

# Comparative Study of Demographic and Phylogenetic Evolution of *Sitophilus Zeamais* Subservient to 2 Host Plants (Millet and Maize) in Senegal (West Africa)

Ngagne Demba Sarr \*<sup>1</sup>

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

Cheikh Thiaw <sup>2</sup>

University of Sine Saloum ELHADJ  
IBRAHIMA NIASS Sing-Sing, BP 55,  
Kaolack, Senegal.

Mbacke Sembène <sup>3</sup>

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

**Abstract:-** The maize weevil is a cosmopolitan insect very adapted to the arid climate. This characteristic predisposed it to the massive destruction of many cereals exploited under this type of climate. In Senegal, this Curculionidae beetle ravages particularly maize and millet, 2 host plants to which it is dependent. Studies on the variability and genetic structuring of *Sitophilus Zeamais* in agroecological and agro-climatic zones in Africa in general have been carried out. But none of them focused specifically on Senegal.

This article aims to highlight the type of demographic signal (positive selection or negative selection) that specifies the population of each host plant and the degree of kinship of their individuals. The reason is to know which host plant is most likely to favor a bottleneck or population expansion of each of their insect populations.

To achieve this goal, 125 insects were harvested of which, 72 are tied to maize and 53 subservient to millet, across the country.

Exploitation of the cytochrome B gene sequences corresponding to these individuals revealed, on the one hand, that the global populations of millet and maize each has undergone a positive selection. Thus the 2 host plants promote the development of *Sitophilus Zeamais*. On the other hand, individuals in the maize population are phylogenetically closer than those in the millet population.

**Key words:** *Sitophilus Zeamais*, Cytochrome B, positive selection, negative selection.

## I. INTRODUCTION

Senegal is characterized by a Sahelian to Sahelo-Sudanese climate. This climatic characteristic is an asset to the exploitation of millet and maize, which are crops adapted to the lack of water, but also a drawback because promoting a massive destruction of the stocks of these cereals, especially maize by *Sitophilus Zeamais*, a Curculionidae beetle also

adapted to arid climatic conditions. The solution to eliminate this pest was the use of pesticides. But the negative consequences that accompany this alternative have prompted the search for other remedies.

This study aims to identify the positive or negative selection that characterizes the population of each host plant and the degree of kinship of individuals. The importance of this study is to explain the differential vulnerability of the insect to these 2 host plants, because positive selection is likely to increase the adaptive capacity of the insect by promoting a population expansion while the negative selection promotes its extinction by creating a bottleneck.

To achieve this goal, 125 individuals of *Sitophilus Zeamais* were sampled, of which 72 on maize and 53 on millet, in agroecological zones.

The corresponding sequences were exploited by population genetics software (Bioedit, DNAsp, Mega, Harlequin ...), in relation to demographic and phylogenetic parameters, related to the aforementioned objective.

## 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<sup>1</sup> represented by the only locality of Bambey (14 ° 42'00" Nord / 16 ° 27'00" Ouest), the AEZ of the SBA<sup>2</sup> 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<sup>3</sup> 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<sup>4</sup> 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.

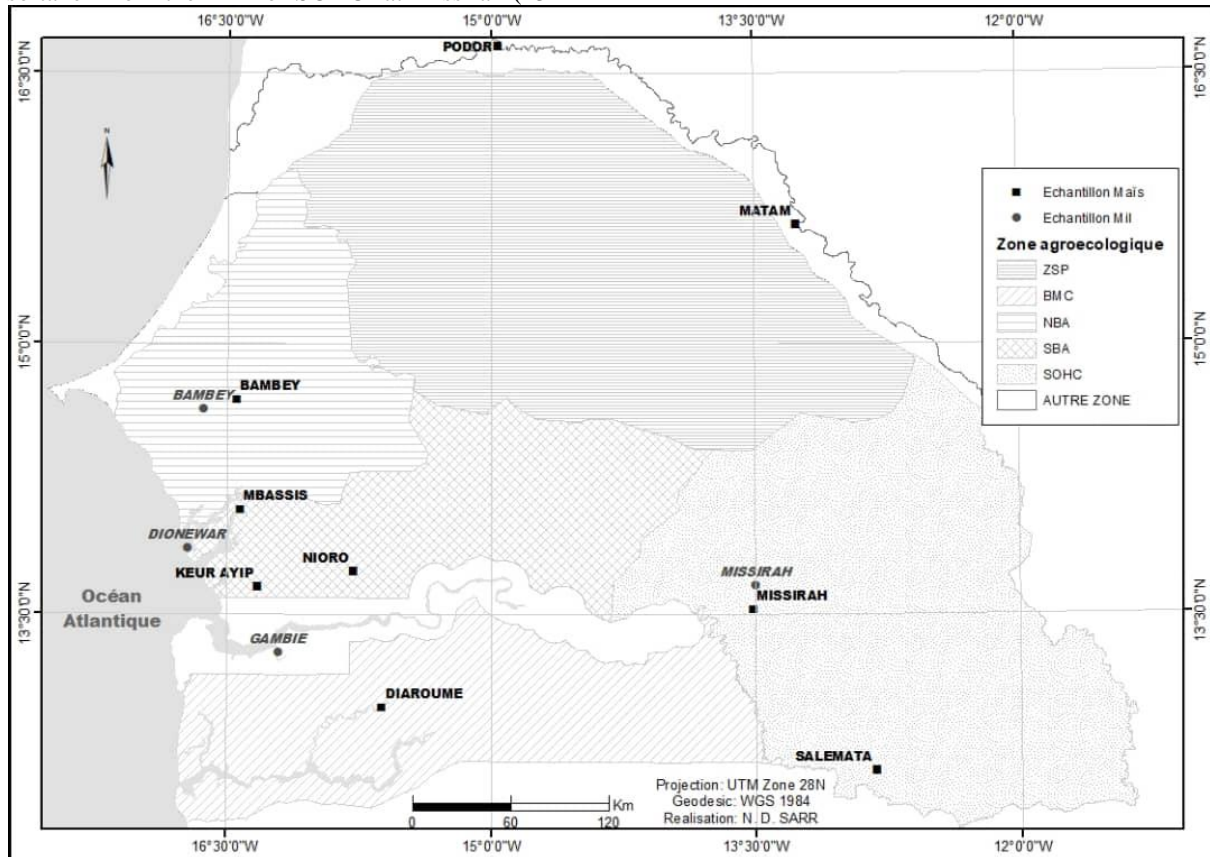


Figure 1 : Sampling locations

### II.1.2. Harvesting individuals

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
<b>NBA</b>	<b>23</b>		
Bambey	12/11	14°42'00''N/16°27'00''W	SMaBa/SMiBa
<b>SBA</b>	<b>47</b>		
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
<b>SOHC</b>	<b>35</b>		
Missirah	12/13	13°41'00''N/16°30'01''W	SMaMi/SMiMi
Salémata	10	12°37'60''N/12°49'00''W	SMaSa
<b>BMC</b>	<b>20</b>		
Gambie	10	13°27'09''N/16°34'40''W	SMiGa
Diaroumé	10	13°03'19''N/15°38'34''W	SMaDi
<b>TOTAL</b>	<b>125</b>		

## 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 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' 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 (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 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 vesion7.0.14 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.

### III. RESULTS AND DISCUSSION

#### III.1. Results

##### III.1.1. Demographic history

##### III.1.1.1. Neutrality tests

The Fay and Wu of H, The Tajima's D, the Fu's Fs of populations of *Sitophilus Zeamais* subservient to maize and subservient to millet are negative (Table 28).

Table 28: Neutrality indices of the global populations of *Sitophilus Zeamais* maize and millet (significant gray values).

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	DEMOGRAPHIC PARAMETERS				
	Tajima's D	Fu's Fs	Fay and Wu's H	SSD	Rag
<b>Maize</b>	-0,94738	-1,17277	-0,0724	0,04469	0,05497
<b>Millet</b>	-1,19703	1,54478	-0,0896	0,03178	0,08448

Local millet and maize populations have some Tajima's D, Fay and Wu of H and to a lesser extent Fu's Fs identical to those of global millet and maize populations, except for Dionewar and Missirah millet populations where Tajima's D, Fu's Fs and Fay and Wu of H are null (Table 29).

Table 29: Neutrality indices of local populations of *Sitophilus Zeamais* subservient to maize and millet (significant gray values).

	DEMOGRAPHIC PARAMETERS				
	Tajima's D	Fu's Fs	Fay and Wu's H	SSD	Rag
<b>Maize Bambey</b>	-0,98739	4,48991	-0,1487	0,40450	0,24862
<b>Millet Bambey</b>	-2,06625	1,83008	-0,2331	0,33889	0,12463
<b>Maize Keur Ayip</b>	1,26914	0,42091	-0,2257	0,04993	0,05556
<b>Maize Mbassis</b>	0,28569	2,77726	0,1629	0,14046	0,20845
<b>Maize Nioro</b>	0,42126	-0,86758	-0,1950	0,05384	0,05669
<b>Millet Dionewar</b>	0,00000	0,00000	0,0000	0,00000	0,00000
<b>Maize Missirah</b>	-1,31115	1,20764	0,1783	0,07634	0,12227
<b>Maize Salémata</b>	-1,56222	-0,45861	-0,2134	0,01116	0,22222
<b>Millet Missirah</b>	0,00000	0,00000	0,0000	0,00000	0,00000
<b>Maize Diaroumé</b>	-1,90129	1,72621	0,2015	0,11536	0,45136
<b>Millet Gambie</b>	-1,11650	1,23730	-0,2190	0,04218	0,10222

##### III.1.1.2. Mismatch distribution

The two global populations of millet and maize have a multimodal distribution (Figure 22). But the SSD and RAG values are significant for maize and not significant for millet.

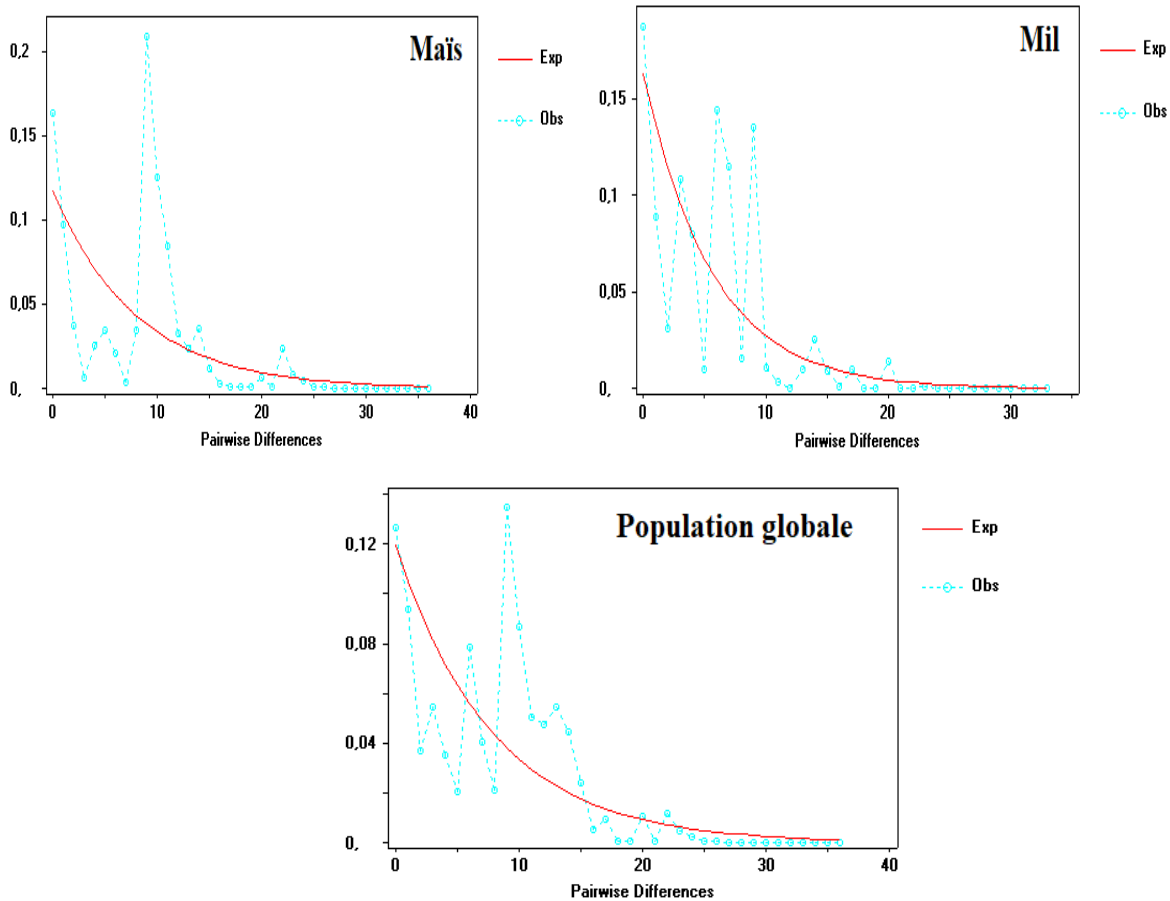


Figure 22: Mismatch distribution of global populations of Millet and Maize.

III.1.2. Phylogenetic trees

The phylogenetic trees according to the Neighbor Joining (A) and Maximum Likelihood (B) methods highlighted a single clade strongly supported at 90%. It covers more than

half of the maize haplotypes. On the other hand, millet-specific haplotypes were not grouped by a single clade, even at low posterior probability value.

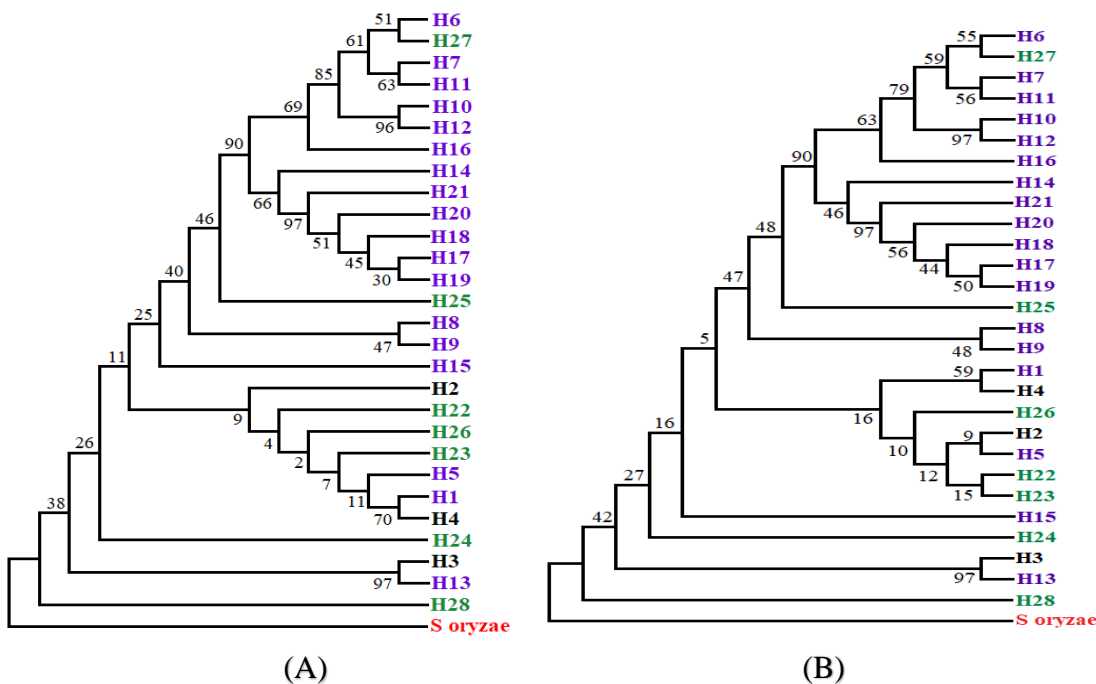


Figure 23: Phylogenetic trees: Neighbor Joining Method (A), Maximum Likelihood Method (B)

### III.2. Discussion

#### III.2.1. Demographic evolution

The starry shape of haplotype networks of maize and millet is the signal for a demographic expansion of these 2 populations. Maize infested insects are characterized by a negative D of Tajima and Fs of Fu. The negativity of these statistical tests suggests within this population the existence of an excess of rare variants, consistent with positive or negative selection. However, the non-significance of Tajima's D, Fu's Fs and the multimodal Mismatch distribution of haplotypes subservient to maize do not confirm this signature.

The negative and very high Tajima's D of the millet population is a sign of a demographic expansion, which is nevertheless challenged by a positive Fs of Fu. However, the non-significance of the SSD and RAG values confirms the multimodality of the Mismatch distribution curve and thus a demographic expansion of the millet population.

The demographic expansion of the maize population attested by the starry shape of the haplotype network, by the demographic tests (D of Tajima, Fs of Fu) but disputed by the non-significance of the Mismatch distribution curve, is still exaggerated by the indices of genetic diversity and the negativity of the H value of Fay and Wu. This population has thus undergone a positive selection.

The millet and maize populations are characterized by high haplotypic diversity and low nucleotide diversity. The high number of haplotypes on both sides suggests that each population initially experienced a bottleneck followed by population growth (Avise, 2000). The negative value of H of Fay and Wu, a sign of a positive selection confirms this beginning of demographic expansion. The demographic expansion may be due to favorable climatic conditions. In fact, Senegal has experienced major droughts which have caused huge losses of cereals and consequently of their pests. For decades, rainfall has become acceptable. The requirements of adaptation to this new climatic situation may be at the origin of the appearance of new rare variants in the maize population and in the millet population. The genetic similarity between haplotypes symbolized by low nucleotide diversity is explained by a lack of time for a high genetic diversity of these populations. The time separating the 2 climatic events being weak. But the genetic approximation between populations can also be justified by a gene flow between the two host plants. The cereal crops are conserved alternately in the same storage means: bag, rack, barrel, rack, attic, store (Guéye et al, 2000). The disadvantage is that insect residues, larvae and eggs may remain and infest the future cereal that will be stored there. In addition, millet and corn are grown in the same fields or neighbors. This neighborhood favors a migration on both sides of the host plants.

#### III.2.2. Phylogeny of individuals

Phylogenetic trees indicate that almost all maize-specific haplotypes share the same ancestor which is from latest generations. This clade is supported by a posterior probability of 90%. This grouping confirms the genetic similarity of the insects subservient to maize.

On the other hand, the trees revealed that the individuals subservient to millet come from different ancestors. Thus, these individuals are phylogenetically distant.

### CONCLUSION

Our study in Senegal on 72 individuals of *Sitophilus Zeamais* subservient to maize and on 53 subservient to millet, in four (4) agro-ecological zones revealed a demographic expansion of the global populations of maize and millet, which is the result of a positive selection. The two host plants then confer adaptability to the insect. She also showed that maize-infested insects, unlike millet infested ones, are phylogenetically close. Since the demographic signal of populations does not explain the differential vulnerability of the 2 host plants to the insect, further studies on the hardness, shape and chemical composition of the grains of each host plant can be conducted.

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