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RESEARCH ARTICLE

DEMOGRAPHIC AND PHYLOGENETIC EVOLUTION OF SITOPHILUS ZEAMAI SUBSERVIENT TO MILLET IN SENEGAL (WEST AFRICA).

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Abstract

Food security remains a major challenge in Senegal. The millet that occupies the most important place in cereal production can play a fundamental role in taking up this challenge.

But it is strongly ravaged by a beetle of the Curculionidae, *Sitophilus Zeamais*, in 4 agroecological zones (AEZ) where it is substantially exploited. The solution required to eliminate this pest is the use of pesticides, despite the horrible consequences that accompany it. It is possible to find healthy solutions.

This article aims to identify the type of selection (positive or negative) that specifies each population and the degree of kinship of their individuals.

The importance of detecting the demographic signal of AEZ populations is to be able to identify agroecological zones that are likely to favor survival or extinction of the insect. Positive selection militates more in favor of the adaptation of individuals than negative selection.

Thus, insects of *S. Zeamais* subservient to millet were sampled in each AEZ.

The exploitation of Cyt.B gene sequences led to the conclusion that the NBA population has undergone a positive selection, while the BMC population was negatively selected. SBA and SOHC populations have not been selected. Thus, the agroecological zone of the NBA is likely to favor the survival of the insect whereas the AEZ of the BMC is likely to favor a bottleneck. Phylogenetic trees have highlighted a close relationship of individuals in each AEZ.

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Introduction:-

Millet is the staple food of rural Senegalese people. It is grown throughout the national territory. Because of the climatic and edaphic specificities of the zones, this cereal is exploited more in the agroecological zones (AEZ) of NBA¹, SBA², SOHC³ and BMC⁴. However, like many other cereals, stocks of millet are deteriorated particularly by *Sitophilus Zeamais*, a beetle of the Curculionidae. The elimination policy of this insect is based fundamentally on

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the use of pesticides, chemicals whose use has a negative impact on living beings and the environment. Otherwise, genetic studies on *S. Zeamais* so far have focused on its phylogeny and its distribution in Africa in general. No study on the genetic effect of the environment and its consequences on the insect targeting the specific case of Senegal has been made.

To avoid the enormous losses caused by the insect, natural solutions can be studied. This article fits into this perspective. It aims on the one hand to identify the agroecological zones naturally likely to favor the survival or the extinction of *S. Zeamais*, by the detection of the demographic signal which characterizes each one of them and on the other hand the degree of kinship of their populations. Indeed, agroecological zones where genetic drift and natural selection exert a positive selection are likely to favor the survival of the insect in contrast to those undergoing negative selection.

Insects of *S. Zeamais* were sampled for this purpose in each of the four AEZs.

The 43 sequences of the Cytochrome B gene corresponding to these individuals were exploited by population genetics software (Bioédit, DNAsp, Mega, Harlequin ...), in relation to demographic and phylogenetic parameters, in relation to the aforementioned objectives.

Material and methods:-

Sampling

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 Bambe (14 ° 42'00"North / 16 ° 27'00"West), from the SBA AEZ at Dionewar (13 ° 52'60 " North / 16 ° 43'60 " West). Samples were also taken from the SOHC ZEA at Missirah (13 ° 41' 00 " North / 16 ° 30' 01 " West) and from the BMC AEZ in The Gambia (13°27' 09" North / 16 ° 34'40"Ouest)). Figure 1 summarizes the study sites in black.

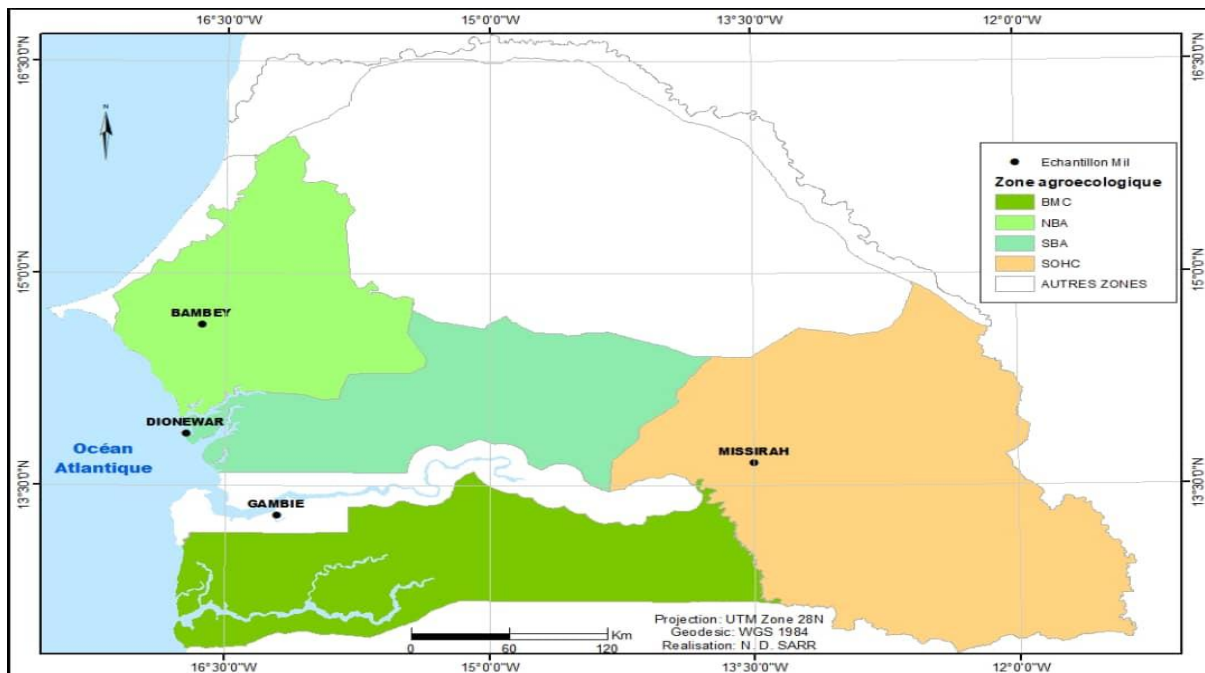


Figure 1:-Sampling locations (in black)

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 Bambej on Mil with the order number 12 is coded as: SMiBa12. if it was on corn from maize, the code would be SMAba12.

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		

Table 1:-Sampling locations

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.

Molecular method of analysis

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.

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.

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:

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.

Bioinformatics Analyzes

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

The demographic history of the populations sampled in the different agroecological zones was apprehended from a "mismatch distribution" analysis of the populations, correlated with the evaluation of the demographic tests of D of tajima, of D^* and F^* of Fu and Li (Fu and Li, 1999), Fu's Fu (Fu, 1997), of Ramos' R_2 and of Fay's and Wu's H. This analysis is accredited by the demographic indices SSD (sums of squares deviations) and RAG, calculated between distributions observed and expected by the software Arlequin 3.5.13 (Excoffier and Lischer, 2010). The values of D of tajima, of Fs of Fu and D^* and F^* of Fu and Li were calculated by software Harlequin 3.5.13. While those of R_2 Ramos and of H Fay and Wu were calculated by DNAsp software.

The phylogenetic reconstruction clarifies existing kinship relationships between haplotypes identified in different agroecological zones. Thus, in our study, we constructed 2 phylogenetic trees, one using maximum parsimony (MP) and the other with maximum likelihood (MC), using Mega version 7.0.14 software (Tamura et al, 2016) and Mr Bayes version 3.12 (2007). The comparison of these 2 trees made it possible to verify the coherence of the interpretation of the phylogeny of the populations.

Results and discussion:-

Results

Demographic history

Neutrality tests

NBA and BMC populations have a significant negative D of Tajima. The corresponding Fu's Fs are positive and insignificant. The D of Tajima and the Fs of Fu of the other 2 populations (SBA and SOHC) are null. (Table 2) The global population has a Tajima D, a positive Fs of Fu, all insignificant.

	AEZ	NBA	SBA	SOHC	BMC	Globale Population
	Tajima's D	-2,0934	0,0000	0,0000	-1,0538	-0,0457
	Fu's Fs	0,63237	0,0000	0,0000	1,02765	0,3466
DEMOGRAPHIC PARAMETERS	Ramos's R_2	0,16198	0,1599	0,1640	0,16058	0,1617
	Fay and Wu's H	-0,0242	0,0000	0,0000	0,15420	
	SSD	0,03346	0,0000	0,0000	0,05915	
	Rag	0,13455	0,0000	0,0000	0,14796	0,0900
	Hd	0,618± 0,16	0,0000	0,0000	0,711± 0,11	
	Pi	0,007 ±0,004	0,0000	0,0000	0,006 ±0,003	

Table 2:-Neutrality indices of Sitophilus Zeamais populations subservient to millet in the different AEZs (significant values in gray).

Mismatch distribution

Populations of NBA and BMC AEZs exhibit a multimodal distribution. The corresponding SSD and RAG values are positive and insignificant. The other ZAEs have not undergone any change.

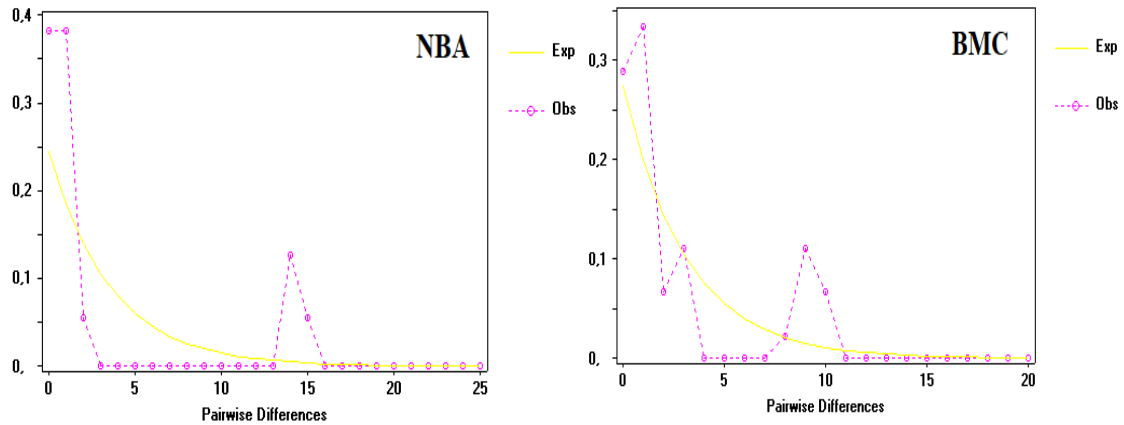


Figure 2:-Mismatch distribution of NBA and BMC Millet populations.

Phylogenetic trees

The phylogenetic trees in Maximum Likelihood and Neighbor Joining of individuals infesting millet reveal 4 clades sustained respectively by posterior probabilities of 33% (clade 1), 62% (clade 2), 99% (clade 3) and 94% (clade 4). But only 3 strongly supported clades are genetically homogeneous : it is the clade 4 regrouping all the individuals of the SBA, the clade 3 regrouping those of SOHC and the clade 2 formed only of individuals of the BMC corresponding to haplotypes H7, H8 and H9. The Clade 1, which is weakly supported by a 33% probability of rearing, is made up of individuals from different agroecological zones.

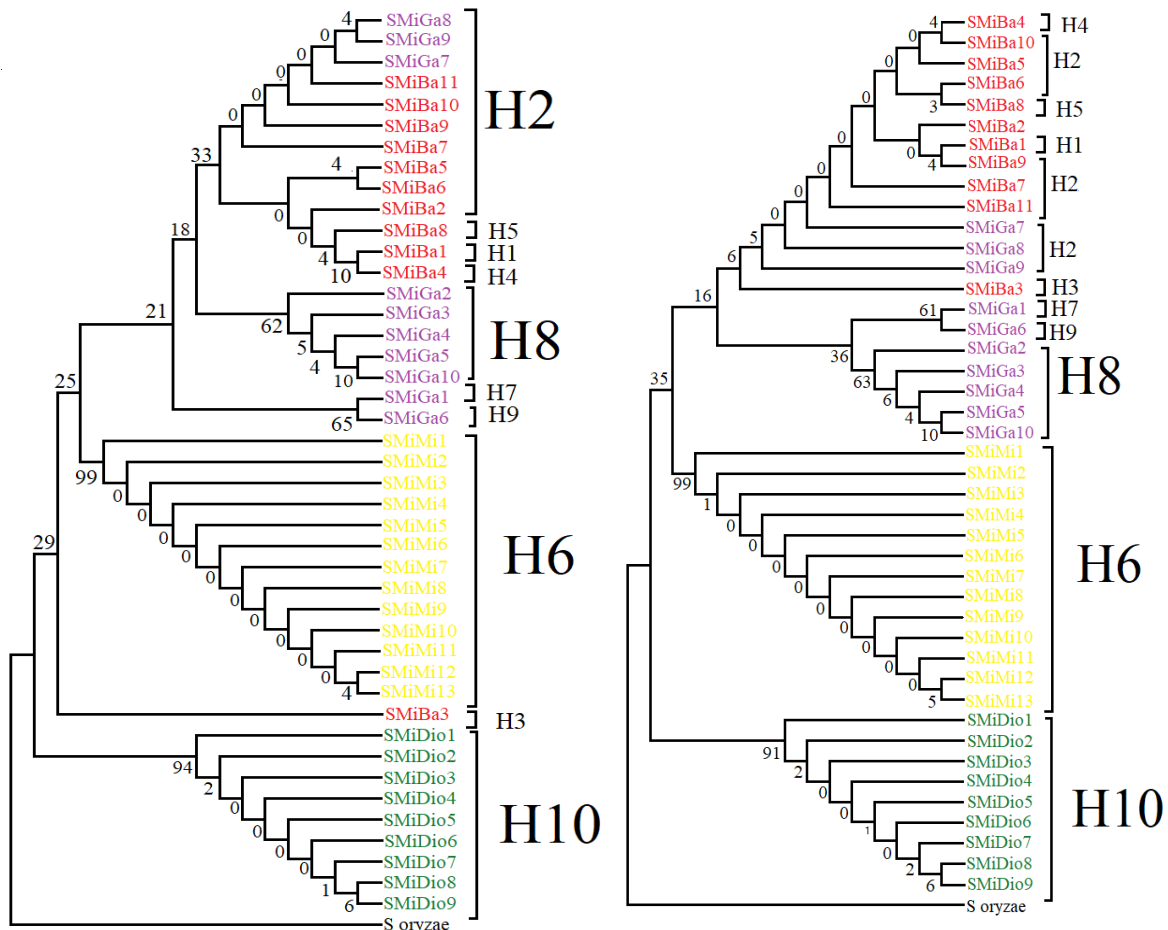


Figure 3:-Phylogenetic trees : Maximum likelihood method (A), Neighbor Joining method (B)

Discussion:-

The NBA and BMC populations are characterized by negative and significant Tajima's D and positive and insignificant F_s of F_u . They are therefore in demographic expansion. This type of evolution is accredited by the non-significance of the multimodal curves of the haplotypes of the 2 AEZs. On the other hand, the high haplotypic and low nucleotide diversity indicate that they are in early demographic expansion. Thus, their respective haplotypes are genetically very close. Based on the negative H values of Fay and Wu, the NBA population was positively selected, unlike the BMC which had a purifying selection, based on its positive H-value. Fay and Wu.

On the other hand, the values of the demographic tests of the SBA and SOHC AEZs are null. The homogeneity of these 2 populations would be the result of a severe bottleneck having caused the disappearance of almost all the haplotypes.

The demographic expansion of millet-specific individuals from NBA and BMC AEZs, symbolized by significantly negative Tajima's D values, may be the expression of the successive advent of 2 events. These populations could be the victim of a bottleneck following a secular episode of drought as that of the years 1903 (Jacques SIRCOULOU, 1976), which would have considerably reduced the population of insects. Then, in favor of the appearance of new better climatic conditions, the individual populations of the NBA and the BMC can know a beginning of blooming. Rainfall trends favorable for decades (Jean LEBORGNE, 1988) comfort this idea. According to the negative value of H of Fay and Wu, the demographic expansion of the NBA population is the result of a positive selection. This is the opposite of the BMC population whose population expansion results from a purifying selection if one sticks to the positive value of H of Fay and Wu. The null values of D of Tajima of populations SBA and SOHC indicate that these populations have not evolved.

Thus, the NBA AEZ with positive selection favors the survival of the insect while that of the BMC which has been negatively selected is conducive to a bottleneck. The other 2 AEZs, namely the SBA and the SOHC, whose populations are characterized by Tajima's D and H values of Fay and Wu null, have no effect on the adaptability of the insect.

The phylogenetic trees (Maximum Likelihood and Neighbor Joining) revealed 3 clades: 2 clades strongly supported by posterior probabilities superior to 90%, formed individually of insects subservient to SBA and SOHC and another weakly supported clade (16%), consisting of both BMC and NBA insects. Insects, those of SOHC and BMC are phylogenetically closer.

Conclusion:-

The study that we made in Senegal on 43 individuals of *Sitophilus Zeamais* subscribed to millet and identified in four (4) agroecological zones revealed on the one hand a demographic expansion of the population of the NBA and the BMC which is respectively the result of a positive selection and negative one, and a complete lack of polymorphism of the SBA population and that of SOHC. The agroecological zone of the NBA thus gives the insect a strong adaptability while that of the BMC which can be at the origin of a bottleneck through the genetic drift and the natural selection, is hostile to the insect. NBA and SOHC AEZs have no effect on the suitability of the insect.

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