

Full Length Research Paper

# Haplotype diversity of *Tribolium castaneum* H. (Coleoptera, Tenebrionidae) pest of stored millet in Senegal

Toffène Diome<sup>1,3\*</sup>, Cheikh Thiaw<sup>2</sup>, Assane Ndong<sup>1,3</sup>, Malick Sarr<sup>2,3</sup>, Mamadou Kane<sup>1</sup> and Mbacké Sembène<sup>1,3</sup>

<sup>1</sup>Centre de Biologie pour la Gestion des Populations ; Institut de Recherche pour le développement. IRD/ Bel-Air Sénégal.

<sup>2</sup>Senegalese Institute of Agricultural Research; Regional Center for the Study on Improvement of Adaptation to Drought (ISRA/CERAAS), P.O. Box 3320 Thies Escale, Senegal.

<sup>3</sup>Département de Biologie Animale, Faculté des Sciences et Techniques, Université C.A. Diop, B.P. 5005 Dakar, Sénégal.

Accepted 8 June, 2012

In Senegal, millet occupies the most important place among the cereal crops. This cereal stocks are attacked by the Coleoptera, such as *Tribolium castaneum*. This beetle attacks grains and causes significant losses on stocks of beaten millet in the Sahelian zone. The objective of this study is to genetically characterize the populations of *T. castaneum* encountered in different agroecological zones to identify the different haplotypes circulating in Senegal. The results showed that there are at least 12 haplotypes of *T. castaneum* in Senegal. High haplotype diversity in the northern groundnut basin and high nucleotide diversity in the south of the groundnut basin were also noted. The cereal in the market of Sandiara was the cause of the wealth of haplotype and nucleotide diversity in the north of the groundnut basin. The high haplotype diversity and the low nucleotide diversity in the basin centered in eastern Senegal, high Casamance may be the result of rapid population growth from an ancestral population at low numbers. There was no significant genetic differentiation between agroecological zones. The strong genetic differentiation of some individuals with multiple mutations not in the haplotype network was due to a structure based on the host plant.

**Key words:** *Tribolium castaneum*, agro-ecological zones, haplotype, genetic diversity.

## INTRODUCTION

Millet is grown for cereal in the Semi-Arid Tropics zone of the Old World (Africa and India) (Tostain, 1998). It is the cereal most tolerant to drought and is the food base of several million people in the Sahel. In Senegal, millet and maize are the two major cereals. Millet occupies among

the cereal crops, the most important place both in terms of sown area (about 75%) and production (60%, MAE, 2001). Food base of 50 million of people, it remains the only crop that matches the conditions of the Sahel and traditional eating habits (Guèye et al., 2011). Current yields of these crops are far below the expectations of the food national coverage indeed, several factors contribute to limiting the increase in the production of millet among them, we have: the constraints of environmental agroecological degradation, those related to low utilization

\*Corresponding author. E-mail: [toffene.diome@ird.fr](mailto:toffene.diome@ird.fr). Tel: +221 77 427 06 13.

of seed and fertilizer, the hardship of millet threshing, problems of financing the needs of producers and constraints common to raising the price to producers of cash crops (Groundnut and Contons) (MAE, 2001). There is also a specific constraint in the crop of millet; it is the phenomenon of pests (ISRA, 1994). Since the beginning of human civilization, foodstuffs have been attacked by insects during their storage (Camara, 2009). According to an analysis by Kouassi (1991), foods are infested mainly by beetles, whose presence is undoubtedly related to the persistently high temperature and humidity to which these grain stocks are subjected. The damage caused by Coleoptera are mainly due to the beetles *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum*. *T. castaneum* (Herbst) is an insect of inventory that is ubiquitous, polyphagous and very formidable. The incidence of the pest is practically zero on the whole cob of *P. typhoid* stored in traditional granaries (Seck, 1983). On whole grain cereals, larvae develop slowly, but the species cannot only be considered as a secondary pest. In addition, certain currently widespread practices of farmers, such as, mechanical threshing millet after harvest are favorable for the development of *T. castaneum*. It causes severe damage on millet stocks beaten throughout the Sahelian zone (Roorda et al., 1982; Seck et al., 1992). Apart from ecological studies, few studies of the populations genetics of insects harmful to crops in general and in particular, species of beetles have been conducted in Senegal. Clearly, in evolutionary biology, differentiation of host races is widely discussed in phytophagous insects (Feder et al., 1988; 2003; Sembène, 2000). The beetle *T. castaneum* is polyphagous and met in different agro-ecological zones. Each zone has its own potential and also its own vulnerabilities to environmental hazards and climate. Would haplotype diversity of the insect arise with variation of agro-ecological zones? The objective of this study is to genetically characterize living populations of *T. castaneum* to identify the different haplotypes circulating in the storage locations of three agro-ecological zones sampled in Senegal.

## MATERIALS AND METHODS

### Study sites

Five localities (Mbam, Djilas, Sandiara Diaroumé, Karang and Koungheul) were sampled for genetic characterization. Djilas (14° 14' 45" N, 16° 38' 04" W) and Sandiara (14° 26' 02" N, 16° 47' 33" W) are in the agro-ecological zone of North groundnut basin, Mbam (14° 07' 06" N, 15° 37' 04" W) is located in the center of the groundnut basin, Diaroumé (12° 59' 08" N, 15° 37' 04" W) is in the agro-ecological zone in eastern Senegal high Casamance, Karang (13° 35' N, 16° 42' W) and Koungheul (13° 59' N, 14° 48' W) are in the South of groundnut basin in each locality, grains of millet and maize are collected in attics of warehouses or of producers or traders. These samples are then brought to the laboratory and preserved in

jars of 17 cm in diameter and 23 cm in height until adult emergence.

### DNA extraction and polymerase chain reaction (PCR) sequencing

The abdomen, elytra and antennae were kept apart to avoid contamination by fungi and nematodes and to allow subsequent morphological observations. To characterize both mitochondrial and nuclear DNA, a partial cytochrome B (CytB) and 28S ribosomal RNA gene regions were PCR-amplified. The primers of cytochrome b were used CB1 (5'-TATGTACTACCATGAGGACAAATATC-3') and CB2 (5'-ATTACACCTCCTAATTTATTAGGAAT-3'). The 25 ml PCR reaction mixture for the cytochrome b contained 18.3 µl water, 2.5 ml enzyme buffer supplied by the manufacturer, 1 µl MgCl<sub>2</sub>, 0.5 µl dNTP, 0.25 µl of each primer, 0.2 unit of Taq polymerase and 2 µl of DNA extract. After an initial denaturation step at 94°C for 3 min, followed by 35 cycles that are repeated distortion at 94°C for 1 min, annealing at 47 °C for 1 min and elongation of the complementary DNA strand 72°C for 1 min, a final elongation at 72°C for 10 mins end the PCR. Sequencing was performed by the company Macrogen in South Korea.

### Data analysis

The genetic changes that are noticed on the mitochondrial marker are the polymorphism of sequences (number and distribution of nucleotide substitutions). The term haplotype which tallies with nucleotide sequence can be common to several individuals, but differs from others by one or more nucleotide substitutions. Sequence alignment was performed using BioEdit. The haplotypes were determined by arlequin31. Different statistical tests: Tajima's D, Fu Fs (Tajima, 1989; Fu, 1997) were used to test the hypothesis of deviation from neutrality. The Fs test of Fu compares the average number of pairwise differences with the number of haplotypes (k) in the population. The D of Tajima is based on the difference between the average number of pairwise differences and the number of polymorphic sites (S). In a population of constant size, the D of Tajima and Fu's Fs are 0. Conversely, a demographic expansion leads to negative values of D and Fs (Excoffier et al., 2005). These parameters were calculated using software ARLEQUIN V3.1 (Excoffier et al., 2005). After the determination of these parameters for each gene, we have combined sequences for phylogenetic reconstructions. A bootstrap procedure (1000 iterations with the same option of heuristic search) was used to establish the score of each node (Felsenstein, 1985) by retaining group compatible with the 50% majority rule consensus. A strict consensus tree was computed whenever multiple equal parsimonious trees were obtained. The resulting data were subsequently used to calculate Kimura 2-parameter genetic distances between groups. The calculation of genetic distances, nucleotide diversity and variable sites as well as the reconstruction of phylogenetic trees by maximum parsimony and the maximum likelihood were performed using the software MEGA5. The sequences of *Caryedon serratus* are considered an out group in phylogenies.

## RESULTS

### Genetic diversity of *T. castaneum*

Our data set contained a total of 12 haplotypes, 54 variable sites, 52 sites of segregation and eight

**Table 1.** The polymorphism of the study population.

Number of sitediversity	Number of haplotypes	Polymorphic sites	Haplotypes
466	12	52	0.689

**Table 2.** Genetic variability of agro-ecological zones.

Genetic variability	North Basin	Central Basin	South Basin	Eastern Senegal / High Casa
sample size	9	18	15	9
haplotypes number	5	4	6	3
Variables sites	7	7	47	5
Genetic distances	0.006	0.004	0.017	0.005

parsimony-informative sites. Haplotype diversity (0.689) is high enough in the study sample (Table 1).

### The haplotypes

The haplotype number one is majority in the sample with 24 individuals and groups all individuals of all agro-ecological zones sampled but the southern area of groundnut is predominant. It is followed by the haplotype 2 containing 16 individuals. The agro-ecological zone of eastern Senegal high Casamance prevails in this haplotype. The remaining individuals are all individual haplotypes, except haplotype 10 which includes two individuals from the northern groundnut Basin. The area south of groundnut basin contains four individual haplotypes. All applotypes and individuals representing them are presented below. The haplotypes are shown in bold and individuals in italic.

**Hap\_1:** 24 [TcDe1 TcDe4 TcDs1 TcDs2 TcDs4 TcDs5 TcDs10 TcDs1 TcMm3 TcMm4 TcKg3 TcKg7 TcKg8 TcKI2 TcKI3 TcKI4 TcKI6 TcKI7 TcMm7 TcMm8 TcMm12 TcS4 TcS6 TcS9], **Hap\_2:** 16 [TcDe2 TcDe3 TcDe5 TcDe6 TcDe7 TcDe8 TcMm2 TcK2 Tckg4 TcKg9 TcMm5 TcMm6 TcMm10 TcMm11 TcS2 TcS8], **Hap\_3:** 1 [TcDe9], **Hap\_4:** 1 [TcDs6], **Hap\_5:** 1 [TcMm1], **Hap\_6:** 1 [TcKg1], **Hap\_7:** 1 [TcKI5], **Hap\_8:** 1 [TcKI8], **Hap\_9:** 1 [TcKI9], **Hap\_10:** 2 [TcS3, TcS5], **Hap\_11:** 1 [TcS7], **Hap\_12:** 1 [TcS10].

### Diversity based on agro-ecological zones

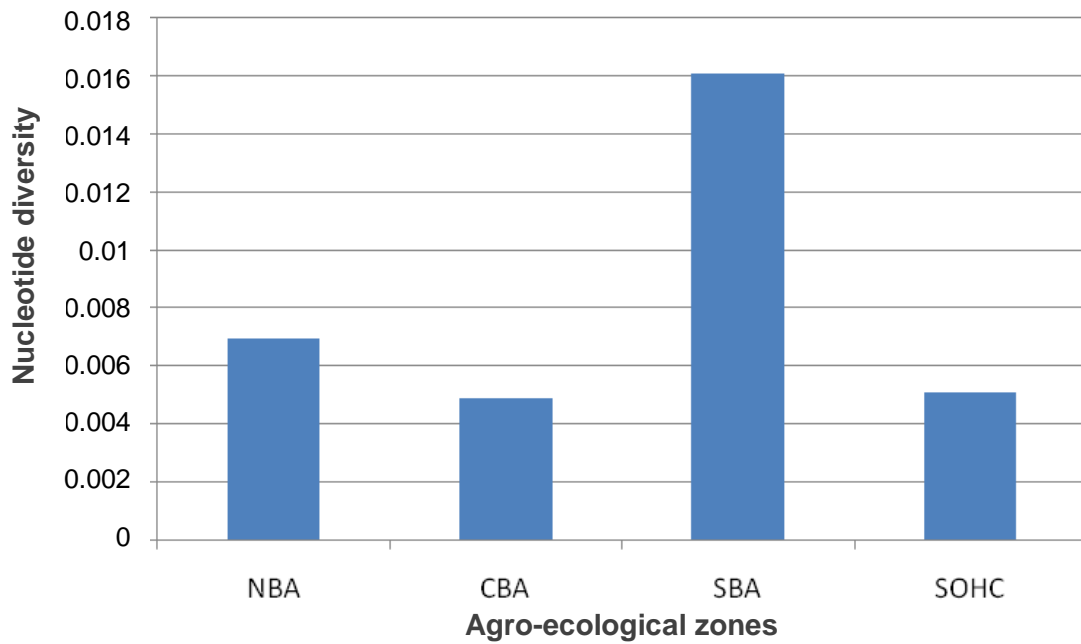
The area south of the groundnut basin contains more haplotypes and is followed by the north groundnut basin. The highest genetic distance as well as the highest haplotype diversity was observed in the northern zone of the groundnut basin and the southern groundnut Basin.

However, the center of the groundnut basin and the area of east Senegal High Casamance have low genetic distances 0.004 and 0.005 respectively (Table 2). South and North areas of the groundnut basin have the highest nucleotide diversity (Figure 1). However, the area south of the groundnut basin contains more variable sites compared to other agro-ecological zones, but the major part of these variable sites are carried by two sequences that are: TcKI5 TcKI9 and individuals representing respectively 5 and 9 of the locality of Koungheul.

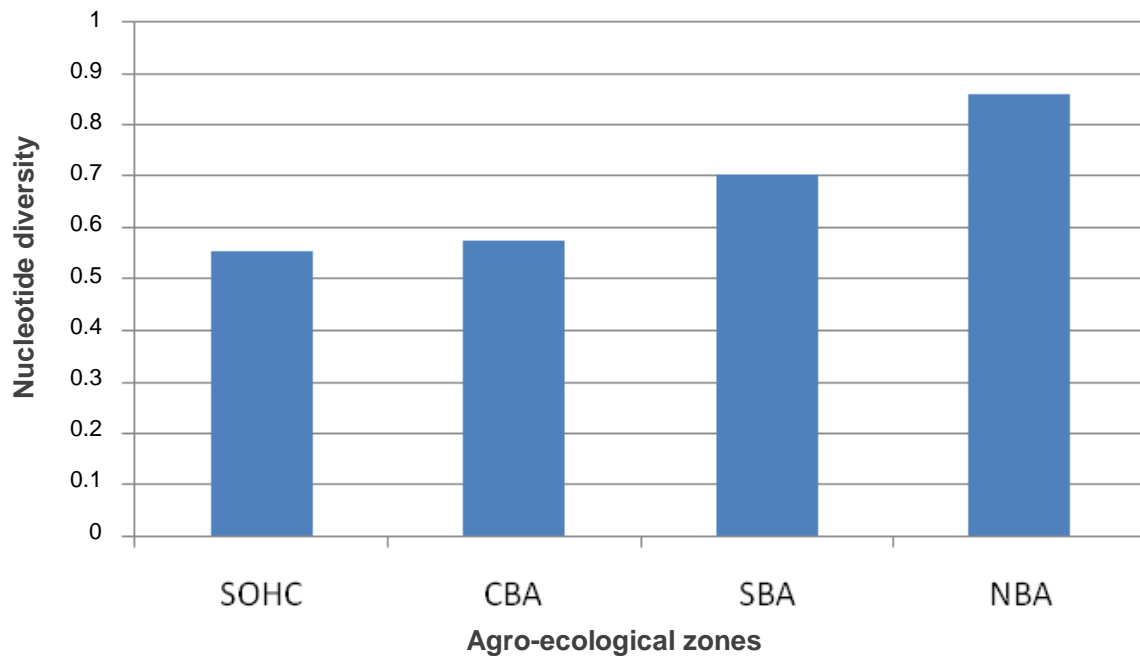
Haplotype diversity is higher in the northern area of the groundnut basin. Southern basin also has a high haplotype diversity (Figure 2). The latter is less important in central groundnut basin and high Casamance. The values of Tajima's D were negative in the center and south of the groundnut basin (Table 3). The values of pairwise population differentiation ( $F_{st}$ ) are not significant; levels of differentiation between these four populations are not similar (Table 4). No genetic differentiation exists between the center and south of the groundnut basin. Genetic differentiation seems to exist between the area of Eastern Senegal high Casamance and the other areas.

### Genetic distances between agro-ecological zones

The highest genetic distance (0.012) was noted in the agro-ecological zone of eastern Senegal High Casamance and South groundnut basin and the other between the second and the North groundnut basin. Between the center and south of the groundnut basin, is a distance of 0.010. The lowest genetic distance was obtained between the North Zone and the Central Groundnut Basin. Between the Eastern Senegal/ high Casamance and central Groundnut Basin and between the second and the area north of the groundnut basin, we have a genetic distance equal to 0.006 (Table 5).



**Figure 1.** Nucleotide diversity of populations based on agro-ecological zones.



**Figure 2.** Haplotype diversity of the different agro-ecological zones.

**The phylogenetic trees of haplotypes**

Phylogenetic trees of haplotypes generated using the

methods of maximum parsimony and maximum likelihood are similar in topology and show three clades. The first clade being born with a rather low bootstrap value (49)

**Table 3.** Tests of neutrality performed on populations of *T. castaneum* (D Tajima DT P-value  $\leq 0.05$ , Fs Fs Fu P-value  $\leq 0.02$ ).

Tests	North Basin	Central Basin	South Basin	Eastern Senegal / High Casamance
Fu's Fs	-0.036	1.662	3.189	1.919
DT	0.54268	-0.00412	-2.12466	0.62496

**Table 4.** Genetic differentiation in pairs of populations of *T. castaneum* (Fst ).

Genetic	SOHC	CBA	SBA
CBA	0.21024	-	-
SBA	0.12988	-0.01457	-
NBA	0.10000	0.02531	0.01271

**Table 5.** Genetic distances between agro-ecological zones.

Genetic	SOHC	CBA	SBA
CBA	0.006		
SBA	0.012	0.010	
NBA	0.006	0.005	0.012

and contains five haplotypes from different agro-ecological zones sampled. This clade is so insignificant because the bootstrap value is less than 50%. The second clade supported with high bootstrap value (100%) of contains also 5 haplotypes from southern and northern groundnut basin and Eastern Senegal high Casamance zone. The third contains a single haplotype (H5) from North of the groundnut basin. This zone is present in all clades and contains the greatest genetic diversity. The phylogenetic tree revealed a close relationship between the lineage of the clade 1 that includes the haplotypes H2, H4, H8, H12 and H10 and the clade 2 containing the haplotypes H7, H9, H6, H3 and H11 (Figure 3).

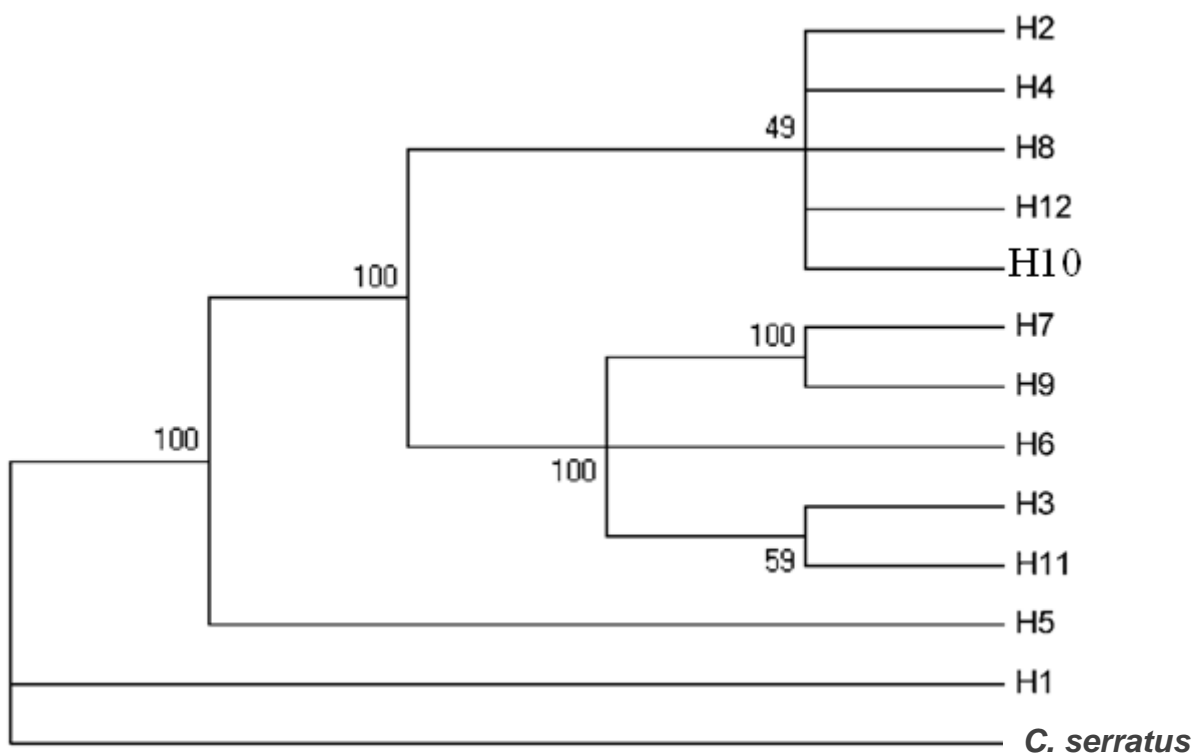
### The haplotype network

A haplotype network by the parsimony method was generated for populations of *T. castaneum* (Figure 4). Each disc represents a haplotype, and their size is proportional to the number of individuals corresponding to the haplotype. The circles correspond to mutational steps between haplotypes. Of the 51 sequences of *T. castaneum* obtained, 12 haplotypes were identified including in the most important thing haplotype (Figure 4), distributed in the four agro-ecological zones: Northern Basin, Central Basin, southern Basin and the area of eastern Senegal / high Casamance. The species

nevertheless has high haplotype diversity in northern groundnut Basin which contains the most divergent haplotypes directly (up to three mutational not) of the ancestral haplotype. We also have two haplotypes from southern groundnut basin and which are very distant from the other although they have widely divergent branches.

### DISCUSSION

The objective of this study is to genetically characterize the populations of *T. castaneum* encountered in different agroecological zones to identify the different haplotypes circulating in Senegal. Populations of *T. castaneum* encountered in Senegal contain different haplotypes. 12 haplotypes were encountered; five are present in northern and six in South groundnut Basin. These two areas contain more haplotypes than the other. However, haplotypic richness of North groundnut basin in which individuals are from the locality of Sandiara would be caused to grain marketing that may cause import of haplotypes from other areas in the market and thus in the locality. And South Basin where individuals come from a commercial shop where a lot of grain were stored to be due to the fact that *T. castaneum* is a polyphagous insect and there would have a passage of individuals with genetically recycled in other cereals such as maize or other legumes such as groundnuts, in the millet. It seems

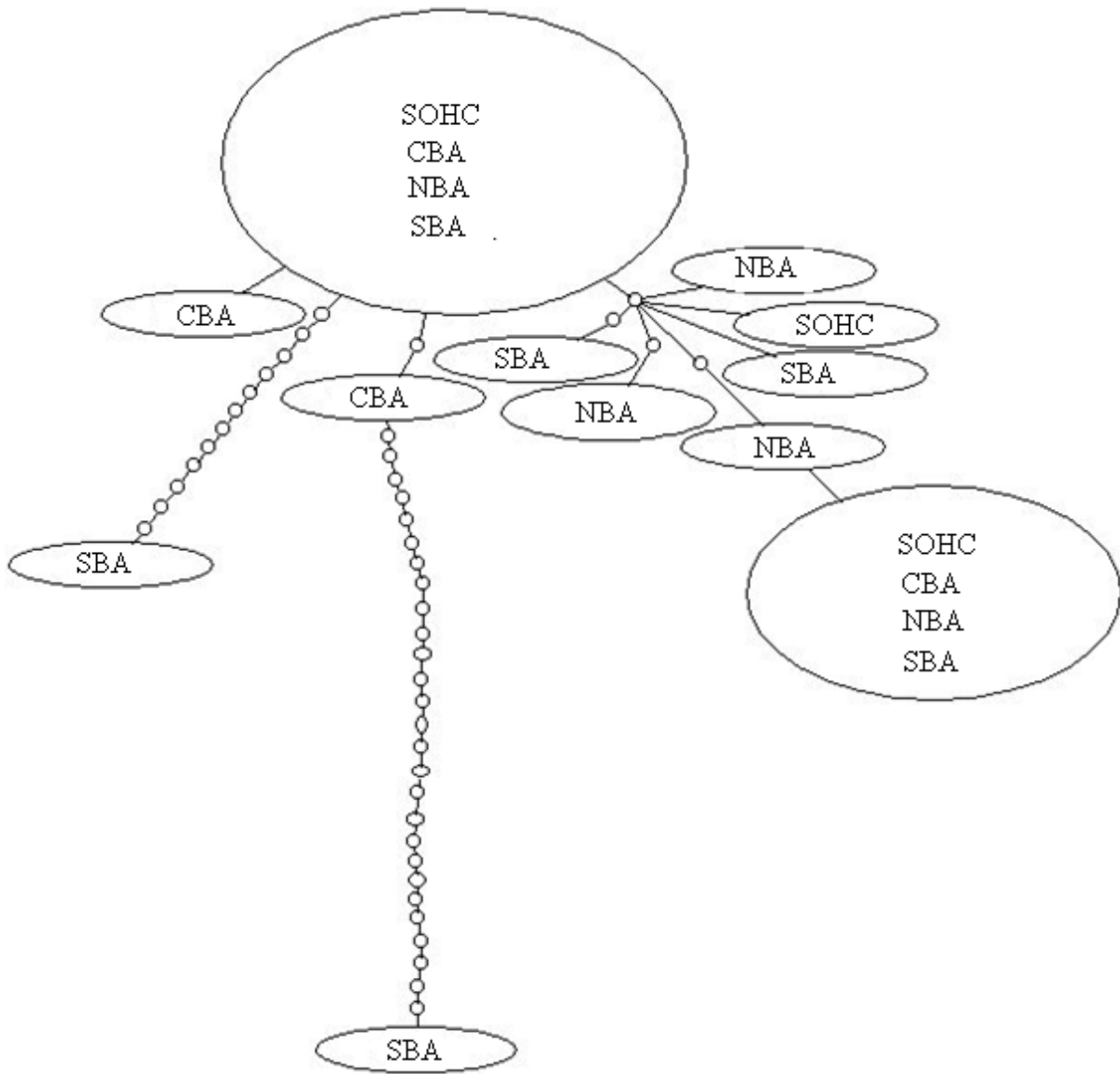


**Figure 3.** Phylogenetic relationships among nucleotide sequences of partial cytochrome b gene (466 pb) of 5 populations of *T. castaneum*. This tree is a tree of maximum likelihood which is similar to the tree of the maximum parsimony. The first numbers above or under branches are MP% bootstrap values (1000 replicates). *C. serratus* is the outgroup.

that there is a structuring of the population of *T. castaneum* based on host plants as was demonstrated by Sembene (2000) in the groundnut seed beetle *Caryedon serratus*. In the southern area of the groundnut basin where the samples came from a commercial shop, there could be also a mixture of millet variety since the area south grows much more Sagno variety than souna variety. What would be the role of variety in the genetic structure of *T. castaneum*? The number of haplotypes encountered in the center of the groundnut basin is due to the fact that millet cultivation is intense in this zone and there would be less import in this area. It is the same for the area of eastern Senegal high Casamance. Of all agro-ecological zones, haplotype diversity is high important. Indeed, a high haplotype diversity and low nucleotide diversity (on mtDNA) can be the result of rapid population growth from an ancestral population at low numbers and for which he has not been enough elapsed time to find a high diversity between haplotypes (Sinama, 2009).

A low haplotypic diversity and a low nucleotide diversity of mitochondrial DNA may be the signal of bottleneck severe and prolonged (Salducci et al., 2004). A case of bottleneck could be noted on all agro-

ecological zones even though the study populations were sampled immediately after the beating, in intact grains that appear to slow the development of *T. castaneum* and therefore growth of the population. High nucleotide diversity was noted in the South Basin and high haplotype diversity in northern groundnut Basin. However, high values of nucleotide diversity found in the population south of the groundnut basin suggest a recovery in this population. Despite being good colonizers, the populations of *T. castaneum* may be founded by genetically related beetles from a single source (Douglas et al., 2009). The values of  $F_{st}$  range from -0.01457 (between the southern and central groundnut basin) to 0.21024 (between the center of the basin and the area of Eastern Senegal upper Casamance). Values (ranging from 0018 to 0149) found by Semeao et al. (2010) are included in this interval. The values range from -0.01457 (between the southern and central groundnut basin) to 0.21024 (between the center of the basin and the area of Eastern Senegal upper Casamance). Values (ranging from 0018 to 0149) found by Semeao et al. (2010) are included in this interval. The values found between southern and central Groundnut Basin shows that there is no genetic differentiation



**Figure 4.** Haplotype network representing the minimum evolutionary relationships between different haplotypes of *T. castaneum*.

between these two zones. The highest genetic differentiation was found between the basin center and the area of Eastern Senegal Casamance high. The values of D of Tajima are negative at the center and south of Groundnut Basin. The populations of *T. castaneum* have saddened a recent demographic expansion in these areas because according to (Muths et al., 2008), negative values of Tajima's D correspond to a recent demographic expansion. The highest genetic distance within area (0.017) is noted in the south of the groundnut basin. Polyphagy of the insect would be the source of genetic diversity observed in the store

marketing of the Koungheul locality. This genetic distance is also higher than those found between other agro-ecological zones in pairs. This shows that there is no genetic structure of an agro-ecological zone to another despite the distance between the localities sampled. It was reported that geographically distinct populations of *T. castaneum* exhibit low levels of genetic differentiation that is not correlated with geographic distance (Semeao et al., 2010). We noted a genetic variation from one area to another. According to Douglas et al. (2009), genetic variation between regional populations of *T. castaneum* is more important than that observed in *Drosophila*

*melanogaster*. Genetic diversity of the insect is higher in trade shops and localities with have market grain marketing. It was noted that males of *T. castaneum* prefer to mate with mature females having made previous mating with other males. These polyandrous matings would have more influence in the nuclear genes to transmission in two-parent than in those mitochondrial whose transmission is uniparental and maternal.

Phylogenetic trees show the existence of three clades that could correspond to different lines. The number of clade was found by Diome et al. (2011) in *Caryedon serratus* populations encountered in the West African sub-region. Genetic diversity is noted in the first two clades that constitute a monophyletic group. In addition, on each clade, there are group of individuals from different localities or different agro-ecological zones. The formation of clades would be due to an admixture of different strains specialized in different grains. This would help in grain marketing in stores where they stock different cereal or in the markets. The disorganization of biological communities poses several problems for people and can greatly affect productivity and profitability of agricultural systems and the conservation of stored by the appearance of species or biotypes from other regions or agro-ecological zones but especially by the increased populations of native species favored by global change. It is therefore important for farmers to avoid the storage of grain from the markets or shops of marketing or other localities with cereals harvested in their specific localities.

## ACKNOWLEDGEMENT

This work was funded by the world bank program of "West Africa Agricultural Productivity Program " (WAAPP) National Centers of Excellence.

## REFERENCES

- Camara A (2009). Lutte contre *Sitophilus orizea* L. (Coléoptères curculionidae) et *Tribolium castaneum* Herbst (coléoptère ténébrionidae) dans les stocks de riz par la technique d'élevage traditionnelle pratiquée en Basse Guinée et utilisation des huiles essentielles végétales. Thèse d'obtention du titre de Docteur en Science de l'environnement, Université du Québec à Montréal. p.173.
- Diome T, Ndiaye A, Ndong A, Doumma A, Sanon A, Ketoh GK, Sembène M (2011). Genetic identification of West African ecotypes of the groundnut seedbeetle *Caryedon serratus* Ol. (Coleoptera, Chrysomelidae). *South Asian J. Exp. Biol.* 1(2):88-93.
- Douglas WD, Ashley LS, Michael JW (2009). Genetic differentiation among wild populations of *Tribolium castaneum* estimated using microsatellite markers. *J. Heredity* 100 (6):732-741.
- Excoffier L, Laval G, Schneider S (2005). Arlequin ver. 3.0: An integrated software package for population ge-netics data analysis. *Evol. Bioinformatics Online* 1:47-50.
- Feder JL, Berlocher SH, Roethele JB, Dambroski H, Smith JJ, Perry WL, Gavrilovic V, Filchak KE, Rull J, Aluja M (2003) Allopatric genetic origins for sympatric host plant shifts and race formation in *Rhagoletis*. *Proc. Natl. Acad. Sci. U. S. A.* 100:10314-10319.
- Feder JL, Chilcote CA Bush GL (1988) La différenciation génétique entre les races hôtes sympatriques de *Rhagoletis pomonella*. *Nat.* 336:61-64.
- Fu YX (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915-925.
- Guèye MT, Seck D, Wathelet JP, Lognay G (2011). Lutte contre les ravageurs des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale : synthèse bibliographique. *Biotechnol. Agron. Soc. Environ.* 15:183-194.
- ISRA (Institut Sénégalais de Recherches Agricoles) (1994). Les cultures céréalières dans le bassin arachidier Motivations et contraintes chez les producteurs. *ISRA-Etudes et Documents* 5(2):26.
- Kouassi B (1991). Influence de quelques facteurs externes sur le cycle de développement et la survie de *Sitophilus orizea* L. (Coléoptères curculionidae). Thèse d'obtention du titre de Docteur en Sciences Naturelles, Université nationale de Côte d'Ivoire p. 92.
- MAE (Ministère de l'Agriculture et de l'Élevage) (2001). La culture et la production du mil et du sorgho au Senegal: bilan-diagnostic et perspectives. *Projet initiative mil/sorgho* p.130.
- Muths D, Tessier E, Bourjea J (2008). Contribution de la génétique des populations à l'étude de la connectivité régionale de certaines espèces de poissons commerciaux présents dans la Réserve Naturelle Marine de La Réunion : étude de faisabilité. *Synthèse finale.* p.23.
- Salducci MD, Martin JF, Pech N, Chappaz R, Costedoat C, Gilles A (2004). Deciphering the evolutionary biology of freshwater fish using multiple approaches—insights for the biological conservation of the Vai-rone (*Leuciscus souffia souffia*). *Conserv. Genet.* 5:63-77.
- Seck D (1983). Study of a pest *cétiales*, *Sitotroea cerealella* OLIV. (*Lep. Gelechiidae*), on-farm in Senegal. *Memory graduation, Fac. Agric. Sci. Gembloux (Belgium)* p.123.
- Seck D, Sidibé B, Fall A (1992). Observations sur le développement de *Tribolium castaneum* Herbst. sur mil (*Pennisetum typhoides* L.), en fonction du taux de brisure dans le substrat. *Mém. Soc. r. belge Ent.* 35:471-475
- Sembène M (2000). Variability of the Internal Transcribed Spacer (ITS) of ribosomal DNA polymorphism and microsatellite loci among the weevil *Caryedon serratus* (Olivier): differentiation and host races infestation of groundnut in Senegal. PhD Thesis State University of Science Cheikh Anta Diop (Sénégal) p.180.
- Semeao AA, Campbell JF, Beeman RW, Whitworth RJ, Sloderbeck PE, Lorenzen MD (2010). Genetic structure of *Tribolium castaneum* populations in mills. *Julius-Kühn-Archiv* 425:85-89.
- Sinama M (2009). Using mitochondrial and nuclear DNA in the identification of genetic structure and population dynamics in *Chondrostoma toxostoma toxostoma* and *nasus nasus* *Chondrostoma* (Cyprinidae). Master thesis Biodiversity and Ecology continental IMEP - UMR 6116 du CNRS p.36.
- Tajima F (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585-595.
- Tostain S (1998). Le mil, une longue histoire: hypothèses sur sa domestication et ses migrations. p. 490.