with a more important genetic affinity (0.00667). It is important to point out a strong genetic differentiation within the *S. zeamais* between the SOU and TRF agro-climatic zones.

Table7. Test of genetic differentiation (F_{st}) between the West African agro-climatic zones

	SAS	SOU	TRF	SOG	SUG
SAS	—				
SOU	0.08141	—			
TRF	0.08632	0.10267	—		
SOG	0.89046	0.88483	0.90985	—	
SUG	0.90669	0.90128	0.92606	0.00667	—

3.6. Haplotypes Trees Built Upon Bayesian Method

The maximum of likelihood tree presents the same topology as that obtained with the Bayesian probabilistic method. Hence, it's less determining in bootstrap values or posterior probability than the Bayesian tree. MR. Bayes's tree will only be presented here, it is obtained after a simulation of 3 000 000 of generations with a perfect checking of hot and cold chains. This phylogenetic reconstruction displays two paraphyletic clades (C1 and C2), each one is composed of several monophyletic (bootstrap from 70 to 100%) sub-clades (SC). These gatherings go in the same way as the previous results that highlight two species (*S. zeamais* and *S. oryzae*) within the *Sitophilus* genus with bootstrap values from 60 to 80%. It is worth pointing out that the values of bootstrap and posterior probability are more significant between the haplotypes inside *S. oryzae* species than haplotypes outside *S. zeamais*. Among the 8 sub-clades, only 2 belong to *S. oryzae*. They are SC7 and SC8 sustained by bootstraps respectively estimated to 89% and 85%. Within the SC7, we find a set of subjected haplotypes as well in Salémata locality as in Diaroumé locality (H26 and H27 sustained to 100%) whereas the SC8 especially shows genetic affinities in each locality (H21, H22 and H23 all the haplotypes of Salémata are sustained in a sub-set to 84%). The sub-clades SC2 (Keur Ayip), SC3 (Labé), SC4 (Keur Ayip) and the sub-set (H17 and H18 of Mbassiss) of SC5 are the obvious results of an intrinsic genetic structure in each locality. SC5 is the symbol of an intra-specific pool of genes since it almost gathers the whole localities. The sub-set of SC5 composed of H13, H14, H15 and H16 haplotypes to 90% is responsible for the manifestation of the probable effect of the original sample variety (maize).

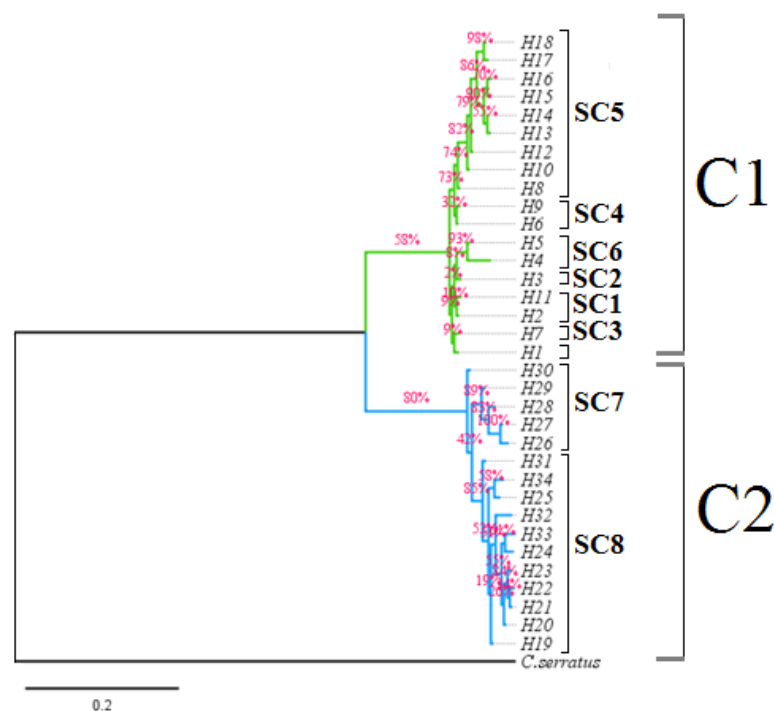


Fig10. Topology of the phylogenetic tree obtained with the bayesian method after an estimation of 3, 000, 000 of generations (Average Gap-type of frequencies equal to 0, 011738 and thus inferior to the comparative value 0,015). A chrysomelidae beetle, *Caryedon serratus* has been used as an out-group so as to deep-root the tree.

3.7. Discussion

The aim of this study is to identify both the different species in the genus *Sitophilus* infesting stored maize in Senegal and Republic of Guinea and the number, polymorphic diversity and genetic structure of haplotypes that exist there through the analysis of gene cytochrome B. On the other hand, it is to determine the genetic impact of storage infrastructure and agro-climatic zones in the affinities between allopatric populations and population dynamics.

The cytochrome B gene amplified for the first time inside this genus *Sitophilus*, shows that it is composed of two primary pest species of maize storages in Senegal and Guinea Konakry. The recognition of the two species is achieved subsequently to a barcoding comparison (100% of

Likelihood) in Bold and blast in Genbank since the Cytochrome Oxidase I (COI) gene has been sequenced for these same individuals. The *Sitophilus oryzae* specie is more polymorphic than *Sitophilus zeamais*. Because upon 49 sequences of 410 bp of *Sitophilus zeamais*, 2 single sites and a rate in difference number of 5.26% have been identified while they are respectively evaluated at 5 and at 6, 68% within 24 *Sitophilus oryzae* sequences having the same length. The genetic difference between these species is correlated to the number of 133 mutations, including the most polymorphic site which is in the 42nd position. The differentiation between *Sitophilus zeamais* and *Sitophilus oryzae* is also noticed around the evolution upon a number of excessively high mutational step (51 steps) and 5 median vectors that indicate a genetic barrier between these two clades. The *S. zeamais* is exclusively found in the middle-west (Bambey), in the centre (Keur ayip, Mbassis), in the East (Missirah) of Senegal and the north of the Republic of Guinea (Labé). The *S. oryzae* exclusively observed in the southern localities of Senegal and not elsewhere. It's about Salémata and Diaroumé localities which are separated from other localities by the Gambian frontier, the Gambian, Senegalese, Guinean river tributaries and other culminating peaks of the relief (mountains) and the Niokolo Koba Parc, in the south-east of the country. So, in Senegal the *Sitophilus* genus is composed of two species (*S. zeamais* and *S. oryzae*) specifically found in their own predilection zones without sympatric cross-checking: it exists then a zoning of species. This would be explained by the fact of allopatric isolation of the southern localities due to natural or artificial barriers. This fact is confirmed by GERAD [16] which shows that southern localities have a pretty thick hydrographic network because of their geographical position and topography. This constitutes a geographic barrier isolating this zone from the central localities of Senegal. The isolation of these localities is stressed from north to west by the national park of Niokolo Koba and the classified forest of West-Niokolo and in the south by the hills (400m) that entail an exacerbated isolation by the reliefs which break the monotony of the landscape and multiply the ecologic habitats. Thus such a situation causes exchange problems manifested in some of these localities by a lack of important market equipment. In fact, in these localities; there is neither bus station nor permanent markets. Seeing that *S. zeamais* flies a bit [17] and the human effect, through the neglectful exchanges, these southern localities are consequently characterized by the absence of this species.

It might be due to the Hopkins principle promoted by eating and ethnical habits in the south of the country and the practices in the storing infrastructures. According to Sembène [18], it exists within the coleopterans a preference of females to lay eggs exclusively on the grain of the host plants of which they are subjugated. This principle is confirmed in the beetles of the *Sitophilus* genus where according to Kranz [17] *S. oryzae* has a preference for the grains of small size like rice whereas *S. zeamais* prefers the grains of big size like maize. Besides, it has been proved that in southern Senegal; the agricultural production that covers only on average 1/3 of the year, is mainly composed of maize, rice, and fonio [19]. Nevertheless, during the times of famine, the food expenditures of the most important household are noticed in the south and south-east localities of the country from 76.2% to 78.3% of all the food purchasing expenditures [19, 20]. Out of this percentage, we have from 22% to 46% of the total expenditures that was meant for rice purchasing. So, one can say the maize presence is temporary, in the stocks of the farmers while that of rice occupies the 2/3 of the year. The *Sitophilus zeamais* that prefers the big grains will a declining demographic dynamics whereas the *Sitophilus oryzae* or rice weevil, specialist of small grains (rice) is favorable to the environmental selective forces, in storing places. This choice is even expanded to the manufactured products because the works of Trematerra [21] show that *S. zeamais* prefers maize pasta to rice, wheat and barley pasta. This justifies its unique presence in the southern part of the country and its important intra-population polymorphism might be associated with the fact that *S. oryzae* is a good flyer. *S. oryzae* is able to attack the stored cereals from the field [18] and adapt itself in different selective environmental obstacles as well diverse as varied while *Sitophilus zeamais* prevails over the part of the country which is situated in the north of the Gambia River where the most important agricultural production is millet. In fact, the produced maize in this locality is stored for a food complement during the times of food shortage. This situation is favorable to the persistence of the habitat and the development supply that are essential to the formation and the maintaining of host races within the phytophage insects [22]. Then within the *Sitophilus* spp., the haplotype number obeys a decreasing gradient of genetic diversity for the two species basing respectively upon storing infrastructures such as trading Store in the market, producer's Store, Attic, Field, Hut and Kitchen. The conclusions of [9] on the

topology of the storage systems and maize conservation in Senegal ratify a difference of the loss level and consequently that of the maize infection between ethnics. According to the same authors, the cause of this loss decrease is related to the performance of the storing infrastructures of each ethnic group and particularly the kind of available material in the respective localities. Therefore, the highest losses noticed at the Dialonke's and Bédick's ethnic groups have repercussions in the department of Kédougou where they are the numerous. The strong genetic diversity observed in the stores might be the result of the food effect because we find there a storage mixture of cereals, leguminous etc. *Besides, it is a critical point in this system of conservation because it brings about a cereals cross-infection.* The weakest infection obtained in kitchen would be due to a bottleneck carried out by the selective pressure of the place's conservation conditions that confined by the almost permanent action of the smoke. The demographic expansion which starts in the field is extended and reaches a high level during the drying in the huts before culminating in the attics. Moreover, H2 is not only a sub-regional haplotype since it is found in all the sample localities up to Guinea, but it also is a haplotype retracing the itinerary of cereals from the production in the field until the consumption (kitchen) or the sale (store) through the drying (hut) and the storage (attics). Yet, the samples of Diaroumé are taken from a living room but according to the farmer, this maize is bought in the market. This is factual because of the haplotypal richness of this locality which is the turntable of the country's southern part by its market and the importance of the communication ways that stem from there to link the 3 regions (Ziguinchor, Kolda and Tambacounda) to the Gambia and Guinea. This is confirmed by Sembène [23, 24] who indicates a differentiation of the stocking stores' populations and attics in relation to the savage population.

The haplotypal diversity (Hd) and nucleotidic (Pi) are high in all the localities, within *Sitophilus spp.*, except that of Labé (high Hd and weak Pi). However, the genetic diversity (haplotypal and nucleotidic) and the genetic flow related to the human transport (the market of Diaroumé and the store of Salémata) are more important within *S. oryzae* species than *S. zeamais*. Populations in admixture and panmictic are mentioned within *S. oryzae* too. This human influence on the scattering of this species is in accordance with the observations of [2] who points out its presence in the temperate zones today via the importation of raw materials from the sub-tropical and tropical regions. Furthermore, the population of Bambey that is characterized by a high haplotypal diversity (Hd: 0.600 ± 0.215 ; Pi: 0.01220 ± 0.00727), a maximal Ewens-Watterson's test (1), a significant and negative D tajima (-1.47247) and an insignificant and positive Fs de Fu (2.95986) that indicates a population resulting from an admixture of two populations who were isolated within the time. The sampling being carried out at the beginning of the storage at farmers' homes, one of the populations would surely stem from the natural population that infects the maize since its maturation in the field. And the other is composed of remaining individuals of the population subjugated to the storing infrastructure. What justifies in this population balanced selection marked by an appearance and rapid spreading of a muting (*succession of polymorphs sites in CCCTTTTA and 35 bases pairs after in CCCTT*) within the population and a maintaining the ancestral and advantageous alleles. This result is comparable to that of Missirah attic, at the difference that in this locality no evaluated parameter is significant. *To prevent from this type of storage infection, the farmers must compulsorily clean the stocking places (Hut and Attic) before putting in the new harvests.*

The Mbassis population is marked by a high diversity (Hd: 0.667 ± 0.141 ; Pi: 0.01463 ± 0.00309), a test of Ewens-Watterson (0.948), an insignificant and positive D Tajima (0.28669) and an insignificant and positive Fs de Fu (2.77726), let appear a population in panmixture with deficit of rare variant with the CC and T mutations at the same position as that of Bambey and Missirah. This result can wholly be corroborated to the obtained data in Keur Ayip with a rare muting distant from the majority which is characterized by a T mutation in two distant sites of 8 bases pairs. *Would the appearance of this rare muting be favorite to the diversity of the development supports (cereals and leguminous) in field by the polyculture of the Mbassis Serere or by the different stored foodstuffs in the trading stores of the Keur Ayip frontier zone?*

The Labé population would explain a bottleneck caused by the selective effect of the kitchen smoke (excess of CO₂ and hardening the grains) and the reduced size of the grains (fragmented maize). This reductive situation of the infection would encounter an insects' resistance by the

adaptation of some individuals incarnated by the appearance and the quick spreading within the population of a muting that resists by substitution of A into G. This result is official via a high Hd, Pi and a weak test of Ewens-Watterson a negative D and a positive Fs. *The phytosanitary protection of the stocks of fragmented maize grains above the housetop or in bothered places by the smoke looks like efficient to prevent from Sitophilus infection for an average duration.* The results of [25] are in accordance with this hypothesis because they show an inability of *Sitophilus*'s development upon the maize transformed in flour.

The populations of Diaroumé and Salémata present the same demographic tendency but with an insignificance of all the tests that are nevertheless positive as for D and Fs in Diaroumé but negative in Salémata. The Diaroumé's population would be characterized by an admixture of sub-populations, with a maintaining of ancestral alleles from the markets followed by bottleneck, probably caused by the stay of the speculum stemming from the market, in the rooms subsequently to conservation measures often carried out by the farmers. The Salémata's population shows a demographic expansion due to the intrinsic characteristics of trading stores.

The weakest (0.007) intra-locality genetic distance (intra-storing infrastructure) within *S. zeamais*, is obtained in Labé (kitchen) while the greatest one (0.015), is got in Mbassis (field) and Keur Ayip (store). The weakest inter-locality distance (0.010) is observed between the samples of Bambey (Hut) and that of Labé (Kitchen) and the biggest one (0,016) is found in between Bambey (Hut) and Keur Ayip (store). These values are similar to the ones obtained by Ndong [26, 27] while respectively characterizing the populations of peanut bruchidae, *Caryedon serratus* and the bean bruchidae, *Callosobruchus maculatus*.

The test of Mantel shows that the genetic distances are negatively correlated to the geographic distance. That is to say; if the latter increases, the genetic distance will decrease. This correlation would probably be due to the positive effect of humidity on the demographic increase of *Sitophilus zeamais*. Because the further we go to the south, the more the isohyets' values augment from 500 to 900 mm and the more the number of generations augments.

Since the bases substitution in the DNA are done according to the times of generation, the appearance of haplotypes that exists in the other localities becomes probable and is accompanied with a coherence phenomenon of the genetic pool that decreases the genetic difference between remote localities. The works of some authors validate a *S. zeamais*'s preference towards high humidity 75 to 80% [28] and their determinative role in the level of these populations [10].

Besides, the AMOVA test confirms a genetic structure of *Sitophilus* genus in Senegal and the Republic of Guinea according to the agro-climatic zones with an insignificant and negative fixation indication (-0.00251). The variation rate found in this direction equals to 85, 22%. So, The Fst certifies a *Sitophilus zeamais* clade that is particularly found in SAS, SOU and TRF zones with a strong genetic differentiation between agro-climatic SOU and TRF zones. Moreover, this situation would result from the climatic factors and the genetic impact of the environment which is intrinsic to the storing infrastructure because we have in the SOU zone: field, attic, and store while we have kitchen, in the TRF zone. It also displays a *Sitophilus oryzae* clade that is subjugated to the SOG and SUG zone, with most important genetic affinities (0.00667). This proves the intensity of the genes' flow between markets and stores. The observed differentiation in some zones would be the effect of selection phenomena but not demographic expansion. In fact, the SAS zone admits a negative D and positive Fs added to the D*, FL-D and FL-F, all insignificant and negative. This shows that we have a phenomenon of selection (sweeping or balanced purifying) that entails an appearance and a rapid spreading of a muting within the population and a maintaining of ancestral and advantageous alleles (Fs>0). The SOU and SOG zones have a negative D and negative Fs, symbolizing for each species a population that undergoes a selection, a demographic or space expansion. The TRF zone having a negative D and positive Fs, evidences a population undergoing selection exerted by the kitchen smoke entailing a bottleneck. The SUG zone that is market by positive values make appear a stable population with an ancestral alleles maintaining in the markets, followed by bottleneck. The phylogenetic reconstructions enters the same wake as the previous results so, it evidences two species (*S. zeamais* and *S. oryzae*) within the *Sitophilus* genus. We also observe sub-clades which are the obvious results of an intrinsic genetic structure in each locality, each storing infrastructure and each agro-climatic zone. The particularity sub-clades found in Salémata (isohyets 1200) and in

Mbassis (insulate zone) confirms a genetic structure that would be entailed by the influence of ecologic factors, especially humidity. The most salient fact would result the probable effect of the original sample variety (maize) because different sub-clades sustained at 90% of posterior probability are obtained in Keur Ayip area on different varieties. Besides, this is illustrated on the same Suwan variety by the differentiation of the sub-clades of Labé and Kossou [29] which point out a resistance difference of the maize grain's shell varieties faced to *S. zeamais*. This variety effect on the polyphage insects has respectively been proved in sensitiveness test and genetic characterization by [30] and [27] on *Callosobruchus maculatus*, pest of stored bean, *Vigna unguiculata*.

4. CONCLUSION

In Senegal the genus *Sitophilus* is composed of *Sitophilus zeamais* and *Sitophilus oryzae* found specifically in areas that are their own and without overlapping sympatric. They are characterized respectively by 18 haplotypes and 16 haplotypes. In addition to the genetic structure of this genus based on agro-climatic zones, genetic distances between populations of *S. zeamais* are negatively correlated with geographic distance. There is a decreasing gradient of genetic diversity based on storage infrastructure starting from stores of traders (market) to the kitchen passing respectively through the producer stores, attics, fields and huts.

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AUTHORS' BIOGRAPHY



Assane Ndong is a PhD student at the University Cheikh Anta Diop of Dakar. He was a secondary school teacher and has a master 2 in Population Genetics.



Pr Mbacke Sembene is a Professor, Teacher-researcher at Cheikh Anta Diop University in Dakar, Faculty of Science and Technology and BIOPASS Laboratory. He teaches Population Genetics, Physiology and Entomology.