



Genetic Variability of *Sitophilus zeamais* Subservient to Millet in Senegal (West Africa)

Ngagne Demba SARR*, Toffène DIOME, Déthié NGOM and Mbacke Sembene

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

*Corresponding Author E-mail: ngagnedembasarr@gmail.com

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ABSTRACT

The millet is a plant very adapted to arid climatic conditions. Thus, it is exploited in many agroecological zones (AEZ) of Senegal where the Sahelian to Sahelo-Sudanese climate prevails. After rice, this cereal is the most consumed by Senegalese households. In addition to this vital function, millet plays an important role in the feeding of livestock and poultry. However, it is constantly under attack by pests like Sitophilus Zeamais, a beetle of the Curculionidae.

To overcome the enormous damage caused by the insect, without resorting to chemicals products whose use regularly harms living beings and the environment, we tried through this study to highlight the agroecological zones hostile or naturally favorable to the survival of S. Zeamais, by the genetic effects they present on its genome.

In fact, populations with low genetic diversity have fewer adaptive potentials than those that are genetically heterogeneous.

Thus, we sampled 43 insects of S. Zeamais subservient to millet on stocks infested in 4 agroecological zones that are: NBA¹, SBA¹, SOHC¹ and BMC¹.

The exploitation of the Cytochrome B gene for sequences corresponding to these individuals has revealed a very high genetic homogeneity of SBA and SOHC insects, and a high genetic diversity of the NBA and BMC populations. Thus, the agroecological zones of the SBA and the SOHC, that are less exposed to genetic drift and natural selection, are more hostile to insect survival than NBA and BMC ones, that genetic heterogeneity caused by Genetic fluctuations contributed to the survival of S. Zeamais.

Keywords: *Cytochrome B, Agroecological zone, Millet, Sitophilus Zeamais, Genetic diversity.*

INTRODUCTION

Millet occupies a substantial place in Senegal's agricultural production. Of all the cereals, it is the most cultivated. A study of the rural economy management centers of the Senegal River Valley (REMC), for the 2012 crop year

revealed that for a production of 1669960 tons of cereals, millet ranks first. The predisposition of millet to evolve in cultivation areas more correctly than other crops without major constraints of growth have both physiological and geographical reasons:

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millet tolerates the lack of water, it also adapts to regions with low fertility soils and high temperatures. These intrinsic characteristics explain the adaptation of millet to the agroecological zones of the NBA, SBA, SOHC and BMC where it is exploited massively. With yields increasing year by year, millet is poised to become an alternative to access to food sovereignty and security. But the very high losses of millet stocks, by insects including *S. zeamais*, may undermine this perspective.

So far genetic studies on this insect have traced its phylogeny and geographical distribution in Africa in general. Senegal has never been specifically studied in this area. Our article aims to propose possible solutions to drastically reduce these losses by identifying genetically homogeneous or heterogeneous agroecological zones.

Indeed, agroecological zones that cause low genetic diversity of insect populations reduce their adaptability while those that promote their genetic heterogeneity confers strong adaptive potential.

To achieve our goal, we harvested populations of insects subservient to millet

from stocks of this cereal in each agroecological zone.

The cytochrome B gene of these sequences has been exploited by genetic population study software (DNAsp, Mega, Harlequin ...) in relation to genetic variability parameters, related to our objective.

MATERIALS AND METHODS

II.1. Sampling

II.1.1. Sampling localities

S. zeamais individuals were sampled in four agroecological zones (AEZ) of Senegal. The choice given to these areas is justified by their vocation naturally agricultural and by ecological and geographical characteristics which specify each of them. This is the AEZ of the North Peanut Basin (NBA) represented by the only locality of Bambey ($14^{\circ} 42'00''$ North / $16^{\circ} 27'00''$ West), from the SBA AEZ at Dionewar ($13^{\circ} 52'60''$ North / $16^{\circ} 43'60''$ West). Samples were also taken from the SOHC ZEA at Missirah ($13^{\circ} 41' 00''$ North / $16^{\circ} 30' 01''$ West) and from the BMC AEZ in The Gambia ($13^{\circ}27' 09''$ North / $16^{\circ} 34'40''$ Ouest)). Figure 1 summarizes the study sites in black.

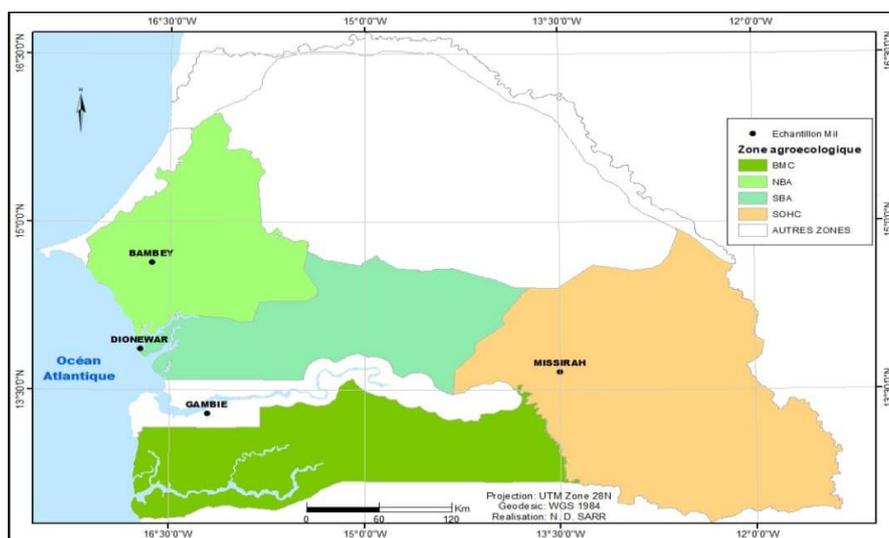


Fig. 1: Sampling locations (in black)

II.1.2. Harvesting individuals

The collection of infested Millet samples in the different AEZs made it possible to isolate individuals of *S. zeamais* for each zone. It has been done in the fields, in storage facilities

where grain is highly vulnerable to infestation, but also in marketing places where there is a high chance of encountering infested millet from different AEZs. After isolation,

individuals from each AEZ are placed in tubes containing 96% alcohol.

To code individuals for their host plant, we capitalized the first letter of the insect's genus name and then 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 (the first letter in capital letters and the second in lowercase), then specify the serial number. Example a

Sitophilus zeamais individual who was harvested in Bambey on Mil with the order number 12 is coded as: SMiBa12. if it was on corn from 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	11		
Bambey	11	14°42'00''N/16°27'00'' W	SMiBa
SBA	09		
Dionewar	09	13°35'00''N/15°36'00''W	SMiDio
SOHC	13		
Missirah	13	13°41'00''N/16°30'01''W	SMiMi
BMC	10		
Gambie	10	13°03'19''N/15°38'34''W	SMiGa
SUM	43		

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 MgCl₂, 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 Bioédit version 7.2.5 program. The evaluation of the sequence diversity was made from certain parameters that the DNAsp software made it possible to calculate. These are, on the one hand, standard indices of genetic variability, such as variable sites, information on parsimony and the number of haplotypes and, on the other hand, Haplotypic (Hd) and nucleotide (Pi) diversity. These two indices

have the distinction of highlighting the diversity and divergence of haplotypes. From the mitochondrial gene Cyt.B, the Network software made it possible to construct the haplotype network according to the maximum parsimony method.

RESULTS AND DISCUSSION

III.1. Results

III.1.1. Genetic variability of sequences

43 individuals of *S. Zeamais* were specifically harvested from millet, including 11 in NBA, 9 in SBA, 13 in SOHC and 10 in BMC. The corresponding sequences, 410 bp in size, have 34 variable sites, 22 singleton and 12 parsimonious and 376 invariable sites.

The populations of SBA and SOHC AEZs present sites, all monomorphic. Then the NBA and BMC AEZs have relatively similar monomorphic and polymorphic sites considered in pairs for each AEZ. (Table 2).

Table 2: Genetic diversity of the *S. zeamais* cytochrome b gene sequences subservient to the Mil in different AEZs in Senegal

Agro-Ecological Zones	Number of individuals	Number of haplotypes	Number of sites	Invariables Sites	Variables Sites	
					Singleton	Parcimony Informative
NBA (Bambey)	11	05	410	393	17	00
SBA (Dionewar)	09	01	410	410	00	00
SOHC(Missirah)	13	01	410	410	00	00
BMC (Gambie)	10	04	410	400	07	03
TOTAL	43	10	410	376	22	12

The 43 individuals are divided into 10 haplotypes. The haplotypic diversity of the global population is very high (0.813 ± 0.031) while the nucleotide is very small (0.001 ± 0.001). (Figure 2).

NBA and BMC AEZs have the highest Hd values while SBA and SOHC are null. The nucleotide diversity of AEZs are similar to those of Hd.

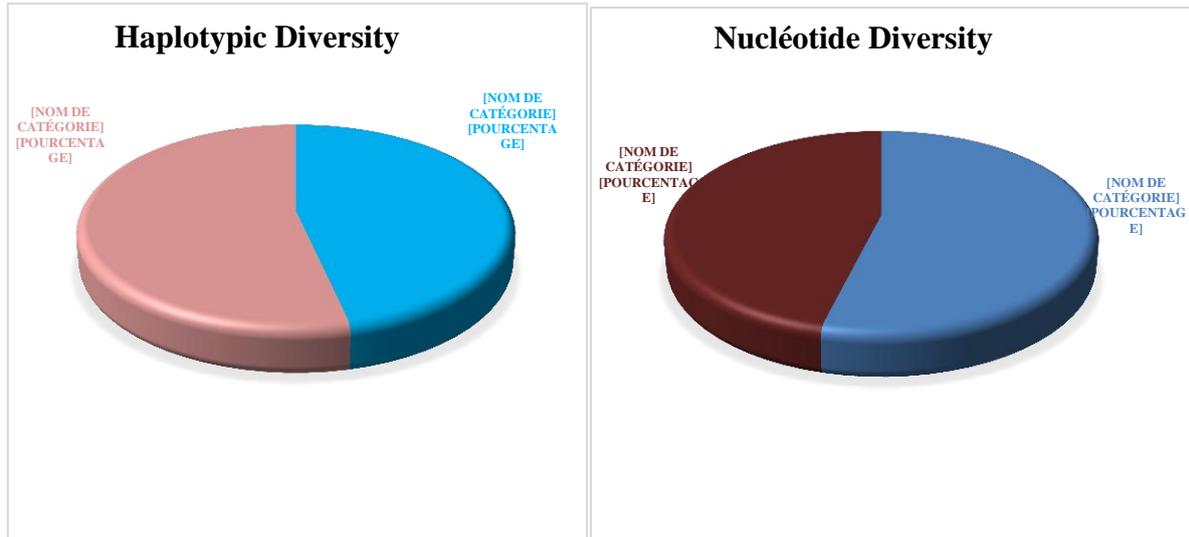


Fig. 2: Haplotype (a) nucleotide diversity (b) of *S. zeamais* subservient to millet

Figure 3 shows the distribution of millet haplotypes in the different agroecological zones. The BMC houses 3 private haplotypes.

These are haplotypes H7, H8 and H9. H3 and H6 haplotypes are specific respectively to NBA and SOHC.

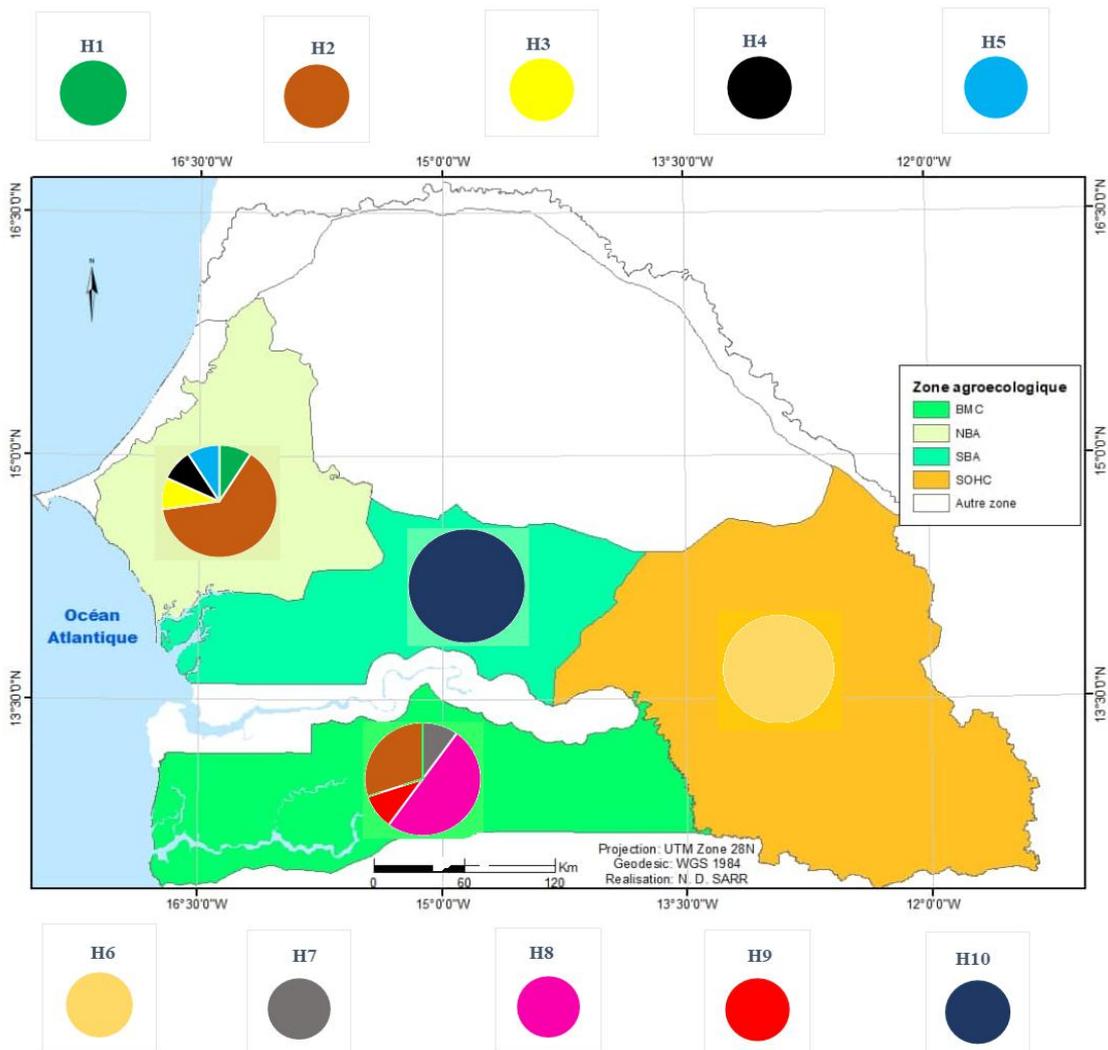


Fig. 3 : Distribution of millet haplotypes in different agroecological zones of Senegal.

III.1.2. Haplotype network

The haplotype network (Figure 4) reveals a star structure with two majority haplotypes H2 and H6 representing respectively 51% and 30% of the dataset. The majority and ancestral haplotype H2 is present in all agroecological zones, except that of BMC. Haplotypes of BMC (H8 and H7), SOHC (H6) and NBA

(H3) are derived from H2. But the BMC haplotypes are phylogenetically closer to H2 than the other haplotypes of the other AEZs, insofar as they diverge from it, at most 3 mutational steps, against 7 for the SOHC haplotype and 15 for the SOHC haplotype. NBA.

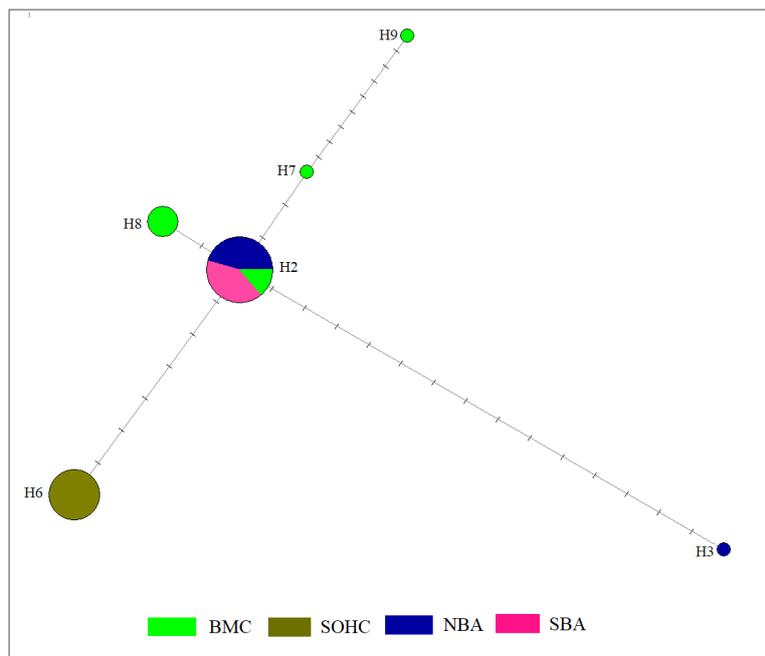


Fig. 4: Haplotype network of *S. Zeamais* subservient to the host plant, Millet. Each disc corresponds to a haplotype, and their size is proportional to the number of individuals corresponding to the haplotype. The traits correspond to mutational steps between haplotypes

DISCUSSION

Of the 4 agroecological zones studied, only the NBA and the BMC have a high haplotypic diversity and a low nucleotide diversity. The populations of the 2 other AEZs namely SBA and SOHC are homozygous.

The NBA is highly agricultural. The favorable edaphic and climatic predispositions that it enjoys have also given it the status of groundnut basin. Thus, it is the seat of important exchanges of cereals, which would be at the origin of the high haplotypic diversity found there. The Republic of The Gambia, a locality of the BMC where an insect harvest has been carried out, is also full of attractive agricultural potential. In addition, it is a crossroads area where farmers from Senegal, from Gambia and Guinea meet regularly for trade in cereals and seeds. Its geographical

position and its agricultural advantages can thus explain its high haplotypic diversity.

The SBA and to a lesser extent the SOHC, like the NBA and the BMC, have agricultural assets. The fact that they do not have the same genetic diversity as the latter, can be explained by sampling. The collection of insects at the SBA and SOHC was done at the end of the dry season, during which the priority of honoring the food demand of the families greatly reduces the stocks of cereals. This coincidence justifies the total absence of genetic diversity in these areas, symbolized by the presence of a single haplotype in each of them.

The high genetic diversity of the NBA and the BMC can be a source of adaptation of the insects of this locality. These areas are then favorable to the survival of the insect. On the

other hand, the agroecological zones of the SBA and the SOHC which are very homogenous genetically are likely to a bottleneck, thus, they are unfavorable to the survival of the insect.

The global population of millet like local populations is characterized by high haplotypic diversity and low nucleotide diversity. The migration of insects, the exchange of cereals between producers and traders can be at the origin of the genetic similarity between haplotypes subservient to millet. But this genetic homogenization is without prejudice in the medium or short term to the adaptability of the corresponding population. For, to believe Luicart and Cornuet, a drastic loss of the size of a population accompanied by a reduction of gene exchanges generally leads to an increase in the overall consanguinity of the population, often reducing the phenotypic aptitude of the offspring.

The haplotype network and the geographical map of their distribution confirm the high haplotypic diversity of BMC and SBA with the presence of several specific haplotypes and the weak one of NBA (only one private haplotype) and SOHC (only one private haplotype).

CONCLUSION

At the end of this study of the genetic diversity of *S. zeamais*, which concerned 43 individuals of insects subservient to the host plant millet in 4 agroecological zones of Senegal, we found that the agroecological zones of NBA and BMC, by promoting a genetic heterogeneity of insects are favorable to their survival. Whereas those of the NBA and the SOHC constrain their development insofar as they provoke their genetic homogeneity.

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