

Diversity and Genetic Structuration of Populations of *Plutella xylostella* (Lepidoptera, Plutellidae), Cabbage Farming Destroyer in Senegal

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Abstract

Plutella xylostella, pointed out as the most dangerous destroyer of cabbage (*Brassica olerarea*), is a cosmopolitan species. In fact, owing to its large capacity of adaptation, *P. xylostella* colonizes any kind of area. This insect can bring about up to 90% of losses on cabbage farming. To control the insect, Senegalese farmers, very often, resort to high dose of chemical pesticides which are repetitively sprayed. The use of these chemical products gives rise to different varieties of resistant insects, which results in the emergence of different haplotypes between populations. It is in such a context that this study has been undertaken. Our objective is then to contribute to the knowledge of the genetic diversity of *P. xylostella* populations in Senegal. To hit the target, PCR-Sequencing method has been applied on samples from the five following localities: Diofior, Malika, Mboro, Santh Ndong, and Sebikotane. *P. xylostella* from Mboro is genetically different from the other populations. This fact could be due to a different way of using pesticides in this area vis-à-vis of the other localities. On the other hand, between populations of *P. xylostella* from Diofior, Malika, Santh Ndong, and Sebikotane, there is no significant genetic difference. In other respects, phylogenetic trees reveal the existence of two clades: one with individuals from Mboro and the other one with individuals from the other four localities. *P. xylostella* from Mboro distinguishes itself from other populations and then, can be regarded as a sub-population. Thus, the phylogenetic trees reveal the existence of two groups of *P. xylostella* in Senegal.

Keywords

Brassica oleracea, Genetic Diversity, Genetic Structure

1. Introduction

Market gardening has become an economic activity efficiently responding to city dwellers nutrition demand. Among market garden produce, cultivation of Brassicaceae is considered, by FAO [1], as yielding the utmost important production in the world. Besides, in agro ecosystems, growing one variety of plant on a vast expanse goes naturally with a great increase of plants bio-aggressors, in particular, making crops not marketable. Thus, Brassicaceae cropped in Senegal, notably in the Niayes area, the Senegal River valley, and the “Bassin arachidier”, are imperiled by manifold natural enemies. Among them, *Plutella xylostella* is regarded as the most dangerous for Brassicaceae cultivated in the world, especially in the subtropical area [2] [3]. It is an insect species confined to this plant family. According to Talekar and Shelton [2], Verkerk and Wright [4], it can breed losses assessed up to 90%. In order to control the insect, Senegalese farmers, very often, resort to high dose of chemical pesticides which are repetitively sprayed. This way of fighting against this pest affects human being health and brings the insect to develop resistance. According to Gnago *et al.* [5], caterpillars of the *P. xylostella*, seem to have developed a certain resistance against the insecticide Kart 500 SP. Odhiambo *et al.* [6] noticed these forms of resistance to insecticides of pyrethrinoid type and DDT (Dichloro-Diphenyl-Trichloro-ethane) too; these are abusively used at high dose by farmers [2]. The use of these chemical products gives rise to different varieties of resistant insects, which results in the emergence of different haplotypes between populations [7]. All the pollutants and insecticides are known for their high capacity of generating mutations; this increases the number of mutations in resistant individuals too. According to Pichon *et al.* [8], *P. xylostella* can be regarded as a species highly polymorphic. This has been confirmed by Marthur *et al.* [9] who indicated a strong genetic diversity in populations of box tree moths from different regions of southern and northern India. Differences in the degree of resistance to insecticides between populations can result from selection of different pressures attributable to the local variation in the way of using insecticides. It is in such a context, that this study has been carried out, to determine the diversity and the genetic structuration of *P. xylostella* populations in Senegal.

2. Materials and Methods

2.1. Sampling

Sampling has been done in Senegal, precisely in five localities of two agro-ecological areas. Samples have been taken on field. It consists in collecting larva of *P. xylostella* living on cabbage plants; see samples on **Table 1**.

Table 1. Sampled localities and corresponding agro-ecological areas.

Sampled localities	Number of individuals	Agro-ecological areas
Malika	10	Niayes
Diofior	10	Bassin arachidier
Mboro	10	Niayes
Sébikotane	10	Niayes
Santh Ndong	10	Niayes

2.2. DNA Extraction, Amplification by PCR and Sequencing

The method was referred to kit Qiagen Dneasy Tissue. The PCR did upon fragments of mitochondrial coding gene: cytochrome b. This gene has been amplified with the help of the primers CB1 (5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3') and CB2 (5'-ATT ACA CCT CCT AAT TTA TTA GGA AT-3'). Amplification has been dealt with in a reactionary volume of 25 μ l consisting of 18.3 μ l of water, 2.5 μ l of tampon 10 \times , 1 μ l of additional MgCl₂, 0.5 μ l of dNTP, 0.25 μ l of each primer, 0.2 μ l of taq polymerase, and 2 μ l extract from DNA. It is done thanks to a cyclic repetition which ensures a multiplication by 2 of the DNA targets at every cycle. It has been carried out thanks to an equipment called thermo-cycler with the following amplification conditions: initial denaturation at 94°C for 3 minutes, then 35 cycles of denaturation at 94°C for 1 minute, followed by 1 minute of hybridation at 47°C, and a bit of DNA elongation additional to 72°C for 1 minute. A final elongation at 72°C for 10 minutes ends the PCR. Sequencing has been carried out in South Chorea.

2.3. Genetic Analysis

2.3.1. Genetic Variability of Populations

Analysis of genetic variability parameters such as the number of polymorphic or monomorphic sites, the number of mutations, nucleotidic and haplotydic diversities have been estimated with the software Mega v.5.05 [10].

2.3.2. Genetic Structure

Genetic distances between *P. xylostella* populations have been calculated by of Kimura 2 parameters method [11]. They have been determined thanks to the Mega software v.5.05 [10]. F_{ST} values, in pairs, have been used to estimate the rate of migration by generation; Nm (N, the effective size of the population multiplied by the migration rate, m), based on the relationship balance $F_{ST} = 1 / (2Nm + 1)$. This parameter has been determined by the Arlequin software version 3.1 [12]. Molecular variance analysis (Amova), for populations, has been done on alleles' frequencies basis, using Arlequin 3.1 [12], to prove more the genetic differentiation of the populations of *P. xylostella* in Senegal. Amova analysis has been implemented using the Arlequin software version 3.1 [12] with 1000 permutations and a significant level of 0.05.

2.3.3. Demographic Evolution

It is constituted by demo-genetic tests and a Mismatch distribution analysis. To detect expansion signs of the breakdown area of *P. xylostella* populations, as well as Tajima D and the F_s of Fu tests have been carried out. Moreover, the test F_s of Fu is very sensitive to demographic expansion, which generally finds expression in negative values. These tests have been worked out using Arlequin v. 3.1 [12]. Mismatch distribution graphs are constructed with the help of the DnaSP v. 5.10 [13]. But the indexes that go with it, particularly SSD and Rag, have been estimated owing to Arlequin v. 3.1 [14].

2.3.4. Phylogenetic Relationships Analysis

For high resolution concern, erection of the phylogenetic trees has been done using two different methods: Neighbor-Joining method where calculation takes into account genetic distances by using the Kimura two parameters method, and the method of maximum probability which even shows us the history of our set of data. Research process of a phylogenetic tree, by using maximum probability, implies finding the typology and the length of the tree's branches that will give us the highest probability to observe the DNA sequences in our data. The most appropriate model to build this tree is that of GTR added to the law of Gama. These two trees have been built, using Mega software v. 5.05 [10].

3. Results

3.1. Genetic Variability

In a total of two hundred eighty-nine (289) sites, we observed ten (10) mutations, hundred (100) of variable sites which were all informative in parsimony, and two hundred seventy-nine (279) invariable sites. Five (05) haplotypes were recorded, distributed as follows: haplotype 1 gathered five (5) individuals, of which four (04) belonged to the population of *P. xylostella* from Diofior and one to the population from Malika; as for haplotype 2, it grouped nine (09) individuals of which, one (01) was from Diofior, six (06) from Mboro, and two (02) from Sébikotane; haplotype 3 concerned the individual M17 which was from Malika; remaining individuals were shared between haplotypes 4 and 5. A strong haplotype diversity (0.761 ± 0.040) and a weak nucleotide diversity (0.01687 ± 0.00134) were noticed in the global sample. Moreover, when we considered separately population from each locality instead of the global one, we remarked that the number of sites of difference varied for each sample, except for the population from Santh Ndong. Value of haplotype and nucleotide diversities was the same with respect to populations considered separately (Table 2).

Estimated under the pattern of Tamura-Nei [15], substitutions of the transition type (41.84) were lower than substitutions of trans-version one (58.16%) (Table 3).

The type of substitution was the same, either it was synonymous or not; consequently, the ratio $k_{ns} (0.0206)/k_s (0.0206) = 1$. Among the five haplotypes, one (h2) was shared between three populations: Mboro, Sebikotane, and Diofior; two

(02) were shared between two populations: h5 between Santh Ndong and Sébikotane, and h1 between Malika and Diofior; haplotypes h3 and h4 were private: H3 was only found in Mboro and h4 in Malika, suggesting a certain degree of isolation between these two populations. Haplotypes h2 and h5 were majority and more abundant in Mboro and Sebikotane. Among the five haplotypes, only one was individual and private; that was haplotype h3, only met in Mboro (Figure 1).

Table 2. Genetic variability of *P. xylostella*.

Parameters	Diofior	Mboro	Malika	Sébikotane	Santh Ndong
Number sites of difference	08	01	04	10	00
Number of haplotypes	02	02	02	02	01
Haplotype diversity	0.4 ± 0.237	0.3 ± 0.196	0.4 ± 0.237	0.4 ± 0.169	00
Average number of nucleotide differences	0.011	0.00059	0.00559	0.01518	00
Nucleotide diversity with JC	0.011 ± 0.00657	0.00059 ± 0.00068	0.00559 ± 0.00328	0.01518 ± 0.00584	00

Table 3. Rates of different types of substitutions.

BASE	A	T	C	G
A	-	11.25	3.29	4.73
T	11.25	-	4.73	3.29
C	11.25	16.19	-	3.29
G	16.19	11.25	3.29	-

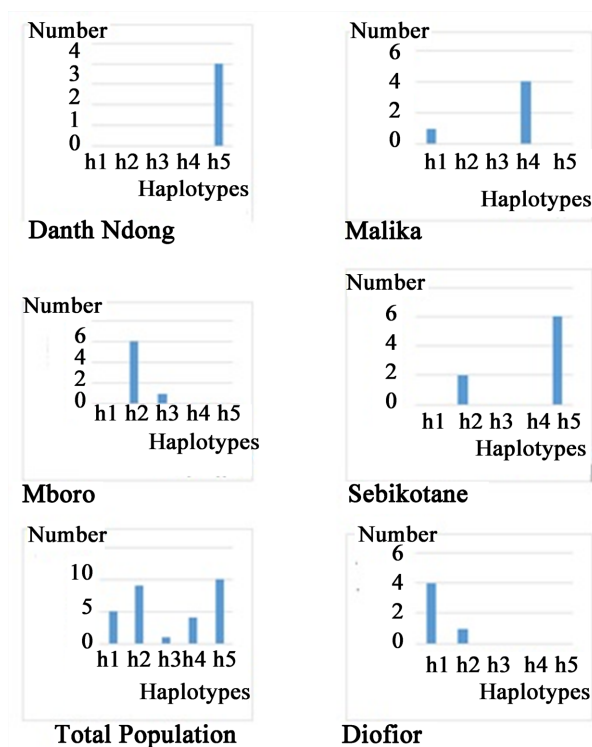


Figure 1. Distribution of *P. xylostella* haplotypes (h1-5) in Senegal.

3.2. Genetic Structure

3.2.1. Genetic Distances

In the table below, we noticed that population from Santh Ndong recorded the smallest absolute value of d (0.000), followed by that from Mboro, where we had $d = 0.0015$, then by those from Malika and Diofior, and finally by that from Sébikotane (Table 4).

When we took into consideration the genetic distance between populations, we noticed a certain proximity between *P. xylostella* population from Diofior with those from Malika, Sébikotane, and Santh Ndong with genetic distances of 0.0166; 0.0127; and 0.0127, respectively. Genetic distances were higher between the population from Mboro and the others. Of all populations, the highest genetic distance, in absolute value, was noticed between Mboro and Santh Ndong, despite their geographic proximity, and the weakest one between Malika and Santh Ndong (Table 5).

3.2.2. Genetic Differentiation

It was based on various factors like the F_{st} mentioned above. Taking into account both the F_{st} and the p -value (Table 6 and Table 7), individuals of *P. xylostella* from Mboro were far from those of Santh Ndong, despite the geographical proximity of the two localities, and those Sébikotane and Malika as well. The genetic differentiation was significant between these different localities (p -value < 0.05), and confirmed the existence of distant genetic groups. On the other hand, between Sébikotane and Diofior, the p -value was non-significant (p -value = 0.24); that showed the existence of closely related populations, with respect to genetic consideration (Table 7).

3.2.3. Space Analysis of Molecular Variance

The AMOVA test implemented (Table 8) allowed us to notice that variation

Table 4. Intra population genetic distances.

Localities	Genetic distance	Standard error
Diofior	0.0149	0.0056
Mboro	0.0015	0.0015
Malika	0.0063	0.0036
Sébikotane	0.0183	0.0065
Santh Ndong	0.0000	0.0000

Table 5. Genetic distances between populations.

Localités	Diofior	Mboro	Malika	Sébikotane
Mboro	0.0305			
Malika	0.0166	0.0324		
Sébikotane	0.0170	0.0316	0.0153	
Santh Ndong	0.0127	0.0419	0.0094	0.0107

Table 6. Comparison of Fst, Nst, Gst and Ks between populations.

Population 1	Population 2	Ks	Fst	Nst	Gst
Diofior	Mboro	1.50	0.73	0.73	0.41
Diofior	Malika	2.40	0.42	0.42	0.35
Diofior	Sébikotane	3.87	0.13	0.12	0.38
Diofior	Santh_Ndong	1.78	0.55	0.55	0.64
Mboro	Malika	0.83	0.88	0.88	0.49
Mboro	Sébikotane	2.42	0.69	0.69	0.37
Mboro	Santh Ndong	0.18	0.98	0.98	0.69
Malika	Sébikotane	3.25	0.16	0.16	0.40
Malika	Santh Ndong	0.89	0.60	0.60	0.64
Sébikotane	Santh Ndong	2.86	0.14	0.14	0.06

Table 7. Comparison of the p-value of Fst between populations.

	Diofior	Mboro	Malika	Sébikotane	Santh_Ndong
Mboro	0.011				
Malika	0.038	0.003			
Sébikotane	0.240	0.002	0.184		
Santh Ndong	0.010	0.003	0.003	0.056	0.547

Table 8. Analysis of *P. xylostella* population structure.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Between populations	4	42.819	1.68988 Va	61.44
Within population	24	25.457	1.06071 Vb	38.56

Fixing Index Fst: 0.61437

occurred generally when populations were separately dealt with (variation between populations estimated at 61.44%).

3.3. Demographical Evolution

3.3.1. Demo-Genetic Test

Tajima D was negative for *P. xylostella* populations from Diofior, Mboro, and Malika localities, and only significant for Diofior; on the other hand, it was positive and not significant for sébikotane. Furthermore, the test was nil for the population of *P. xylostella* from Santh Ndong. For the locality of Mboro, the Fs of Fu was not significant (Table 9).

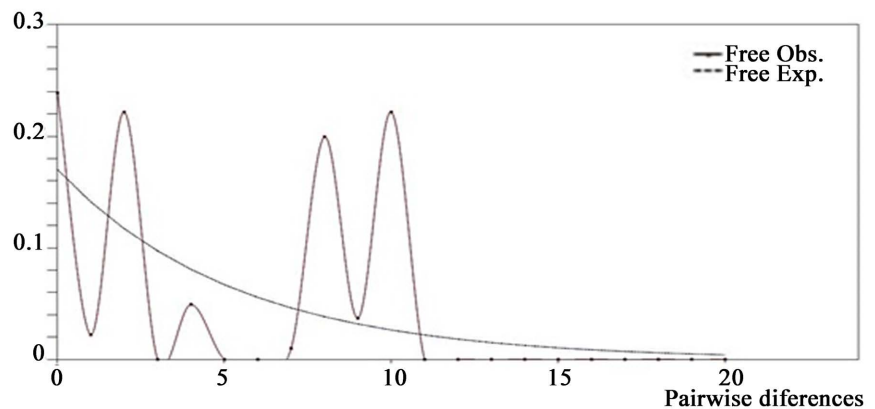
3.3.2. Analysis of Mismatch Distribution

Analysis of Mismatch distribution showed a multimode curve for the global population (Figure 2).

Comparison table of the SSD or the Sum total of square deviations and the Rg

Table 9. Neutrality test.

	Diofior	Mboro	Malika	Sébikotane	Santh Ndong	Mean	s.d.
D	-1.17432	-1.00623	-1.09380	0.55061	0.00000	-0.54475	0.69388
p-value	0.04290	0.23720	0.11320	0.63280	1.00000	0.40522	0.36065
Fs	3.67939	-0.09474	2.20237	6.05901	0.00000	2.36921	2.32565
p-value	0.95630	0.22810	0.83320	0.99640	N.A.	N.A.	N.A.

**Figure 2.** Distribution mismatch curve.**Table 10.** Comparison of SSD and Rg by locality.

Locality	SSD	P-value	Rg	P-value
Sébikotane	0.25	0	0.68	0.3
Santh Ndong	0	0	0.69	0.5
Diofior	0.21	0	0.68	0.6
Mboro	0.18	0	0.26	0.7
Malika	0.213	0	0.28	0.25

(Index of Irregularity) indicated that *P. xylostella* populations were composed by some well-differentiated groups. Values of SSD p-value (0.000) were significant (Table 10).

3.4. Analysis of Phylogenetic Relationships

The maximum probability tree showed two clades: the first one contained individuals of *P. xylostella* from four localities, namely: Santh Ndong, Sébikotane, Diofior and Malika. This clade presented a sub-clade only composed of individuals from Malika, and another one containing individuals from Diofior, Sébikotane and Santh Ndong. Individuals from Diofior distinguished themselves from others in this sub-clade. The second clade reassembled all the individuals from Mboro, two from sébikotane, and one from Diofior (Figure 3 and Figure 4).

4. Discussion

In spite of the weakness of our sampling, which was materialized by five populations

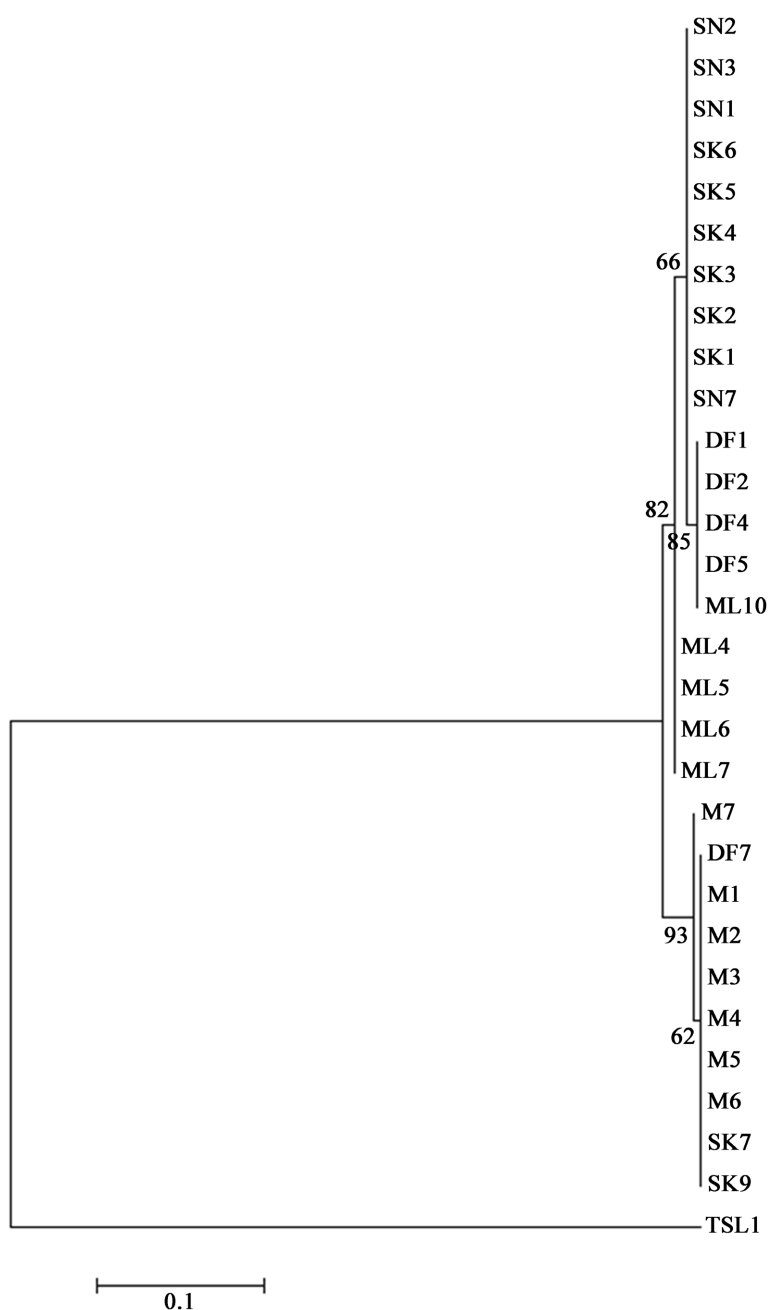


Figure 3. Neighbor-Joining (NJ) tree for *P. xylostella* individuals.

from five localities, with about twenty-nine sequences obtained (29), results revealed the existence of five (05) haplotypes. Our result was really important compared to that found by Saw *et al.* [16], who got only three (03) haplotypes out of forty-two (42) butterflies largely distributed in Australia. Sequence analysis of the mitochondrial gene COI, with 681 pb, from eighty (80) individuals gave sixteen (16) haplotypes [17]. In China, from ten (10) populations, thirty-two (32) haplotypes were detected in a sample of 149 adults [18]. In our study, most of the haplotypes were individually and essentially found in two localities; this could be an evidence of the *P. xylostella* scattering capacity. This result was akin to

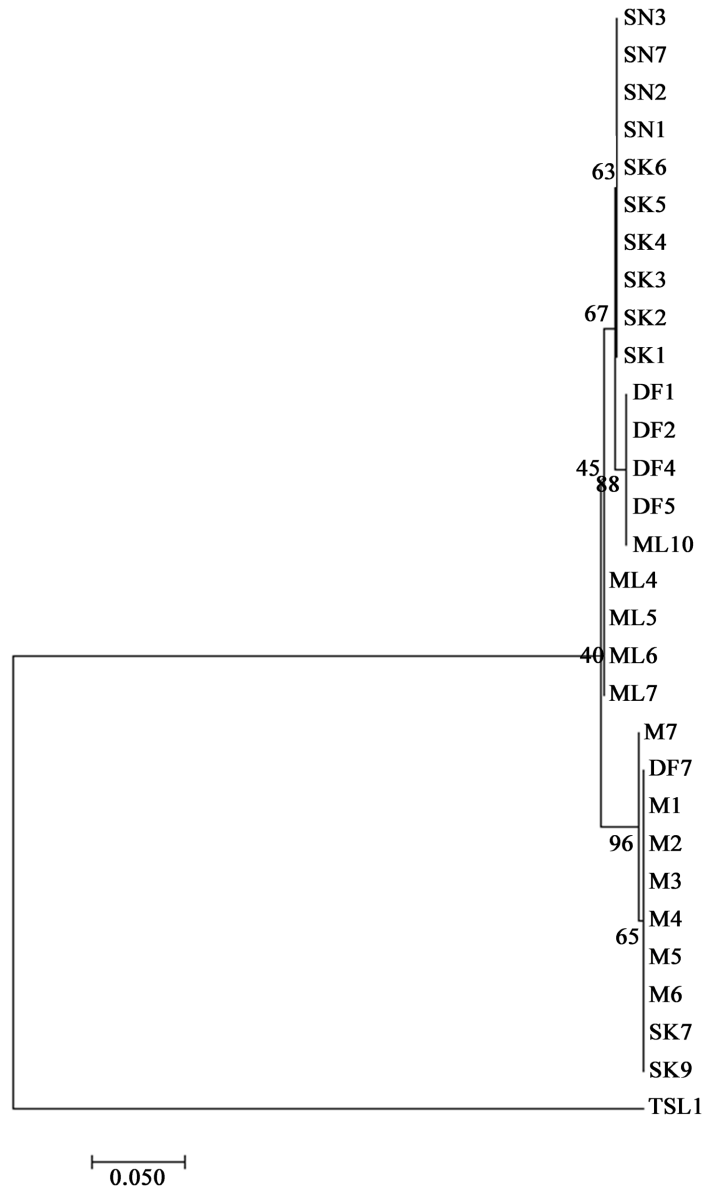


Figure 4. Maximum likelihood tree of *P. xylostella* individuals.

that of Niu *et al.* [18]. Analysis of the haplotype netting showed that all the two dominant haplotypes were scattered in Mboro and Sébikotane for the first one, and in Sébikotane and Santh Ndong for the other; those four localities belonged to Niayes area. We could then assume the existence of a high rate of migration between Mboro, Santh Ndong and Sébikotane localities. Radar data upheld intense migrations about *P. xylostella* [19], too. Moreover, studies, by Caprio and Tabashnik [20] showed that an important proportion of individuals (about 7.8% a day) migrated; the distance could be estimated at about several thousand kilometers [21]. A strong haplotype and weak nucleotide diversities were noticed in the global sample, too. This is in accordance with Wei *et al.* [22] who showed a big haplotype diversity and a weak nucleotide diversity in populations of *P. xylostella* in China. In analyzing COI data, Yang *et al.* [23] determined that popu-

lations of *P. xylostella* from China presented a big mitochondrial and nucleotidic haplotypes diversities. The difference observed between our study and that by Yang *et al.* [23] could be explained either by the weak size of our sample, or the short distances between localities in our study. The nucleotide diversity of the Australian populations of *P. xylostella* was particularly weak unlike that detected in the sample from Kenya [16]. The genetic structure showed that *P. xylostella* population from Mboro was set apart from all other populations, with significant degrees of genetic differentiation. This differentiation may be justified by a local variation of resistance against pesticides. This could be supported by Caprio and Tabashnik results [23], which indicated that the local variation about the resistance against insecticides, for the Pyrale of Diamond Hawaii population, was not an indication of a restricted genetic flow, but probably due to a local variation of selection. Other studies revealed that the largest distances between *P. xylostella* populations reflected probably the mass and the recurrent use of insecticides [9]. A genetic difference were noticed between strains of *P. xylostella* that resisted against pesticides and sensitive ones [24], but between populations at different temperatures and altitudes, too [25]. From information above mentioned, we could assume that *P. xylostella* populations would have a strong capacity of adaption and resistance against pesticides. Migratory capacities and the increasing resistance to insecticides in many populations, made the management of the species more and more hypothetical [10]. However, there was no significant genetic differentiation between *P. xylostella* populations from Diofior and Sébikotane, on one hand, and those from Sébikotane and Malika, on the other. There would have recurrent exchanges of individuals between these populations, either by migration or more likely by means of marketing. Analysis of the genetic flow of *P. xylostella* populations from China, by Wei *et al.* [21], revealed that the number of emigrants, by generation in populations from northern region, was very high, whereas that of the southern region was rather weak. With few genetic differentiation ($F_{st} = -0.038, -0.309$) and a high rate of females, migration ($Nm = 1.117$ -infinite) between the Chinese populations, suggested that the scattering over long distances was a factor of demography of this species [17]. Molecular variance analysis showed that 61.44% of variation was due to a variation between populations (localities). That further consolidated the assumption that *P. xylostella* populations were structured according to pressure from pesticides. In contrast, our results were not in accordance with those of Roux *et al.* [9] who found that most of variability occurred within populations themselves (AMOVA: 73.71%). Mismatch neutrality tests revealed that the population of *P. xylostella* in Senegal were stable. Phylogenetic trees showed the existence of two clades with a discrimination of individuals from Mboro in one of the clades; which one's more pointed out the peculiarity of that population. In the other clade, populations split up in two sub-clades. These two clades revealed the presence of two groups of *P. xylostella* in Senegal. Our results, were in agreement with those of Wei *et al.* [21] who indicated the existence of two groups of Diamond ringworm in China and worldwide.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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