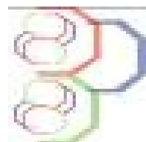

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Identification of *Tribolium castaneum* (Herbst) haplotypes, the pest of stocked millet in Senegal.

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ABSTRACT

Objective: Millet is the staple food of millions of people living in the Sahelian zone and the tropical countries of West Africa. It occupies an important place in the Senegalese alimentation. The major damages to this cereal are caused by the beetle *Tribolium castaneum* Herbst (Tenebrionidae) whose adults give a repulsive smell of stored food and making them unusable. The objective of this study is to identify the different haplotypes of *T. castaneum* and their distribution in Senegal.

Methodology and Results: To achieve this, a sampling was performed in seven (7) localities of Senegal belonging to four agro-ecological zones. The DNA of each individual was extracted and the cytochrome b was amplified with Qiagen protocol and then sequenced. The results showed the existence of nine (9) haplotypes in Senegal and the haplotype Hap_1 prevailed among them and was present in all sampled areas. Haplotypes Hap_4 of Mbam, Hap_3 of Diaroumé, Hap_5 of Koungheul and Hap_7, Hap_8 and Hap_9 of Sandiara were individuals. Sandiara had a market for cereals, which may be a plausible reason for getting the largest number of haplotypes. The Hap_5 comes in all networks as the central Haplotype. Changes of nucleotide composition did not affect the amino acid composition of the haplotypes. Thus, all the mutations were synonymous.

Conclusion and application of results: From 2012 to 2015, 9 haplotypes on 12 of *Tribolium castaneum* imposed themselves in these localities. 9 haplotypes were identified in Senegal including 6 (six) of them are individual. The locality of Sandiara has the largest number of haplotype of *T. castaneum* because of the very strong marketing of cereals and vegetables in that area. The mutations are synonymous; Haplotypes therefore would have the same behaviour towards biopesticides.

Keywords: *Tribolium castaneum*, Cytochrome b, haplotype, Senegal, identification, distribution

INTRODUCTION

The world population has been growing significantly since the end of World War II while giving birth to an imbalance between demographic evolution and food self-sufficiency in many regions through the world (Rosenzweig & Parry, 1994) But it becomes more and more difficult to increase that agricultural production due to climate changes that can cause drought, floods and the emergence of new pests jeopardizing the most vulnerable species or plant varieties. In Africa, especially in the Sahel region, food security is mainly based on cereal and some farming activities for private income; among them production of millet, which occupies a very important place (Saidou *et al*., 2009). Millet, *Pennisetum spp*, is the most tolerant cereal to drought. Its cultivation covered more than 30 million hectares in 1994, and was distributed mainly in arid and semi arid areas of Africa. Millet, being the staple of the people, is squarely the most important cereal in Senegal for both the planted area and for the production (MAE, 2001). Millet represents on average more than 60% of cereal production in Senegal (about one million tons / year) mainly from the groundnut bassin and Tambacounda. Pearl millet (scientific name) is the type almost exclusively used in Senegal as human food and/or animal feed (Diakhaté, 2013). Farming average yields varies between 0.5 and 0.6 t / ha. These low yields that vary between 0.5 and 0.6 t / ha, result from a combination of abiotic constraints: mainly drought, and biotic constraints: diseases (mildew, coal and ergot) and insects (beetles, drillers and cantharides). Many studies are focused on the attack and the loss of stocked cereals and legumes by insects (Philogène *et al.,* 1989; Ratnadass *et al*., 1989; Ashamo, 2006). They are responsible under the tropics of losses ranging from 30% (Gueye *et al.,* 2011) to 44% (Fouabi 1989 in Sezonlin, 2006). The main pest of millet is the beetle *Tribolium castaneum* (Herbst). It is a primary pest, even if the development of its larva upon the whole seeds is very slow (Diome *et al.,* 2013). The insect caused major damages on beaten stocked millet throughout the entire Sahel region (Roorda *et al.,* 1982). Then it would be necessary to seek effective methods to fight against that pest in accordance with the environment standards and public health. It is therefore essential to perfectly know the insect *T. castaneum* and to find an integrated fight against this pest of millet, which is the main cereal of Senegal. This study aims to identify the haplotypes of *T. castaneum* and to know their distribution in Senegal's territories. For this, we seek to determine the polymorphism of the *T. castaneum's* population; to identify its haplotypes and individuals, which constitute them, and to study the geographical distribution of these haplotypes in Senegal.

MATERIAL AND METHODS

Sampling: The study has been conducted in seven localities of five regions of Senegal (Figure 1): Kaffrine (Koungheul and Keurbouki), Fatick (Djilas and Mbam), Thies (Sandiara) Kaolack (Karang) and Sédhiou (Diaroumé). In each locality, 205 grams of millet was taken from the fields and the resulting samples were placed in a plastic packet of 500 grams of transparent colour with closing and opening device at the end of the bag. Then they were brought back to the laboratory and were transferred into daily monitored jars. In the laboratory, the samples were distinguished then encoded according two criteria: the studied insect (*T. castaneum*) of which we used the first letter of the genus' name in uppercase and the first letter of the species' name in lowercase; and the geographical origin by taking the first letter and the last letter of the area's name, if there were two localities beginning with the same letter and if the locality is written with two words, the first letter of each word was taken/ considered. Seven populations were defined according the fields and each of them was composed of several individuals of *T. castaneum* from the same locality as shown in Table 1.

Figure 1: Location of sampled areas in the regions of Senegal

According to agro-ecological zones, Djilas (14 ° 14 '45"N, 16 ° 38'04"O) and Mbam (14 ° 07' 06"N, 15 ° 37 '04"O) are in the center of the groundnut basin, Sandiara (14 ° 26'02"N, 16 ° 47 '33"O) is in the agro-ecological zone of the North of the groundnut basin, Keur Bouki (14 ° 08' 14"N, 15 ° 49 '44' '), Karang (13 ° 35'N, 16 ° 42'W) and Koungheul (13 ° 59'N, 14 ° 48'W) are in the south of the groundnut basin and Diaroumé (12 ° 59' 08"N, 15 ° 37 '04"O) belongs to the agro-ecological zone of eastern Senegal / Upper Casamance.

Table 1: Number of individuals, each individual code , localities, agro-ecological zones and places of storage of sampled individuals

Populations	Regions	Localities	Places of storage	Agro-ecological zones	Insect Code	Sampled individuals number
Kl	Kaffrine	Koungheul	Commerce store	South of the groundnut basin	TcKl	9
Kb		Keur_Bouki	Attic		TcKb	9
Kg	Kaolack	Karang	Producer store		TcKg	8
Ds	Fatick	Djilas	Producer store	center of the groundnut basin	TcDs	9
Mb		Mbam	Commerce store		TcMm	9
Sa	Thiès	Sandiara	Producer store	North of the groundnut basin	TcSa	9
De	Sédhiou	Diaroumé	Producer store	Eastern Senegal/Upper Casamance	TcDe	7

Extraction of total DNA, PCR and sequencing of Cytochrome b: DNA extractions of *T. castaneum* were done by using the Qiagen DNeasy Tissue Kit. The insect was dissected first. Only the thorax and legs were used for extraction. After grinding they were placed in a 1.5 ml tube, wherein was added 180 µl of ATL digestion buffer that contained detergents, which caused dissociation of tissues and an individualization of cells. 20 µl of K proteinase were added to degrade all proteins after incubation at 55 ° C for 3 h. Tissues debris were removed after a quick centrifugation and recovery of the supernatant. In the case where the sample was not completely digested, the mixture was centrifuged again and the liquid was decanted into a new tube to eliminate cells debris. To that solution, 200 µl of cell lysis buffer was added then an immediate passage to the vortex and incubation for 10 minutes at 70 ° C. After this incubation, ethanol 96-100% was added to the mixture, placed in a column and then centrifuged at 1361.357 rad.s-1 (13,000 rpm) for one minute to retain the DNA at the column's membrane. The DNA being negatively charged was absorbed by

ionic interaction on the column's membrane (positively charged). Proteins, lipids and polysaccharides were not retained by the membrane and will end up at the bottom of the collecting tubes and will be discarded. The DNA, fixed on the column was then purified in order to remove any traces of contaminants. This washing was carried out by adding successively two wash buffers (500 µl) which passed through the membrane by centrifugation at 1361.357 rad s-1 (13,000 rpm) for respectively 1 and 3 minutes. The column was then placed in a 1.5 ml tube. Due to the small size of these insects, two elutions of 40 µl and 20 µl were carried out respectively with Buffer AE previously incubated at 70 ° C to increase the yield from 15 to 20%. After this elution, the DNA was conserved at -20 ° C. The PCR was performed on fragments of the mitochondrial gene coding: the cytochrome b. CB1 primers (5'-GTA TAT CTA CCA TGA GGA CAA ATA TC-3 ') and CB2 (5'-ACA ATT CCT CCT GGA TTA AAT TTA AT-3') were used for the amplification of the gene of cytochrome b. The amplification was performed in a reactionnal volume of 25 µl containing a mixture of 21 µl and16.3 µl of milli water Q; 2.5 µl of 10x buffer; additional 1 µl of $MgCl_2$; 0,5µl dNTP; 0.25 µl of each primer (forward and reverse); 0.2 µl of Taq polymerase and 4 µl of DNA extract. It was done by the repetition of cycles that ensures a multiplication by 2 of the target DNA in each cycle. This was carried out in an equipment known as Eppendorf thermocycler with the following amplification conditions: initial denaturation at 94 ° C for three minutes, followed by 35 cycles of denaturation at 94 ° C for 1 minute, annealing at 48 ° C for 1 minute, an elongation of the complementary DNA strand at 72 ° C for 1 minute and a final elongation at 72 ° C for 10 minutes ended the PCR. This technique consisted in determining the nucleotide sequence of one DNA fragment. It allowed, by comparing the sequences of the same gene of different individuals, to bring out point mutations. Sequencing was based on a PCR reaction using, in addition to the usual compounds (matrix DNA, polymerase, primers, dNTPs, Mg^{2+}), modified nucleotides: the dideoxyribonucleotides (ddNTPs). These ddNTPs had the distinction of being coupled to fluorochromes: green-ddATP, red-ddTTP, blue-ddCTP and yellow-ddGTP but also of lacking an OH group at the extremity 3' of the deoxyribose. The ddNTPs incorporation by polymerase blocked the elongation of the complementary DNA molecule being copied and resulted in generated fragments of different sizes. The gel reading was effectuated by the scanning of an automatic laser, which allowed the detection of the different coupled fluorochromes to the 4 ddNTPs.

Genetic Analyses:

- The analysis of polymorphism and the genetic variability of *T. castaneum* population. The sequences were manually corrected and aligned first by using the Clustal-W algorithm (Thomson *et al*., 1997) with the Bio Edit 7.0.8.0 software (Hall, 1999). Variables sites such as singletons or uninformative sites and parsimony informative sites were determined with DnaSP software. The nucleotide composition depending on the codons'position, the synonymous and non-synonymous mutations were analyzed using the software MEGA Version 06 (Tamura *and al.,* 2011).

-Identification of Haplotypes: The DnaSp software enabled determination of the number of haplotypes of the overall population. Thus, the DnaSp software also determined the number of individuals within each haplotype. Polymorphic sites were manually identified and grouped by haplotype in a new alignment with Bio Edit to determine the nucleotide notable differences between one haplotype to another and thus identify haplotypes, which had more mutations. The amino acid composition of each haplotype was also determined using the Bio Edit software. The MEGA6 (Tamura *et al.,* 2011) Software was used to elicit the nucleotide frequencies and the amino acid composition with the frame 3 (no stop codon) by haplotype. The minimum haplotype network was traced with the Network software v 4.6.14 through the Median-Joining method (Bandelt *et al.,* 1999). For its achievement, each haplotype was represented by a single sequence to see the exact location of the mutational steps and the haplotype, which had the most similar sequence among the others. Phylogenetic trees of haplotypes with the maximum parsimony and the maximum likelihood method were built by the MEGA6 software (Tamura *et al.,* 2011). The Kimura-2-parameter model was used for the method of distance and the Corrected Akaike Information Criterion (CAIC), which measured the quality of a statistical model. It allowed us to estimate the best model of evolution for the maximum likelihood tree and Bayesian inference. Thus, the employed evolution model uses the substitution matrix HKY (Hasegawa-Kishino-Yano) model for both probabilistic methods. The robustness of nodes for the maximum likelihood method was evaluated for 1,000 repetitions. The MEGA6 software built the maximum likelihood tree. The tree of the Bayesian approach was built by the Mrbayes software 3.1.2 (Huelsenbeck & Ronquist, 2001). This is an approach that uses the concept of

posterior probabilities, which could be estimated, by using the Monte Carlo method with the Markov Chains (MCMC). This method used simultaneously four channels (three were "heated" gradually) using the HKY model. 10 000 000 generations were performed for each channel by sampling the various parameters every 1000 generations. Generations effectuated during the ignition period were eliminated from the analysis. Conservatively, the first generations of 2500000 were eliminated (25%) and inferences were then realized on the 7500000 next generations. The reconstructions were rooted with a sequence of *Tribolium confusum* (Coleoptera, Tenebrionidae).

-Distribution of haplotypes: The distribution of haplotypes was studied by using the haplotype network traced initially according to localities and secondly according to agro-ecological zones with the Network software v. 4.6.14 and with the Median-Joining method (Bandelt *et al*., 1999). Each haplotype is represented this time by the whole set of sequences that determine them.

RESULTS

Polymorphism and genetic variability: After the cleaning and the alignment of sequences, no deletion or undefined nucleotide were observed in this dataset. 60 sequences, each having 398 sites of which 97.73% were invariable or monomorphic and 2.27% were polymorphic or variables by two variants induced by 9 mutations (Eta equal to 9). Among the polymorphic sites, 1% were singletons at positions 9, 24, 45 and 203, the remaining 1.27% were informative sites in parsimony at positions 63,212,273,302 and 395. Polymorphic sites with more than two variants were not observed. (Table2)

Table 2: Total number of scanned sites and percentage of polymorphic and invariable sites.

Parameters / Population	Total number of sites	Monomorphic Sites	Polymorphic sites	
			Singletons Sites	Informative Sites in parsimony
Total Population	399	97,73%	1%	1,27%

Identification of haplotypes: After the alignment, the 60 obtained sequences were divided in nine (9) haplotypes (Table 3). Among them, the haplotype Hap_1 prevailed with 36 individuals or 60% of the sample, followed by the haplotype Hap_2 with 15 individuals representing 25% and the haplotype Hap_6 with 3 individuals or 5%. The remaining haplotypes such as Hap_3, Hap_4, Hap_5, Hap_7, Hap_8 and Hap_9 were individual and each represented 1.67% of the overall population.

Table 3: Different haplotypes and the individuals within them

Haplotypes	Individuals
Hap_1	(36) TcDe1TcDe4TcDs1TcDs2TcDs3TcDs4TcDs5 TcDs6TcDs8TcDs9TcDs10 TcKb1TcKb2TcKb3TcKb4 TcKb5TcKb7TcKb8TcKb9TcKb10TcMm3 TcMm7 TcMm8 TcMm12 TcKg3TcKg7 TcKg8TcKg10 TcKl2TcKl3TcKl4 TcKl5TcKl6Tckl7TcS6TcS9
Hap_2	(15)TcDe2TcDe3TcDe6TcDe8TcMm2 TcMm5 TcMm6 TcMm10 TcMm11 TcKg4TcKg5 TcKg9TcS1 TcS2TcS8
Hap_3	(1)TcDe9
Hap_4	(1)TcKg1
Hap_5	(1)TcKl8
Hap_6	(3)TcK19TcK110TcS10
Hap_7	(1)TcS3
Hap_8	(1)TcS5
Hap_9	(1)TcS7

For the all haplotypes, the mutations were substitutions between purine bases in one hand and pyrimidine bases on the other. Therefore, all mutations were transitions. The haplotypes hap_2 hap_9 had more

transitions than others did if we took as reference the haplotype hap_1, which had the majority (Table 4).

Table 4: Different mutations between haplotypes

Sites variables / Haplotypes	9	24	45	63	204	213	274	303	396
Hap_1	C	T	A	T	C	C	C	A	G
Hap_2	C	T	A	C	C	T	T	G	G
Hap_3	C	C	A	T	C	C	T	A	G
Hap_4	C	T	G	T	C	C	T	A	G
Hap_5	C	T	A	T	C	C	T	A	G
Hap_6	C	T	A	C	C	C	T	G	G
Hap_7	C	T	A	T	C	C	T	A	A
Hap_8	T	T	A	T	C	C	T	A	A
Hap_9	C	T	A	T	T	C	T	A	G

Frequencies of nucleotides according to positions: Frequency in nucleotides for all the sequences showed that, at the first, the second and even the third position of codons, Thymine and Adenine were more represented with higher frequencies than Guanine and Cytosine. Globally, the percentage of A-T was greater than that of C-G (1st, 2nd and 3rd position of the codon). Taking into account the double link between Thymine and Adenine with higher frequencies and the triple link between Guanine and Cytosine, the Cytochrome b of the *T. castaneum* species was exposed to changes and these will result in most cases to an amino acid change (Table5) if that mutation was localized in the first two positions of codon.

Table 5: Nucleotide frequencies according to the positions on the triplet of the total population

	Positions	**T**	**C**	**A**	**G**
Average	Total	33,4%	25,5%	29,8%	11,3%
	1st P	27%	25,3%	26,3%	21,1%
	2nd P	41%	24,8%	24,1%	9,8%
	3rd P	32%	26,3%	38,9%	3,2%

Amino acid composition: The amino acid composition was obtained according to the FRAME 2 (2nd reading frame), which revealed less stop codon (any stop codon for Cytochrome b of *T. castaneum*). It revealed that, despite the transitional substitutions observed between haplotypes, they all had the same protein chain. Therefore, mutations leading to the appearance of these haplotypes were not synonymous. Minimum network of haplotypes: Each ellipse represented one haplotype. The separate lines between haplotypes were mutational steps. (Figure 2).

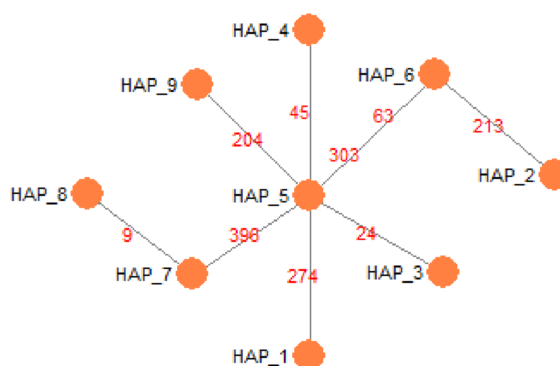

Figure 2: The Minimum Network of haplotypes

The network of haplotypes revealed that Hap_5,being a private haplotype of Koungheul, was also the central haplotype. Hap_1, which had the majority, stemmed from Hap_5 with the transitional substitution of Thymine by Cytosine. Haplotypes Hap_3, Hap_4, Hap_9 and Hap_7 stemmed also from Hap_5 with the respective transitional replacements of Thymine by Cytosine at the 24th position, of Adenine by Guanine at the 45th position, of Cytosine by Thymine at the 204th position, and of Guanine by Adenine at the 396th position. The Hap_6 came from Hap_5 after two mutational steps to positions 303 and 63 with respective transitions of Adenine to Guanine and of Thymine to Cytosine. Haplotypes, Hap_8 and Hap_2 came respectively from transitional substitutions of Cytosine by Thymine of haplotypes Hap_7 at position 9 and Hap_6 at the 213th position.

Phylogenetic trees of haplotypes: The obtained samples in seven localities in Senegal were divided in four agro-ecological zones. Sampled individuals were divided into 9 haplotypes. Trees were rooted with an individual of the species *Tribolium confusum* (Figure 3). The phylogenetic tree of maximum likelihood (ML) accented the existence of three haplogroups and the Bayesian inference (BI) revealed only two. With posterior probabilities of 81% with ML and 100% with BI, haplotypes Hap_2 and Hap_6 formed the first haplogroup. The second, with the posterior probabilities, smaller than those of the first haplogroup, with 67% with ML and 71% with BI was formed by haplotypes Hap_7 and Hap_8. The third haplogroup, revealed by the ML reconstruction, was composed by haplotypes Hap_5 and Hap_ (Figure 3).

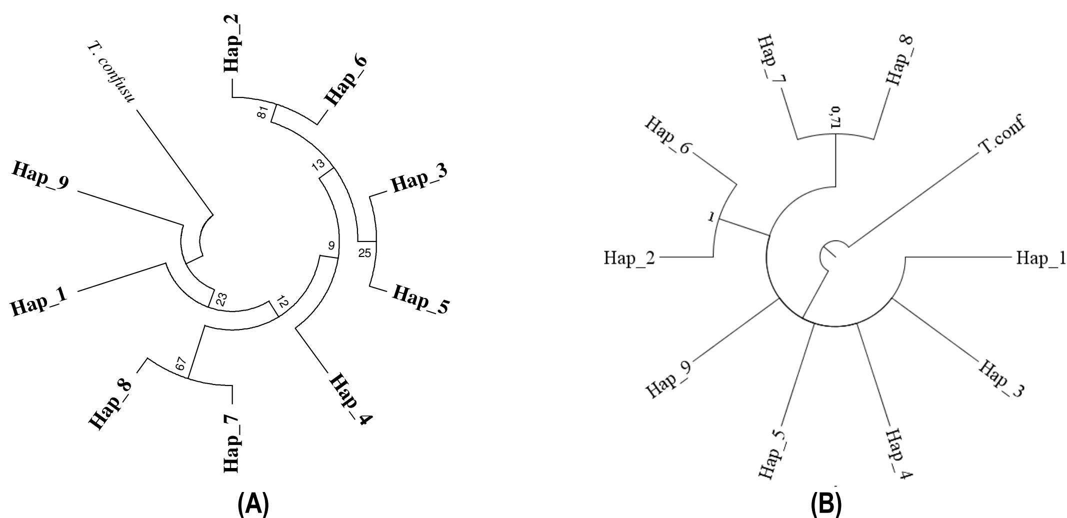

Figure 3: Phylogenetic trees of haplotypes by Maximum Likelihood method (A) and Bayesian Inference (B).

Distribution of haplotypes in Senegal: Distribution based on the sampled localities**:** According to localities, the network of haplotypes showed that the haplotype Hap_1which had the majority was represented in all sampled areas (Figure 4). It was the only haplotype present in the localities of Djilas and Keur_Bouki. Hap_2 was absent from these two localities, but was present in Diaroumé, Sandiara, Karang, Koungheul and Mbam. After Hap_1, the haplotype Hap_2 was the most distributed. Hap_7, Hap_8 and Hap_9 were private haplotypes of the locality of Sandiara. Haplotypes Hap_4, Hap_3, were private respectively in Mbam and Diaroumé. Hap_5, being the central haplotype of the network, was a private haplotype in the locality of Koungheul (Figure 4).

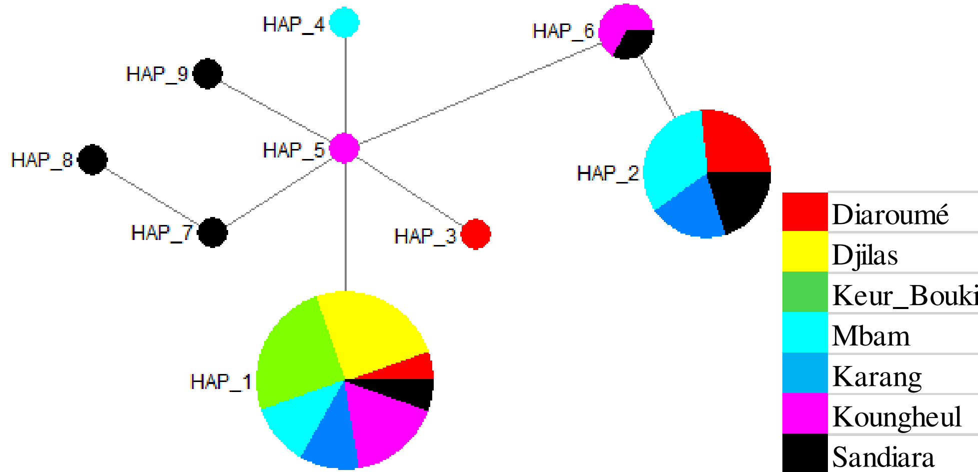

Figure 4: The haplotypes network based on sampled localities in Senegal

Distribution based on sampled agro-ecological areas: The distribution of haplotypes according to agro-ecological areas showed that Hap_1 and Hap_2 were the most distributed haplotypes; the most shared and they were found in all sampled agro-ecological areas (Figure 5). Hap_1 was much more common in the south and in the Center of the Groundnut Basin (SBA), less common in the North of the Groundnut Basin (NBA) and in the area of the Low Casamance (BA). The haplotypes number was higher in the North of the Groundnut Basin (6 identified haplotypes: Hap_1, Hap_2, Hap_6, Hap7, Hap_8 and Hap_9) and in the South of the Groundnut Basin (5 identified haplotypes: Hap_1, Hap_2, Hap_4, Hap_5 and Hap_6). Haplotypes Hap_7, Hap_8 Hap_9 were private in the Northern Zone of the Groundnut Basin, haplotypes Hap_4, Hap_5 were private in the southern area of the groundnut basin and the haplotype Hap_3 was a private one in the Low Casamance. The Center of the Groundnut Basin (CBA) had no private haplotypes and had only haplotypes Hap_1 and Hap_2 (Figure 5).

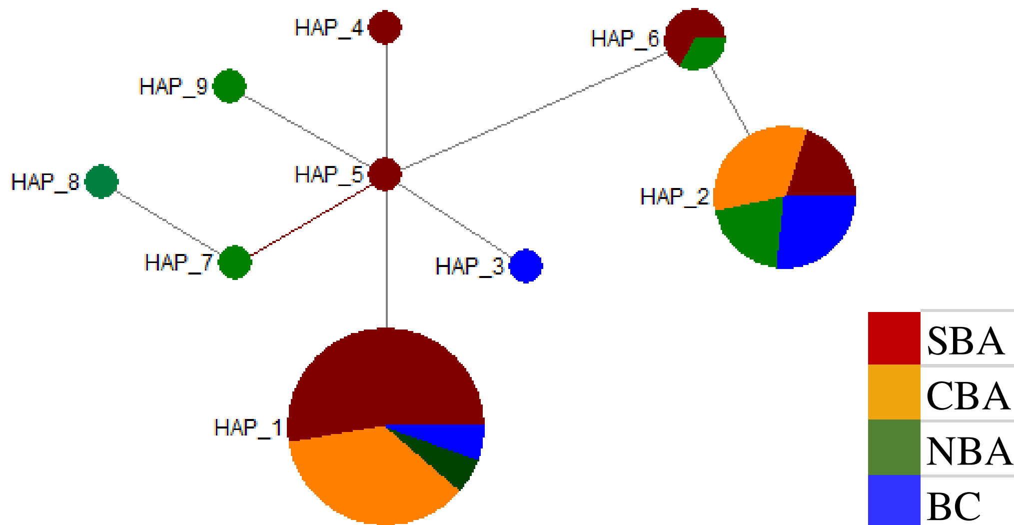

Figure 5: Haplotypes network based on sampled agro-ecological areas in Senegal: *SBA: South Groundnut Basin*; *CBA: Center of the Groundnut Basin*; *NBA: North of the groundnut Basin*; *BC: Low Casamance.*

DISCUSSION

The objective of this work was to firstly identify the different haplotypes of *T. castaneum*, pest of millet in stock, and secondly to see its distribution in the different agro-ecological zones and in the sampled localities of Senegal. After a careful alignment of the 60 sequences in seven localities belonging to four agro-ecological zones, we noticed a degree of polymorphism with a rate of 2.27% of variable sites. These variables sites were caused by 9 mutations, which were all transitional substitutions, that gave birth to 9 haplotypes. These results confirmed those of Dia *et al.* (2014) who had found 9 haplotypes with 71 sampled individuals in Senegal. It confirmed also the decrease of 3 haplotypes from 2012 to 2014 based on the results of Diome *et al.* (2012), which had recorded 12 haplotypes in Senegal while those of Dia *et al.* (2014) found only 9.The haplotypes had the same protein chain, it implied that the observed transitional substitutions were synonymous. This was due to the redundancy of the genetic code. Therefore, these changes would have any effect in the role of the cytochrome b, on the insect physiology and phenotype. The space of development cycle between individuals of Djilas and Karang in one hand and Diaroumé and Djilas on the other, which was revealed by the studies of Diome *et al.* (2015), would not be determined then by the variability of cytochrome b. In terms of numbers, the haplotype Hap_1 had the majority with 60% of individuals and was found in all the sampled localities and agro-ecological zones. This haplotype would be national in Senegal and this result confirmed those of Dia *et al.* (2014) and Diome *et al.* (2008), according to them the haplotype Hap_1 was fixed and national. Hap_2 was the most different haplotype of Hap_5, which was considered as the central haplotype, with three nucleotide replacements that occurred for its formation. Despite this difference, Hap_2 was found in all agro-ecological zones and in almost all localities except in Djilas and Keur_Bouki. According to Diome *et al.* (2012), Isman (2012) and Dia *et al*. (2014), the trade flow between these areas might be the cause of such a distribution of haplotypes Hap_1 and Hap_2. They showed that the marketing of cereal was the cause of the genetic variability of *Tribolium castaneum* in Senegal. The Hap_5 is identified in the locality of Karang and, according to the minimum haplotype network, it is the central haplotype, according to the minimum network haplotype which originate from other haplotypes by some transitional nucleotide substitutions .In addition, the haplotype Hap_1 was the most distributed and the only one that was identified in Djilas (Closed population (Isman, 2012)) and in Keur_Bouki (No differentiation due to a founder effect, or their way of traditional storage in granary which influenced the proliferation of the insect thus its genetic diversity (Seck *et al.*, 1992)). This allowed us to think that Djilas and Keur_Bouki could not be infested by Hap 5, which would be the first insect's strain in Senegal. Since the appearance of the first haplotype of *T. castaneum* in Koungheul until the occurrence of one mutation of Cytosine by Thymine giving birth to Hap_1, this pest would not attack millet of Djilas and Keur_Bouki. This allowed us to think that the proliferation of this insect would coincide with the birth of the haplotype Hap_1. That is the reason why haplotype Hap_5 was not distributed in any other parts of Senegal. With trade flows Hap_1 would be scattered almost everywhere in Senegal but with this topology revealed by all haplotype networks, despite having the majority, it could not be the ancestral haplotype as the work of Diome *et al.* (2012) and Dia et *al.* (2014) had revealed. The low diversity observed in Djilas and Keur Bouki due to the fact that they had no markets and they grew millet in large quantities, that's why these communities rarely import millet (Diome, 2014), therefore rare importation of new haplotypes. The Haplotype Hap_4 observed in Karang mighty come from Gambia because they share some borderlands. *T. castaneum* haplotypes might be then imported with products from Gambia by the inhabitants of this town and increased the haplotype diversity (Dia *et al.,* 2014). These same causes would be the cause of the specific presence of the haplotype Hap_3 in Diaroumé (belonging to the low Casamance), which is close to Guinea Bissau. Mbam is an open population and that would be probably the cause of the presence of Hap_1 and Hap_2, therefore they would have been imported. The largest number of haplotypes was observed in Sandiara (town hosting a large market where farming products were mixed in trade stores), Diaroumé (town hosting a large market where farming products were mixed in trade stores) and Mbam (locality where farmers stored their products (millet, maize, rice, groundnuts and black-eyed peas in warehouses). They were hosting the largest number of haplotypes, some were private to them but some among the biggest part of the individuals belong to shared haplotypes between all localities and all agro-ecological areas, we can mention among them haplotypes Hap_1 and Hap_2. In addition, obtained trees with the method of Maximum Likelihood and

Bayesian Inference revealed that haplotypes Hap_2 and Hap_6 were the closest phylogenetically with strong posterior probabilities and showed also a topology of Haplogroups grouping haplotypes from different localities and different agro-ecological zones. This tally with the results of Dia *et al.* (2014), according to them, the phylogenetic groupings and the distribution of haplotypes were done neither by different localities, nor by agro-ecological zones of Senegal. Grain Marketing would be the cause of such a distribution of haplotypes.

CONCLUSION

This work allowed us to identify the different haplotypes of *T. castaneum* Herbst and their location in the Senegalese territory by using the mitochondrial gene cytochrome b. Thus the genetic characterization of *T. castaneum* shows that the haplotypes of this insect are (9), 6 (six) of them are individual. The locality of Sandiara has the largest number of haplotype of *T. castaneum* because of the very strong marketing of cereals and vegetables in that area. Haplotype Hap_1 is the most distributed and is located in all areas and in all sampled agro-ecological zones in Senegal but it cannot be considered at this level as the ancestral haplotype. This study also allowed us to see that the distribution of haplotypes was not done according to localities and sampled agro-ecological zones despite the large number of individual haplotypes compared to the total number of haplotypes. However, for a better understanding of this insect population, it would be important to expand the sampling in Senegalese territory up to Ferlo, the Senegal River Valley and the total area of Casamance, in order to better know its distribution throughout the national territory. This study will be considered as a gap in the geographical distribution of this insect in Africa and all around the world in order to know its phylogeography.

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