American Journal of BioScience
2019; 7(3): 71-76
http://www.sciencepublishinggroup.com/j/ajbio
doi: 10.11648/j.ajbio.20190703.12
ISSN: 2330-0159 (Print); ISSN: 2330-0167 (Online)

Comparative Study of the Genetic Variability of Sitophilus Zeamais Subservient to 2 Host Plants (Millet and Maize) in Senegal (West Africa)

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To cite this article:
Ngagne Demba Sarr, Dethie Ngom, Mbacke Sembene. Comparative Study of the Genetic Variability of Sitophilus Zeamais Subservient to 2 Host Plants (Millet and Maize) in Senegal (West Africa). *American Journal of BioScience.* Vol. 7, No. 3, 2019, pp. 71-76.
doi: 10.11648/j.ajbio.20190703.12

Received: August 21, 2019; **Accepted**: September 20, 2019; **Published**: September 30, 2019

Abstract: Millet and maize, because of the importance of their yields, constitute a reliable alternative to the question of sovereignty and food security. These cereals are exploited in all agroecological zones of Senegal. But their stocks, especially those of maize, are deteriorated by Sitophilus Zeamais, a beetle of the Curculionidae. It is therefore necessary to find natural solutions that are healthier than the use of pesticides which is harmful to living beings and the environment. This article makes a comparative study of the genetic diversity of the populations of this insect individually subservient to maize and to millet. The importance of this study is to find a genetic explanation for the differential vulnerability of these host plants to Sitophilus Zeamais, because the genetic diversity influences the adaptability of the individual and consequently its development. For this, insects Sitophilus Zeamais subservient on the one hand to maize and on the other hand to millet were collected in each agroecological zone. The exploitation of 125 sequences of the Cytochrome B gene corresponding to the individuals, by software of study in population genetics (Bioedit, DNAsp, Mega, Arlequin…) compared to parameters of genetic variability, revealed an approximately similar and high genetic diversity of the 2 populations of millet and maize. Thus, millet and maize genetically have the same effect on the adaptability of Sitophilus Zeamais.

Keywords: Cytochrome B, Maize, Millet, Sitophilus Zeamais, Genetic Diversity

1. Introduction

In Senegal, maize and millet constitute the food base of households, especially rural households. These 2 cereals occupy respectively 28% and 11% in rural areas against respectively 19% and 5% in urban areas [14]. But their stocks are altered by a powerful pest, a coleopterous beetle of the Curculionidae, named Sitophilus Zeamais. According to Ngamo and Hance, losses can range from 25% to 40% in six months of storage [14]. Thus, the extent of the damage threatens the socio-economic function that these cereals can play in a current context of poverty reduction.

The search for solutions to drastically reduce these losses has always been to find the most effective pesticide to eliminate the insect. No genetic study to correlate host plants populations with the extent of damage which they are individually victim has been done.

Our study aims to identify the host plant most favorable to the survival or extinction of Sitophilus Zeamais, by the comparative exploitation of the genetic diversity of the 2 populations of millet and of maize. Indeed, homogeneity and genetic heterogeneity affect the adaptive potential of the insect.

To achieve our objective, we sampled in each agroecological zone insects subservient on the one hand to millet and subservient on the other hand to maize, on stocks and fields widely spaced. A total of 125 individuals were harvested, of which 72 from maize and 53 from millet.

The sequences of the Cytochrome B gene corresponding to these individuals have been exploited by population genetics software (Bioedit, DNAsp, Mega, Harlequin...), with respect to parameters of genetic variability (haplotypic and

nucleotide diversity, number of haplotypes, number of parsimony or singleton variable sites and invariable, average number of nucleotide differences...), in relation to our objective.

2. Material and Methods

2.1. Sampling

2.1.1. Sampling Localities

Individuals of Sitophilus zeamais were sampled in 4 agroecological zones (AEZ) of Senegal, on 2 host plants (Millet and Maize). The choice given to these zones is justified by their vocation naturally agricultural and by ecological and geographical characteristics which specify each of them. As for millet and maize, they were chosen for their socio-economic functions and their very high vulnerability to the insect. These agroecological zones are constituted by the AEZ of NBA (Nord Bassin Arachidier) represented by the only locality of Bambey (14°42'00" Nord/16°27'00" Ouest), the AEZ of the SBA (Sud Bassin Arachidier) to Keur Ayip (13°36'00" North/15°37'00" Ouest), to Mbassis (14°04'60" Nord/16°25'60" West), to Nioro (15°13'55" North/09°35'37" West) and Dionewar (13°52'60" North/16°43'60" West). Samples were also taken from the AEZ of SOHC (Senegal Oriental Haute Casamance) at Missirah (13°41'00" Nord/16°30'01" Ouest) and Salémata (12°37'60" North/12°49'00" West). The other AEZ sampled is BMC (Basse Moyenne Casamance) in The Gambia (13°27'09" North/16°34'40" West) and Diaroumé (13°03'19" North/15°38'34" West). Figure 1 summarizes the sampling sites and their respective AEZs.

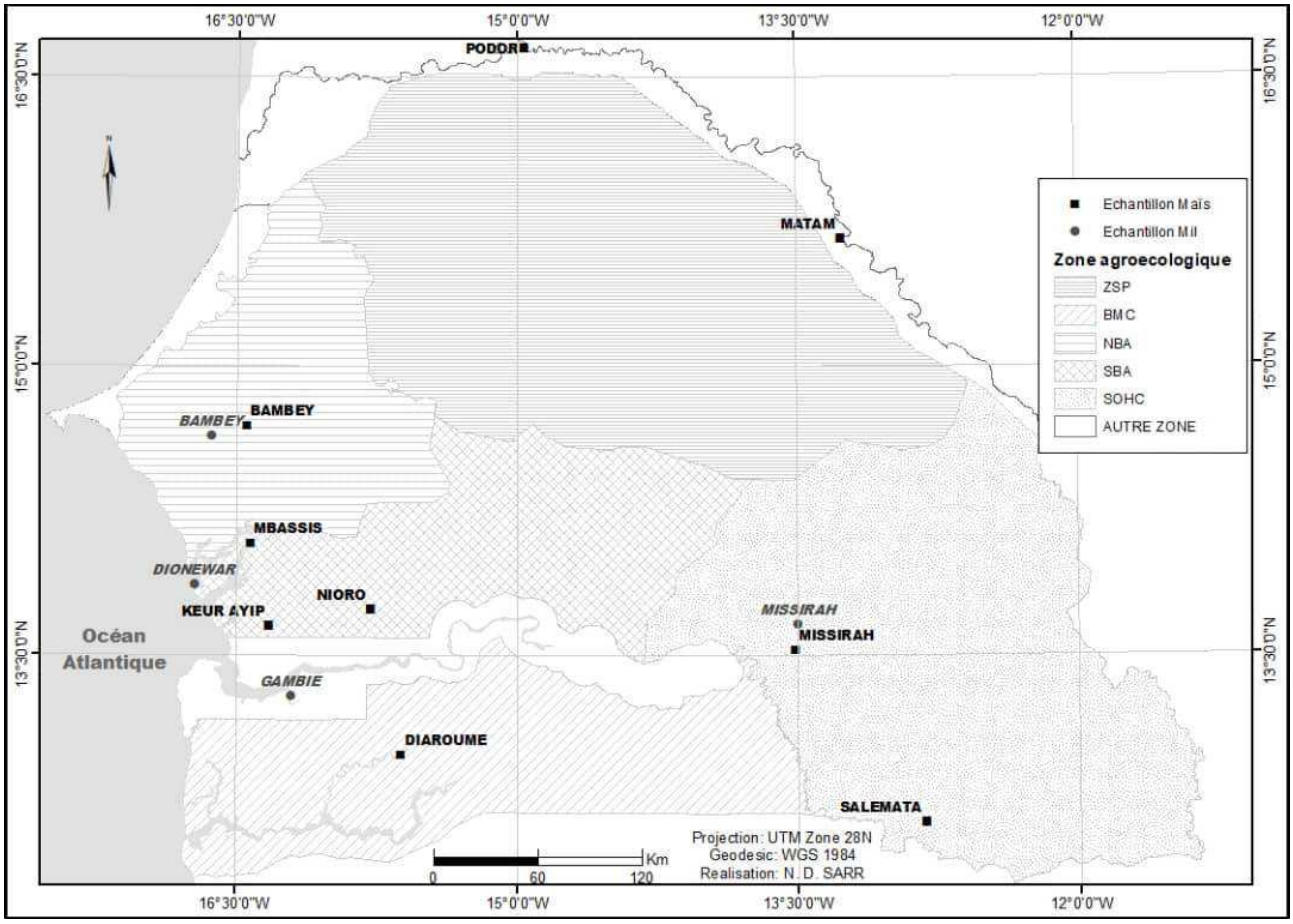

Figure 1. *Sampling Localities.*

2.1.2. Harvesting Individuals

Collecting maize and millet samples infested in the different AEZs, allowed to isolate individuals of Sitophilus zeamais for each zone and each host plant. It has been used in storage facilities where grain is highly vulnerable to infestation, but also in marketing places where there is a high chance of encountering infested maize from different AEZs.

After isolation, individuals from each AEZ and each host plant are placed in tubes containing 96% alcohol.

To code individuals compared to their host plant, we capitalized the first letter of the insect's genus name and then we specified the type of host plant of the individual using the first two letters of the plant (The first letter in upper case and the second in lower case), we have specified the locality of origin by 2 letters too (the first letter in upper case and the second in lowercase), then specify the serial number. Example a Sitophilus zeamais individual who was harvested in Bambey on Millet with the order number 12 is coded as:

SMiBa12 if it was on maize, the code would be SMaBa12.

Table 1 summarizes the localities of the AEZs where the harvests took place, the number of individuals sampled for each AEZ, the geographical coordinates of the localities and the codes of the individuals.

Table 1. *Sampling locations.*

Agro-Ecological Zones	Number of individuals	GPS	Sampling code
NBA	23		
Bambey	12/11	14°42'00''N/16°27'00''W	SMaBa/SMiBa
SBA	47		
Keur Ayip	19	13°35'00''N/15°36'00''W	SMaKa
Mbassis	12	14°04'60''N/16°25'60''W	SMaMb
Nioro	07	15°48'55''N/13°45'37''W	SMaNi
Dionewar	09	13°52'60''N/16°43'60''W	SMiDio
SOHC	35		
Missirah	12/13	13°41'00''N/16°30'01''W	SMaMi/SMiMi
Salemata	10	12°37'60''N/12°49'00''W	SMaSa
BMC	20		
Gambie	10	13°27'09''N/16°34'40''W	SMiGa
Diaroume	10	13°03'19''N/15°38'34''W	SMaDi
TOTAL	125		

2.2. Molecular Method of Analysis

2.2.1. DNA Extraction

The extraction is the DNA release technique of the cell. It includes the individualization of cells (digestion) and the destruction of their plasma and nuclear membranes (lysis).

The digestion of the cells consisted of placing their paws and prothorax into tubes containing ATL buffer and K proteinases. After incubation, the tubes were centrifuged to separate the supernatant from cell debris.

To destroy the cell membranes, first cell lysis buffer (AL) was added, then some ethanol (96%) after incubation into the tubes. Then the tubes are transverse in silica membrane columns. Finally, the centrifugation of the tubes allowed to retain the DNA on the siliceous membranes of the columns because negatively charged.

2.2.2. DNA Purification

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

2.2.3. PCR of the Mitochondrial Gene Cytochrome B

The PCR of the mitochondrial gene Cyt. B was carried out by two primers CB1 (5'TATGTACTACCATGAGGACAAATATC-3') and CB2 (ATTACACCTCCTAATTTATTAGGAAT-3'). For each sample (tube), the amplification was made from a total volume of 25µl, of which a mixed volume of 23µl and a volume of 2µl of DNA extract. The mixed volume was constituted by: 18.3µl of milli water, 2.5µl of 10 × buffer, 1µl of additional $MgCl_2$, 0.5µl of Dntp, 0.25µl of each primer and 0.2µl of Taq polymerase.

The conditions under which the PCR was performed are as follows:

1. The DNA strands were first separated with a temperature of 94°C for 3 minutes. This first denaturation was followed by 35 denaturation cycles of 1 minute at the same temperature.
2. The synthesis of complementary strands (elongation) was made at 72°C for 10 minutes. After amplification, the fragments are sent to a South Korean company for sequencing.

2.2.4. Bioinformatics Analyzes

The sequences were corrected and aligned by the Clustal software implemented in the Bioédit version 7.2.5 program.

The evaluation of the sequence diversity was made from some parameters that the DNAsp software made it possible to calculate. These are on the one hand, standard indices of genetic variability, such as parsimonious or singleton variable sites, the number of invariable sites, and the number of haplotypes, and on the other hand Haplotypic diversity (Hd) and nucleotide diversity (Pi). These two indices have the distinction of highlighting the diversity and divergence of haplotypes.

3. Results and Discussion

3.1. Results

72 individuals were harvested specifically on maize and 53 exclusively on millet. Our dataset thus counts 125 sequences of size 410bp. The exploitation of these sequences revealed overall a similarly low polymorphism rate of the two global populations of Millet and of Maize, with respectively 8% and 12% of polymorphic sites.

Insects subservient to maize have more haplotypes (21), sparingly variable sites (34) than the population insects subservient to millet, which are characterized by 10 haplotypes, and more invariable sites (376) and singleton sites (22). The substitution rates of the two populations are similar (Table 2).

Table 2. *Variability Parameters of Cyt. B of the two populations of Millet and of Maize.*

Host Plants	Number of individuals	Number of haplotypes	Number of sites	Invariables Sites (%)	Variables Sites		Substitution Rate
					Singleton (%)	Parcimony informative (%)	
Maize	72	21 (29%)	410	88%	12% 33%	67%	0,938
Millet	43	10 (23%)	410	92%	8% 65%	35%	0,949

The haplotypic diversity (Figure 2a) is approximately equal and high for the two global populations of millet and of maize, with respectively values of 0.837 and 0.813. There is also no significant difference in nucleotide diversity (Figure 2b) (Maize: 0.18, Millet: 0.13). Nevertheless, haplotypes subservient to millet are genetically more convergent because their nucleotide diversity is lower than that haplotypes subservient to maize.

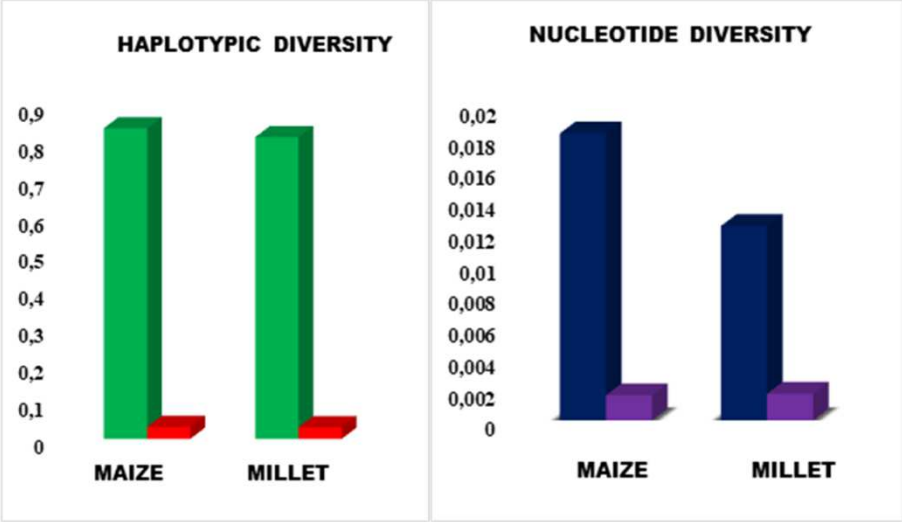

Figure 2. *Genetic diversity of the two populations of Millet and of Maize.*

If we compare the genetic diversity of the populations of Millet and Maize in the agroecological zones (AEZ), we find that those of the populations of the NBA and of the BMC are relatively similar, except the Hd which is weak for the maize population of the BMC. In contrast, Sitophilus zeamais populations of host plants are highly divergent in AEZs of SBA and of SOHC, where Hd and Pi populations of Mille are null while those of Maize are high (Table 2).

Table 2 indicates that haplotype and nucleotide diversity, haplotype numbers, average number of nucleotide differences in millet and maize populations are approximately identical in NBA and in BMC AEZs. This is not the case in other AEZs.

Table 3. *Genetic diversity of populations of Millet and maize in agroecological zones.*

AEZ	PGV HP	Number of individuals	Number of haplotypes	Haplotypic Diversity	Nucléotide Diversity	MPD
NBA	Maize	12	4	0,561±0,154	0,015±0,005	6,182
	Millet	11	5	0,618±0,164	0,007±0,004	3,091
SBA	Maize	12	5	0,667±0,141	0,014±0,003	6
	Millet	9	1	0,000±0,000	0,000±0,000	0
SOHC	Maize	10	3	0,378±0,181	0,001±0,002	0,6
	Millet	13	1	0,000±0,000	0,000±0,000	0
BMC	Maize	10	3	0,378±0,181	0,004±0,002	1.8
	Millet	10	4	0,711±0,117	0,006±0,003	2,64

AEZ=AgroEcological Zone. PGV=Parameters Genetic Variability. HP=Host Plants. MPD=Mean number of Paire Differences.

3.2. Discussion

The maize population has a low number of haplotypes, a low polymorphism and substitution rate. The millet population expresses these same characteristics. This similarity reflects a genetic convergence between millet-dependent insects and maize-specific insects. Similar values of haplotypic diversity and to a lesser extent of the nucleotide diversity of the 2 populations would be a guarantee that the host plants are subservient by the same haplotypes or genetically very close haplotypes. No genetic study of this insect on its 2 host plants has been done, either in Senegal or in Africa in the broad sense of the term. Nevertheless, a study of the genetic distribution of an insect named Caryedon serratus according to 5 host plants (Arachis hypogaea, Bauhinia rufescens, Cassia sieberiana, Piliostigma reticulatum) was carried out. She revealed besides a genetic structuring of the insect according to these 5 host plants [11].

The existence of an ancestral and majority haplotype (H2) shared between the host plants accredits this sharing of genes between millet and maize. Farmers tend to farm millet and maize in the same fields or in neighboring fields. This cultural practice favors the infestation of host plants by the same insects. This infestation is favored by the use of the same storage means. Indeed, the transfer of grain from one AEZ to another can be accompanied by the transfer of larvae, cocoons or even adults able to flourish in an AEZ that is not previously infested [15]. The high Hd and low Pi values of the 2 populations may be the result of rapid growth in their small ancestral populations for which there was not enough time for high diversity between haplotypes. Exchange of individuals between host plants has not impacted the strong haplotypic and low nucleotide diversity of the global population, as it is the same insects that mingle with each other. In some agro-ecological zones such as SBA and SOHC, genetic convergence between millet and maize populations is not verified. The millet populations of these localities are individually formed from the same haplotypes. This situation is the result of sampling. Indeed, it was carried out at the end of the dry season, a lean period during which the need to satisfy family food needs considerably reduces the availability of cereals, not to mention the fall in cereal harvests in recent years because of a deficit rainfall. The corollary is therefore a reduction in the number of haplotypes in these areas. The decrease in the genetic diversity, especially nucleotidic populations of some agroecological zones and the global population is an asset in the fight against this pest of millet and maize, because it can lead to the loss of potential adaptations of the insect to environmental changes.

4. Conclusion

At the end of this study of the genetic diversity of Sitophilus zeamais, which concerned 53 individuals of insects subservient to the host plant millet and 72 subservient to the host plant maize, in 5 agroecological zones of Senegal, we have found that global and local populations of maize and millet have approximately the same genetic characteristics. Therefore these plants have the same influence on the adaptive potentials of the insect. Since no host plant has more genetic influence than the other, other studies can focus on grain characteristics, or the influence of the agroecological zones, to try to explain their differential susceptibility to Sitophilus Zeamais.

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