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Demographic and phylogenetic evolution of *Sitophilus zeamais* subservient to maize in different agroecological zones in Senegal

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Abstract

In Senegal, maize is mainly exploited in the agroecological zones of the NBA¹, SBA², SOHC³, BMC⁴, and ZSP⁵. Where it is heavily ravaged by a beetle *Curculionidae*, *Sitophilus zeamais*. The extent of the damage requires the search for alternatives. This article aims to detect the type of selection of populations in agroecological zones and their degree of kinship. The advantage of demonstrating a population demographic signal is to recognize the agroecological zones exposed to a bottleneck or a positive selection, therefore respectively constraining or naturally favorable to the adaptation of the insect. To achieve this goal, 89 insects were harvested in the different agroecological zones. The study of cytochrome b gene sequences revealed that the NBA, SBA and SOHC populations has undergone a positive selection. On the other hand, those of the BMC and the ZSP are victims of a negative selection. The individuals in each zone are closely related. Between two to two populations, BMC and SOHC insects are more closely related.

Keywords: *Sitophilus Zeamais*, maize, agroecological zone, cytochrome B

1. Introduction

Located in West Africa, Senegal is bordered on the north by Mauritania, on the east by Mali, on the south by Guinea Bissau and Guinea Conakry and on the west by the Atlantic Ocean. Its climatic and edaphic assets predispose it to promising agricultural exploitations. Thus, many cereals, particularly maize, are grown in the agroecological zones (AEZs)^[6] of the NBA, SBA, SOHC, BMC and ZSP. However, maize stocks are heavily damaged mainly by an insect called *Sitophilus zeamais*. Ngamo and al estimate that post-harvest damage can range from 25% to 60% in 6 months of storage. Some solutions have proved effective against this pest. But the negative repercussions on living beings and the environment oblige necessary to look for some other remedies.

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.

This study fits in this perspective. It aims to identify agroecological zones where the extinction or survival of the insect is likely, by detecting the demographic signal of populations in agroecological zones. Indeed, the type of selection (Positive or negative) may be a natural advantage or constraint to the development of the insect. But also, to highlight the degree of kinship of the individuals of the AEZs.

To achieve this goal, insects were collected from each AEZ, in infested maize corn stocks, then placed in alcohol tubes. A total of 89 individuals were obtained.

The sequences of the cytochrome b gene of these individuals have been exploited by population genetics software (DNAsp, Mega, Bioédit, Harlequin...) in relation to demographic and phylogenetic parameters, related to our objective.

2. Material and Method

2.1 Sampling

2.1.1 Sampling localities

The individuals of *Sitophilus zeamais* were sampled in 5 agroecological zones (AEZ) of Senegal. The choice given to these localities is justified by their vocation naturally agricultural and by ecological and geographical characteristics which specify each of them. This is the

AEZ of NBA, represented by the only locality of Bambey (14° 42'00"North / 16° 27'00" West), from the AEZ of the SBA to Keur Ayip (13° 36'00 " North / 15° 37'00"West), to Mbassis (14° 04'60"Nord / 16° 25'60 " West) and to Nioro (15° 13'55') North / 09° 35'37 " West). Some samples were also taken from the SOHC AEZ, at Missirah (13° 41'00"Nord

/ 16° 30'01"Ouest) and Salémata (12° 37'60 " North / 12° 49'00). The other 2 AEZs sampled are the BMC, only at Diaroumé (13° 03'19"Nord / 15° 38'34"Ouest) and the ZSP at Matam (15° 06'18) " / 13° 38'30 West) and Fouta (Podor: 16° 40'00 " N / 16° 27'00"W). Figure 1 summarizes the study sites in red.

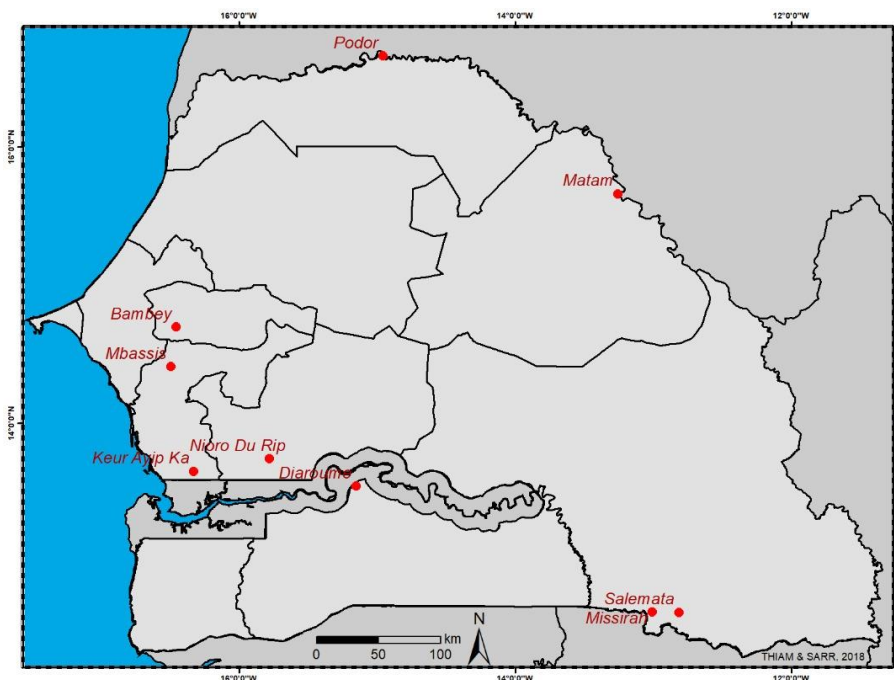


Fig 1: Sampling locations (in red)

2.1.2 Harvesting individuals

The collection of infested maize samples in the different AEZs made it possible to isolate individuals of *Sitophilus zeamais* for each zone. It has been done in the fields, in storage means 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 are placed in tubes containing 96% alcohol.

Individuals are coded using the first letter of the genus name

in uppercase followed by the first two letters of the locality of origin (The first letter in upper case and the second letter in lower case) and finally the serial number. Example the code SDi₁₀ designates a *Sitophilus* individual from the locality of Diaroumé with the order number 10.

Table 1: Summarizes the localities of the AEZs where the harvests take place, the number of individuals sampled for each AEZ, the geographic coordinates of the localities and the codes of the individuals.

Table 1: Sampling locations

Agroecological Zones	Number of individuals	GPS	Sampling code
NBA	12		
Bambey	12	14°42'00"N/16°27'00" W	SBa
SBA	28		
Keur Ayip	19	13°35'00"N/15°36'00" W	SKa
Mbassis	12	14°04'60"N/16°25'60" W	SMB
Nioro	7	15°48'55"N/13°45'37" W	SNi
SOHC	22		
Missirah	12	13°41'00"N/16°30'01" W	SMi
Salémata	10	12°37'60"N/12°49'00" W	SSa
BMC	10		
Diaroumé	10	13°03'19"N/15°38'34" W	SDi
ZSP	17		
Matam	8	15°06'18"N/13°38'30" W	SMa
Fouta	9	16°40'00"N/16°27'00" W	SFo
SUM	89		

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'TATGTACTACCATGAGGACAAATA TC-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.

2.2.4 Bioinformatics analyzes

The sequences were corrected and aligned by the Clustal software implemented in the Bioédit version 7.2.5 programs. 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's Fu, of Ramos' R2 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 . 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 R2 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 vesion 7.0.14 software and Mr Bayes. The comparison of these 2 trees made it possible to verify the coherence of the interpretation of the phylogeny of the populations.

3. Results and Discussion

3.1 Results

3.1.1 Demographic history

3.1.1.1 Neutrality tests

Table 2: Neutrality indices of AEZ populations (Non-significant gray values).

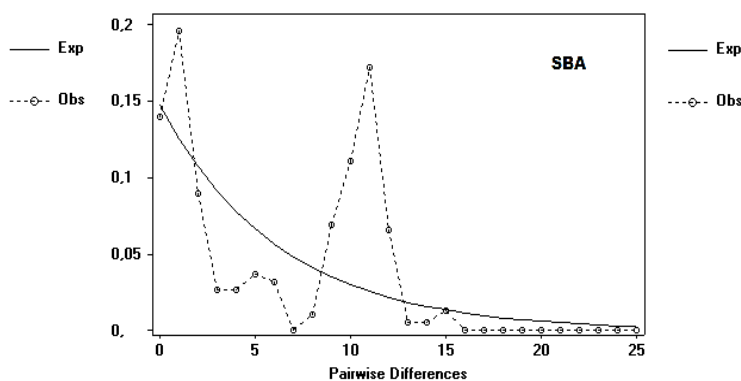
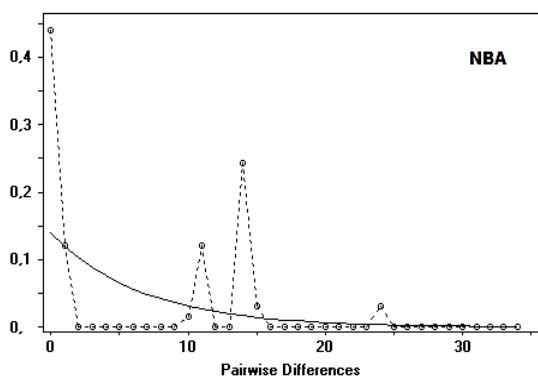
	AEZ	NBA	SBA	SOHC	BMC	ZSP
	Tajima's D	-0,98739	0,43864	-0,75535	-1,90129	-2,02155
	Fu's Fs	4,48991	0,76622	1,21229	1,72621	-0,94243
Demographic Parameters	Ramos's R ₂	0,16131	0,16022	0,16064	0,16187	0,16048
	Fay and Wu's H	-0,1232	-0,0642	-0,1265	0,4352	0,1167
	SSD	0,40450	0,84079	0,08002	0,11536	0,22178
	Rag	0,24862	0,04359	0,14552	0,45136	0,23648

The Tajima D values of NBA and SOHC are negative, that of SBA is positive. None of these values is significant. In contrast, the Tajima D values of BMC and ZSP are negative and significant. The values of Fs of Fu of all AEZs are positive except for the ZSP, but they are not significant. The Fay and Wu's H values are negative for the NBA, SBA and SOHC. (Table 2).

3.1.1.2 Mismatch distribution

The data simulated according to the population expansion model give a unimodal curve while the data observed for the entire population and for the 5 AEZs show multimodal curves. (Figure 2).

The SSD values are significant for all AEZs except for the BMC.



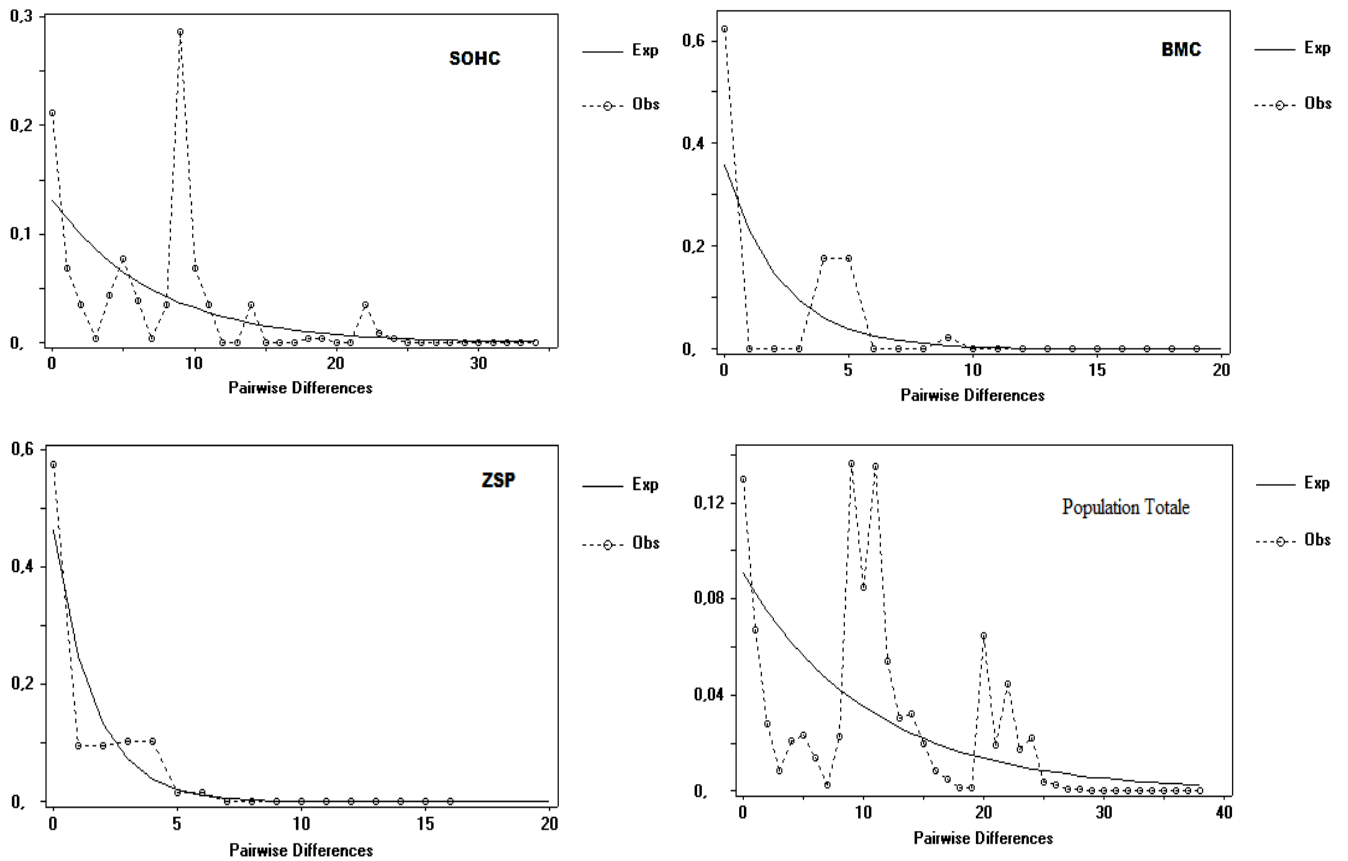


Fig 2: Distribution number of mismatch differences of *Sitophilus zeamais* in AEZs

3.1.2 Phylogenetic trees

Maximum likelihood phylogenetic trees and Bayesian approaches highlighted 3 clades (C1, C2 and C3). The first clade C1 is composed of private haplotypes of the ZSP. The second C2 is made up of haplotypes of the BMC. Finally, the

third clade C3 includes haplotypes of the SBA. Phylogenetic trees also show clusters based on AEZs except the H15 haplotype that exist in both SBA and SOHC and the H1 haplotype found in both NBA and ZSP.

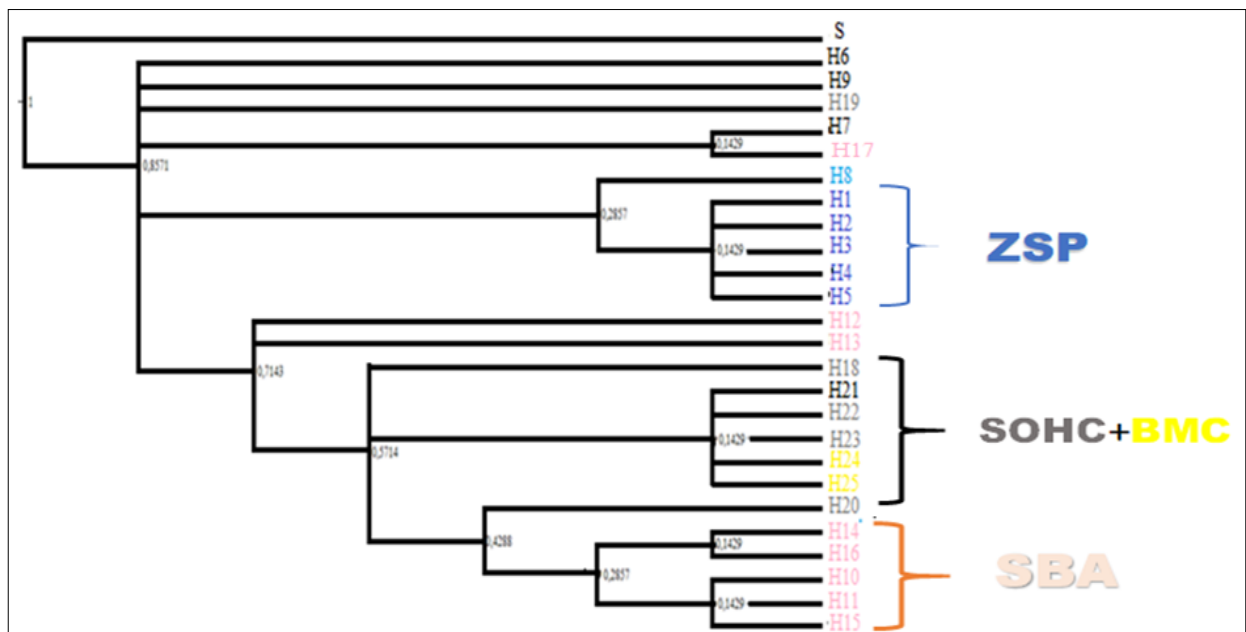


Fig 3(A)

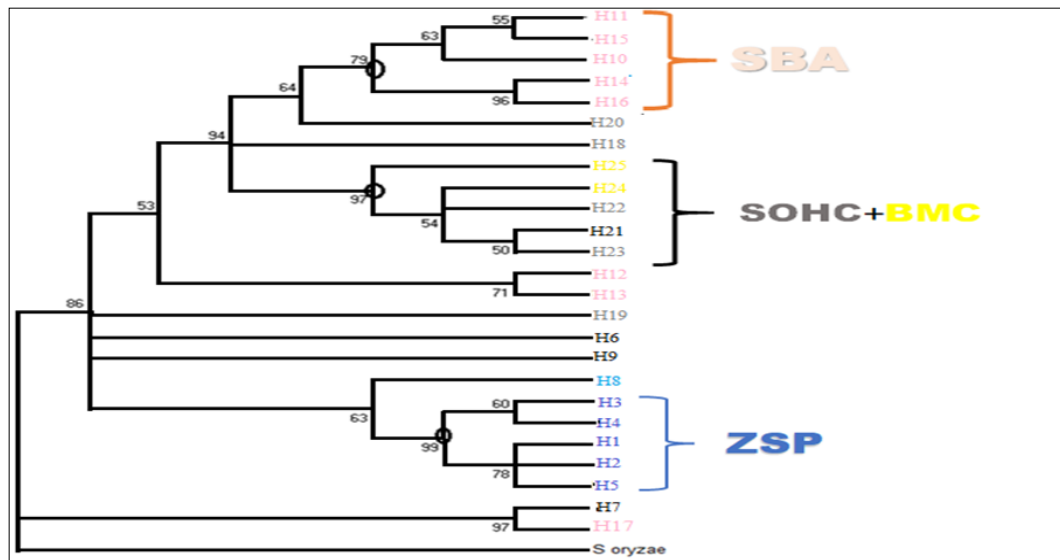


Fig 3(A, B): Phylogenetic Trees: Maximum likelihood method (A), Bayesian approach (B)

3.2 Discussion

Our study has been to detect the demographic signature of the populations of *Sitophilus Zeamais* for agroecological zones [7]. And their degree of relationship. We have tried to achieve these objectives by studying populations of this insect in different AEZs in relation to demographic and phylogenetic parameters.

3.2.1 Demographic evolution

The haplotype network shows a star structure, characteristic of population growth. However, the demographic tests, the mismatch distribution and its indices do not confirm this type of evolution for all AEZs.

The Tajima D of the NBA, SBA and SOHC (even if it is negative for the first 2 AEZ) and the Fu's Fs of these 3 zones are not significant. The populations corresponding to these zones are therefore in demographic equilibrium. The multimodal Mismatch distribution for these 3 AEZ, the SSD significance and the very high Hd and Pi values confirm the demographic stability of NBA, SBA and SOHC agroecology zones. Although the Tajima D values of these populations are not significant, the fact that they are negative indicates that the NBA, SBA and SOHC populations show rare variants, consistent here with a positive selection, according to their negative values of Fay and Wu H.

The Tajima D of the BMC is negative and significant. The population of this AEZ is thus expanding. The insignificant SSD value confirms this demographic expansion of the BMC population and rejects the demographic balance that emerges from Mismatch's multimodal distribution. The high value of Hd (0.378 ± 0.181) and low Pi (0.004 ± 0.0028) in BMC also accredit this type of evolution.

The ZSP with a significantly negative Tajima D and a negative and insignificant Fs of Fu is expanding population. The significance of the SSD does not confirm this result. On the other hand, it is attested by the high value of Hd (0.426 ± 0.147) and low of Pi (0.002 ± 0.0012). The population expansion of the ZSP would be in its infancy. Indeed, according to the positive value of H of Fay and Wu, it is preceded by a purifying selection.

Finally, the AEZs of NBA, SBA and SOHC whose populations have undergone a positive selection are more resistant to the effects of genetic drift and natural selection. While those of ZSP and BMC which have sustained a

negative selection are more vulnerable.

3.2.2 Phylogeny of individuals

Phylogenetic trees show the same topology and reveal 3 clades C1, C2 and C3 strongly supported respectively by a posterior probability of 99%, 97% and 79%. Their existence clearly shows a grouping of *Sitophilus zeamais* according to agroecological zones. The first clade C1 is constituted only of haplotypes subservient to the ZSP. The existence of these genetically close individuals, specifically in this area, indicates that they did not experience the presence of non-native individuals. The geographical distance that separates the ZSP from other AEZs may be the reason for this genetic isolation. But the drastic ecological conditions (high temperature, low rainfall) can also lead to the disappearance of foreign individuals with weak adaptability and thus create a genetic homogenization of the area. The disadvantage of this standardization is the high susceptibility of this ZAE to being a victim of a bottleneck. The second clade C2 is the set of haplotypes of BMC and SOHC, whose genetic convergence has already been highlighted by the haplotype network and other determinants of genetic variability. The genetic similarity between the haplotypes of these AEZs can be explained by the geographical distance. The proximity of Diaroumé (only component of the AEZ of the BMC), to the high Casamance can facilitate the exchange of the same haplotypes between these 2 AEZ.

The third clade is represented by haplotypes all from the SBA and genetically close.

Thus, each AEZ is characterized by genetically close individuals. If we compare populations with each other in terms of kinship, the BMC and SOHC populations are phylogenetically closer.

4. Conclusion

Our study in Senegal of 25 haplotypes of *Sitophilus Zeamais* identified in five (5) agroecological zones revealed a demographic expansion of the NBA, SBA and SOHC populations that is the result of positive selection. These 3 agroecological zones thus confer on the insect a strong adaptability. On the other hand, populations of the ZSP and BMC that have suffered a bottleneck (negative selection) are sheltered by ZAEs vulnerable to genetic drift and natural selection, and therefore hostile to the insect.

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