International Journal of Advanced Research and Review

www.ijarr.in

GENETIC STRUCTURATION OF SITOPHILUS ZEAMAIS ACCORDING TO 2 HOST PLANTS (MILLET AND MAIZE) IN SENEGAL (WEST AFRICA)

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ABSTRACT

Cereals play a very important economic and social function. However, they are deteriorated by several insect pests including Sitophilus Zeamais, a beetle of the Curculionidae, subservient to 2 host plants, millet and Maize. The genetic studies carried out so far have focused on the genetic variability of Sitophilus Zeamais and its genetic structuring according to agroecological and agro-climatic zones.

This article aims to highlight a possible genetic structuring of Sitophilus Zeamais according to 2 host plants, millet and maize.

The importance of highlighting a genetic differentiation of the insect according to these 2 host plants is to be able to apprehend after the adaptability of the insect, from the effect of each host plant on the genetic diversity of the plant population of the insect.

To achieve this goal, insects were harvested from each agro-ecological zone specifically on maize and on millet.

The exploitation of 125 sequences of the Cytochrome B gene revealed the lack of genetic structuring of Sitophilus Zeamais according to the two host plants, millet and maize. So, these are the same insects that roam between them.

KEYWORDS: Cytochrome B, Sitophilus Zeamais, millet, maize, genetic structuring.

I. INTRODUCTION

The fight against poverty is a major challenge in developing countries. In Senegal, one of the strategies to meet this challenge is the promotion of agricultural development. So many cereals are substantially exploited across the country. Millet and maize especially occupy an important place with respectively 40% and 14% of total cereal production. However, their stocks are singularly deteriorated by a beetle of the Curculionidae, known under the scientific name of Sitophilus Zeamais. According to Ngamo and Hance (2007), post-harvest damage can range from 25% to 40% in 6 months of storage. The preferred solution to eliminate this pest has been the use of pesticides. In the genetic domain, research focuses mainly on its variability and structuring according to the climatic and edaphic characteristics of the zones.

This article aims at highlighting a possible genetic structuring of Sitophilus Zeamais according to 2 host plants, millet and maize.

The advantage of highlighting such genetic differentiation is to be able to know after the genetic diversity of each host plant population and consequently its adaptive capacity.

To achieve this goal, insects of Sitophilus Zeamais, on the one hand subservient to millet and on the other hand subservient to maize were sampled in each agroecological zone.

The 125 sequences corresponding to the totality of the individuals were exploited by software of study in population genetics (Bioedit, DNAsp, Mega, Harlequin ...), compared to parameters of genetic structuring (genetic distance, Fst ...), in relationship with our goal.

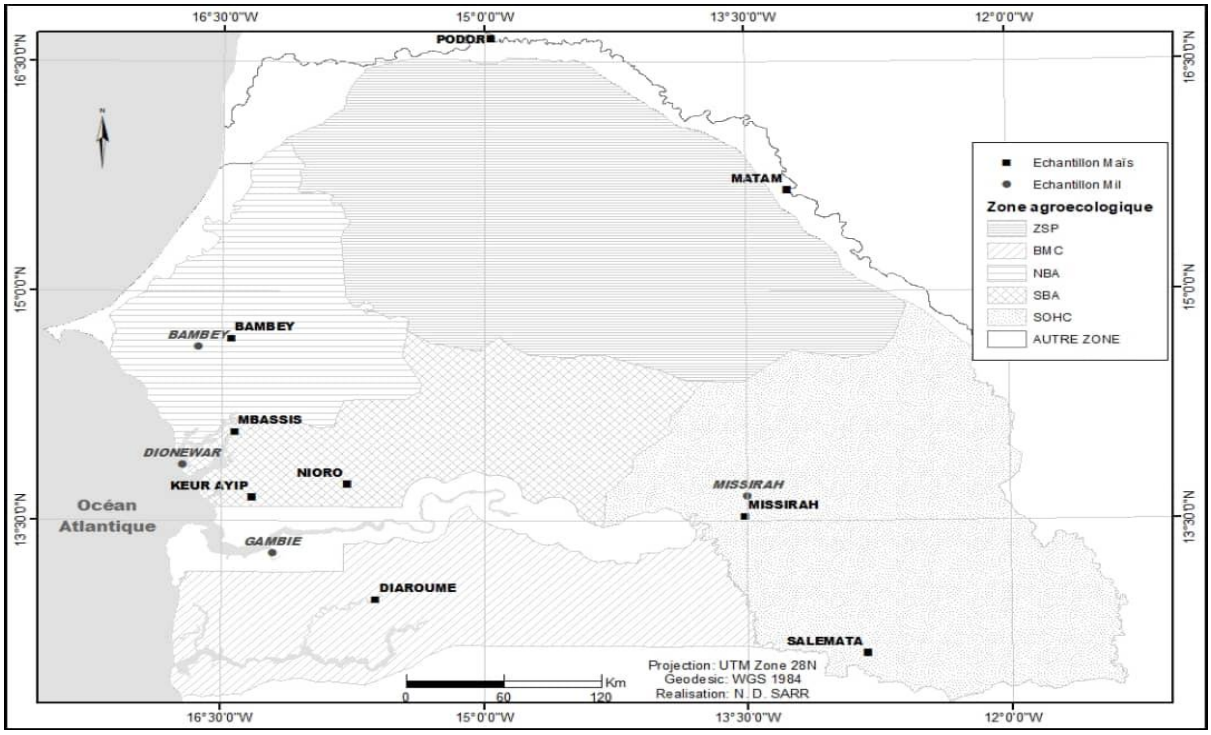
II. MATERIAL AND METHODS

II.1. Sampling

II.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 [1] represented by the only locality of Bambey (14 ° 42'00"Nord / 16 ° 27'00"Ouest), the AEZ of the SBA[2] 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[3] 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[4] 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 locations



II.1.2. Harvesting individuals

[1] Nord Bassin Arachidier
[2] Sud Bassin Arachidier
[3] Sénégal Oriental Haute Casamance
[4] Basse Moyenne Casamance

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
Salémata	10	12°37’60’’N/12°49’00’’W	SMaSa
BMC	20		
Gambie	10	13°27’09’’N/16°34’40’’W	SMiGa
Diaroumé	10	13°03’19’’N/15°38’34’’W	SMaDi
TOTAL	125		

II.2. Molecular method of analysis

II.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.

II.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.

II.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 25 µl, of which a mixed volume of 23 µl and a volume of 2 µl of DNA extract. The mixed volume was constituted by: 18.3 µl of milli water, 2.5 µl of 10 × buffer, 1 µl of additional MgCl 2, 0.5 µl of Dntp, 0.25 µl of each primer and 0.2 µl of Taq polymerase.

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

-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.

-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.

II.2.4. Bioinformatics Analyzes

The sequences were corrected and aligned by the Clustal software implemented in the Bioedit version 7.2.5 program (Hall, 1999).

The genetic structuring of Sitophilus Zeamais according to the host plants was apprehended with respect to genetic differentiation parameters. It's about the genetic distance, the Fst, the Gst and the Amova Test. The genetic distance between Agroecological zones was calculated by the Mega 7 software version 7.0.14 (Tamura et al, 2016), the global Fst and Gst indices by the DNAsp software while the Fst values between populations were calculated by the Arlequin software version 3.5.1.3 (Excoffier and licher, 2010). The indices Fst and Gst are assumed to be similar but they make it possible to check the coherence of the results. The AMOVA test, on the other hand, made it possible to know the part of the variations between host plants in the genetic structuring of the insect.

From the mitochondrial gene Cyt.B, the Network software (Bandel et al., 1999) made it possible to construct the haplotype network according to the maximum parsimony method. The importance of the construction of this network is to check if the possible genetic structuring has appeared there

III. Results and discussion

III.1. Results

III.1.1. Spatial structuring of sequences

III.1.1.1. Genetic distances (GD)

Genetic distances within host plants are low and are quite similar (Table 21): Insects subservient to millet are genetically divergent to the order of 13% and those subservient to maize to the order of 18%.

Table 2: Genetic distance (in black) within Sitophilus zeamais populations dependent on maize and millet and standard errors (in blue) calculated from the MEGA software for Cyt. B.

Table 2: Genetic distance (in black) within Sitophilus zeamais populations subservient to maize and to millet and standard errors (in blue) calculated from the MEGA software for Cyt. B.

Host Plants	GD	SE
Maize	0,018	0,004
Millet	0,013	0,003

Compared with genetic distances between populations encountered in our dataset, up to 0.053, for an average distance of 0.030, the DG between the overall population of maize and millet is low (GD = 0.020) (Table 3).

Table 3: Genetic distance (below the diagonal) obtained by comparing the two Populations of Sitophilus zeamais subservient to maize and millet and the standard errors (above the diagonal) calculated from MEGA software for Cyt. B.

Host Plants	Maize	Millet
Maize	__	0,004
Millet	0,020	__

III.1.1.2. Genetic differentiation

The fixation index (Fst) between the global populations of millet and maize is equal to 0.19 (pV = 0.140) 9 (Table 4).

Table 4: Genetic differentiation value (Fst) between global maize and millet populations from the ARLEQUIN software. The value is not significant.

Host Plants	Maize	Millet
Maize	__	
Millet	0.19648	__

If we compare the value of this index with the significant values of Fst very high overall, between populations of millet and maize, of the different localities where the samplings were made, ranging from 0.12 to 0.98, the Fst between the population of Sitophilus Zeamais subservient on the one hand to millet and on the other hand to maize is not only insignificant but weak.

III.1.1.3. The AMOVA Test

According to the AMOVA test (Table 5), there is no genetic structuring of Sitophilus zeamais according to the 2 host plants. The index of fixation between the global populations of millet and maize was low and not significant (FCT = 0.106 PV = 0.107). On the other hand, there is a strong genetic differentiation between local populations of host plants. This genetic variation is corroborated by a high and significant Fst (FSC = 0.523, PV = 0.000) and is symbolized by a percentage variation of 46.77%. Within populations, individuals are also genetically distinct if we refer to the value of Fst high and significant (Fst = 0.574 PV = 0.000).

Source of variation	Fixation Index	D.f.	Sum of Squares	Components of Variance	Percentage of variation
Between host plants	0.10630	1	46.813	0.44398 Va	10.63
Between populations within host plants	0.52339	9	198.602	1.95361 Vb	46.77
Inside populations	0.57405	104	185.020	1.77903 Vc	42.59

Table 5: Molecular variance test (AMOVA) between host plant populations and between local populations of millet and maize (only gray values are significant).

III.1.1.4. Haplotype network

The haplotype network (Figure 2) shows a star structure with 2 major haplotypes H2 and H17 from which other haplotypes derive. Among the majority haplotypes, only the haplotype H2 is subservient to both millet and maize. The other haplotype H17 is specific to maize. Of the 28 haplotypes identified at the host plant level, 18 are maize-specific (H1, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, H18, H19, H20, H21). 7 are subservient to millet (H22, H23, H24, H25, H26, H27, H28). While 3 haplotypes (H2, H3, H4) are shared between millet and corn. There is no obvious spatial pattern appearing in the haplotype network since the majority haplotype H2 is present at both host plants.

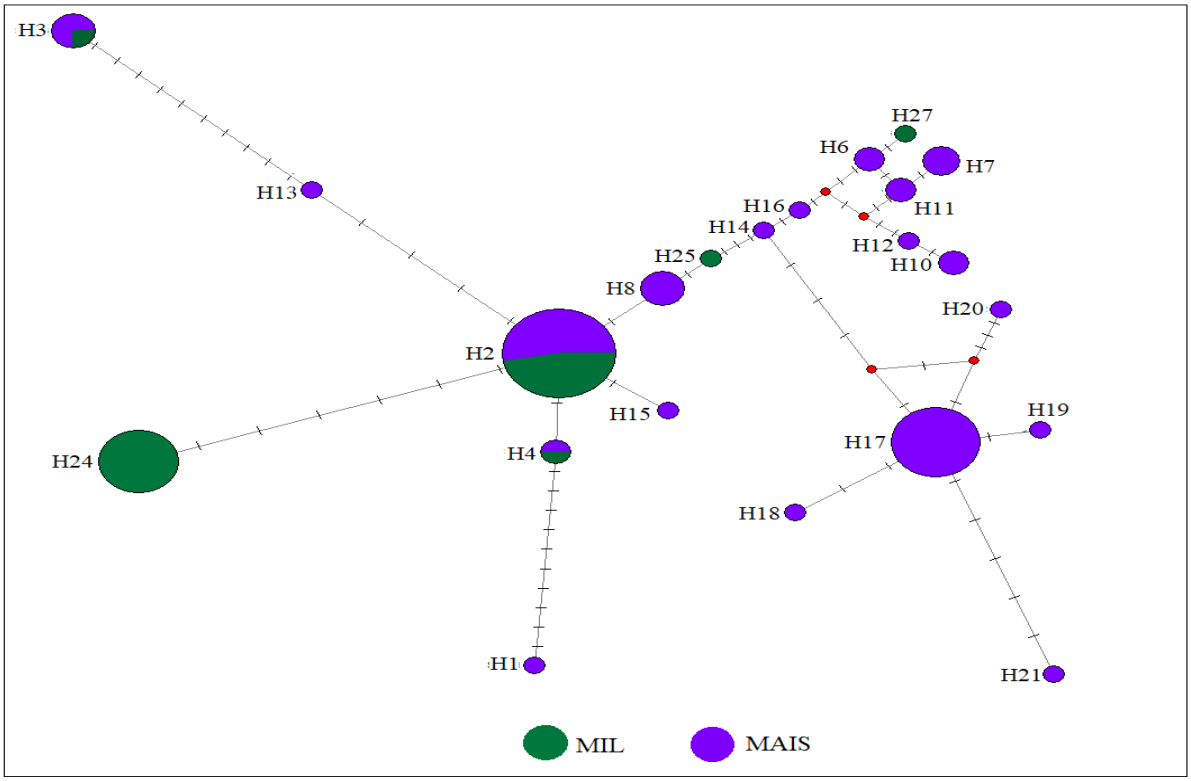


Figure 2: Haplotype network of Sitophilus Zeamais subservient to the host plants, Millet and maize. Each disc corresponds to a haplotype, and their size is proportional to

the number of individuals corresponding to the haplotype. The traits correspond to mutational steps between haplotypes.

III.2. DISCUSSION

Genetic distances within the global millet and maize populations are 0.013 and 0.018, respectively. These low values attest to an individual genetic homogeneity of these 2 populations. They are as convergent with each other, if we refer to the low value of the genetic distance between maize and millet populations (DG = 0.02).

The non-significant attachment index also validates the genetic similarity between millet-dependent insects and maize-specific insects. The lack of genetic differentiation between private haplotypes of millet and maize is finally corroborated by the AMOVA Test. Based on the value of Fst, which is low and non-significant (Fst = 0.016, PV = 0.107), there is no genetic structuring of Sitophilus Zeamais individuals according to the two host plants (maize and millet). On the other hand, the Fst between local populations of millet and maize is high and significant. Thus, these local populations are genetically differentiated, certainly by the specific climatic effects of the zones.

The lack of genetic differentiation between the Sitophilus Zeamais population infesting millet and that infesting maize has an essentially agricultural explanation. In Senegal, especially in the countryside, where agriculture is the main food and commercial activity, the fields that house the farms are very close. This proximity promotes a very easy movement of the same insects between the host plants. The consequence is necessarily a genetic homogenization of the global population of Sitophilus Zeamais.

But the genetic convergence of the global population can be an asset to the protection of cereals. In the context of calling into question the chemical means of fight against insect pests by their harmful effects on living beings and the environment, it can constitute a natural and healthy remedy offer. Because the low genetic diversity can have a negative impact on insects by a low adaptability to extinction factors. Hoffmann et al (2003) reported that a reduction in the genetic diversity of a population leads to a loss of its potential for adaptation to sudden climate change. Homogeneous populations are not only vulnerable to natural selection or genetic drift, but are also easier to find effective codified pesticides.

The genetic differentiation that exists between the local populations of millet and maize confirms the genetic structuring of Sitophilus Zeamais according to the agroecological zones demonstrated in the previous parts.

CONCLUSION

According to the study that we conducted on 125 individuals of Sitophilus Zeamais concerning its distribution as a function of 2 of its host plants, there is no genetic structuring of the insect according to the host plants millet and maize. Studies can be conducted to determine the degree of genetic homogeneity of each host plant population, to determine whether their genetic diversity is likely to affect the adaptability of the insect.

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