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Genetic diversity of *Tribolium castaneum* (Herbst) population in storage infrastructures and agro ecological zones in Senegal

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Abstract

The stocks of millet are attacked by the beetle among them *T. castaneum*. This devastator attacks the grain and causes important losses as for as the stocks of ground millet in the sahel. The aim of this study is to evaluate genetic diversity of *T. castaneum* population meet in the stocking infrastructures and the different agroecological areas with the intention to identify the different haplotypes that walk around. Portions of the cytochrome b and 28S ribosomal genes of *T. castaneum* were sequenced, using samples from that represent storage infrastructures and agroecological areas in Senegal. The results reveal a strong haplotype diversity in the stores of Sandiara (0.889 ± 0.127) and Diaroume (0.556 ± 0.165). The population of *T. castaneum* meet in the stores who supply themselves locally as that of Djilas show a weak haplotypique and nucleotidic diversity. The cereals marketing at the markets such as the Sandiara one would be at the origine of the wealth haplotypic observed in the store taken as an exemple in this locality. The pourcentage (0.16%) of molecular variance analysis (AMOVA) shows that there is not a significant genetic differentiation between agroecological areas. The test of Mantel shows that there is no correlation between geographical distance and genetic differentiation.

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Introduction

It is no longer to prove that the preservation of the corps allows ensuring the availability of food resources which is one of the key factors of food security of a country. The stoking makes possible the availability of foodstuff in peasant (rural) area and ensure also. The seeds for the coming agricultural campaign more than 30% of the production are lost from harvesting to consummation. This proportion is stronger in sahelian region due to a long period of stocking (Ngamo and Hance, 2007). These losses are caused ofently by the coleopterans which have an important impact on agriculture (destructive). Among these Coleoptera, we can cite *T. castaneum* wich attacks just as well the grains and the cereals flour *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae) has had a long association with human stored food and can be an important destructive in anthropic structures used for the treatment and the stocking of products cereals based. (Campbell and Runnion, 2003). *T. castaneum* is an important devastator of stocked products and is observed in several basis products and can provoke serious economical losses if it is not well enough controlled because it has a very high growth rate of it population (Khasaveh *et al*, 2009). Taking in to account the qualitative losses caused by insect (present d'escuvies some left corps in the area secretion of persistent foul smelling) losses can increase during a long period and the extent of quantitative damages, one must consider the *T. castaneum* as a real harmful insect which must be the object of a rational struggle in order to protect the grain during the stocking and the preservation (Bekon and Fleurat, 1989). On the entire cereal grain the larvas just develop slowly but the species can nevertheless be considered only as a secondary devastator. According to Gueye *et al.* (2012) *T. castaneum* is capable to develop itself in the beaten millet just after the harvesting and to a short cycle. In Senegal, the insect attacks a large number of find it into different places of stocking (granary, stores of producers and dealers) located in different agroecological area. It is easy for farmers and their families to forecast the availability of their cereals from now to the coming harvest but it is more

difficult for them to measure the loss of quality of foodstuffs. To better struggle against the devastators of stocks, particularly against *T. castaneum*, a better knowledge of the biology and genetics of its population is essential. To do this, some genetically approaches have used to study dispatching population around agro ecological areas of Senegal. The specific aim of this study is to identify the different haplotypes of *T. castaneum* encountered on stocking places and some agroecological areas of Senegal and to establish if necessary a genetical variation between agroecological areas.

Materials and methods

Stocking infrastructures

The stocking places of millet in Senegal are the producers store, granaries and dealers store. In rural area we can distinguish different types of stocking.

The traditional granaries where grains are preserved in ear or loose. The fact of being infested by the insect is commonplace and that exact the producers to cope with at by mixing the grains with insecticide powder, with ash a leaves of nime. The capacity differs from 1 to 3 Tons.

The rooms or shops

Currently producers and dealers preserve their foodstuffs in some stores. These stores have generally a rectangular form. The foodstuffs are kept there into backs. despite the phytosanitaries treatments which are sometimes applied the infestation by the insect is aften present. The capacity of these stores goes from 5 to 10 Tons. Granaries and stores don't ensure a good phytosanitary protection.

Agroecological areas

The study delt with three ecological areas among seven ones. The North area of groundnut basin, the South of the groundnut basin and Eastern area of Senegal upper Casamance.

Groundnut basin (with its Northern and Southern areas) constituted by the of Thies, fatick

and Kaffrine, with an important demographic weight and its agricultural and rural communities have been seriously affected by the groundnut crisis. The areas have been marked by a persistent drought during the last decades. The climatic conditions have speeded up the deterioration of the ecosystem and have intensified the tiresome of the heritage land (fertility of soils and timber resources) and the weak soils regeneration, the acidification of highland soils (tanned) and the salinization of bottom funds like the coast's side also, the marin invasion in saloum's river, the degradation of the mangroves and the marin pollution (DLICC, 2010).

The Eastern area of Senegal/ upper Casamance gather the departments of Kedougou, Tambacounda, Velinngara and Kolda goes through a remarkable rural poverty despite the enormous agricultural and pastoral potential and a strong pressure as for as natural resources are concerned.

The low and medium Casamance is characterized by the acidification of the bottom soils, water erosion, the loss of forest diversity (because of bush fires) the increasing of the salinity rate, the acidity and toxicity of the iron and aluminium of rice fields the same goes for the considerable degradation of mangroves in the estuary of Casamance.

The sites studied

The localities which are taken are: Djilas (14°14'45"N, 16°38'04"W) and Mbam (4°07'06"N, 15°37'04"W) lacate itself at the northern agroecological area of the groundnut basin. Keur Mbouky (14°08'14"N, 15°49'44"W), Karang (13°35'N, 16°42'W) and Kounghoul (13°59'N, 14°48'W) in the southern area of the groundnut basin and Diaroume (12°59'08"N, 15°37'04"W) can be found at the eastern agroecological area of Senegal/ upper Casamance. The different stocking places are announced on the Table 1.

Extraction, PCR-sequencing

The abdomen, elytra and antennae of samples were kept apart to avoid contamination by fungi and nematodes and to allow for morphological observation. A partial CytB and 28S ribosomal gene region was amplified by PCR to characterize mitochondrial and nuclear DNA of *T. castaneum*. The primers of the cytochrome b used were CB1 (5'-TATGTACTACCATGAGGACAAATATC-3') and CB2 (5'-ATTACACCTCCTAATTTATTAGGAAT-3'). The 25 ml PCR reaction mixture for the cytochrome b contained 18.3 µl water, 2.5ml enzyme buffer supplied by the manufacturer, 1µl MgCl₂, 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 comprising repeated distortion at 94 ° C for 1 minute, annealing at 47 ° C for 1 minute and elongation of the complementary DNA strand at 72 ° C for 1 minute, a final elongation at 72 ° C for 10 minutes ended the PCR. The PCR of the 28s ribosomal gene consists of an *in vitro* selective amplification of a particular sequence of DNA matrix via the extension of two primers: D2CFD45F (5'-TAC CGT GAG GGA AAG TTG AAA 3') and D2CR D45R (5'-AGA CTC CTT GGT CCG TGT TT-3') by a polymerase DNA. The amplification was performed by a repetition of cycles, which assured a multiplication of the target DNA at every cycle (2³⁵). This was carried out in a 25 µl volume of reaction, containing 18.525 µl ultra-pure water, 2.5 µl of non coloured buffer solution (10x), 1 µl MgCl₂, 0.5µl dNTP, 0.175 µl of each primer, 0.125 µl of Taq and 2 µl of DNA extract. The PCR began with a preliminary denaturing at 94°C (3min), followed by a repetition of 35 cycles of initial denaturing at 92°C (30 s), after which hybridization occurred at 55°C (30 s) and elongation of the complementary DNA strang 72°C (1 min), ending in a final phase of extension at 72°C (10 min). Sequencing was performed by Macrogen (South Korea).

Data analysis

The haplotype term correspond to a nucleotidic sequence which can be common to several

individuals, but differ from the other haplotypes by one or several substitution of nucleotide. The sequences have been aligned by the BioEdit software and the DnaSp 5.10.01 (Rozas *et al.*, 2010) has been used for the determination of the haplotypes. The indication such as the d of Tajima and f_s of Fu (Tajima, 1989; Fu, 1997) have been used in order to test the difference of the neutrality hypothesis by the Arlequin software V3.1 (Excoffier *et al.*, 2005). The same software has been also used to perform a test of Molecular Variance (AMOVA) between agroecological areas. In a constant population, these indication are zero. On the other hand, a demographic growth tally with a D of Tajima and a negative F_s . The test of f_s of Fu compares the average number of difference two to two ($\Theta\pi$) and the number of haplotypes (h) in the population. The D of Tajima is based on the difference between the average number of difference two to two ($\Theta\pi$) and the number of polymorphic sites (s). Different statistic test: d of tajima, f_s of fu (tajima, 1989; Fu, 1997) have been used in order to test the difference of neutrality hypothesis. The test of f_s of fu compares the average number of difference two two ($\Theta\pi$) with the haplotypes number (k) in the population. The nucleotide diversity (P_i) and haplotypic (hd) have been calculated by the DnaSp

software. The haplotype Network has been built by the TCS software (Clement *et al.*, 2000). This method produces an estimation of the plausibility of the links between the haplotypes on the tree which must be at the very least 95% to be represented. The correlation test between the geographical and genetic had been built by the XLSTAT software (www.xlstat.com/fr).

Results

Mitochondrial Marker: cytochrome b

Polymorphisme of cytochrome b of the beetle *T. castaneum*

The total mitochondrial genome is composed of 15881 pb. The cytochrome b is laceded between between 10280 and 11419pb namely 1140 pb. We have sequenced the region of cytochrome b located between 10773 and 11131pb namely 399pb. The number of invariable site amount to 390, 9 variable sites which 4 lonely ones and 5 sites which are passimonious informative. The singleton sites are located in the position 462, 477, 498 and 657 pb and passimonious informative sites in the position 516, 666, 727,756 and 849 pb of the cytochrome b. the region going from the position 517 to the position 656 of the gene of cytochrome b is preserved in the sample studied.

Table 1. samples analyzed.

Regions	Localities	Code	Places of stock	Zones Agroecological
Kaffrine	Keungheul	TcKl	Trade Shop	South groundnut basin
	Keur bouki	TcKb	Grenier	South groundnut basin
Fatick	Djilas	TcDs	Store of producer	Center groundnut basin
	Mbam	TcMm	Trade Shop	Center groundnut basin
Thies	Sandiarra	TCS	Store of producer	North groundnut basin
Border Senegalo-Gambien	Karang	TcKg	Store of producer	South groundnut basin
Sédhiou	Diaroume	TcDe	Store of producer	Eastern Senegal Casamance high

Table 2. Percentage of AT in the region of the cytochrome b between 10733 and 11131 pb of the species *T. castaneum*.

	%A	%T	%C	%G	%AT
H1	29.82	33.33	25.56	11.28	63.16
H2	29.57	33.58	25.31	11.53	63.16
H3	29.57	33.33	25.56	11.53	62.91
H4	29.57	33.58	25.31	11.53	63.16
H5	29.82	33.58	25.31	11.28	63.41
H6	29.82	33.33	25.56	11.28	63.16
H7	30.08	33.58	25.31	11.03	63.66
H8	30.08	33.83	25.06	11.03	63.91
H9	29.82	33.83	25.06	11.28	63.66

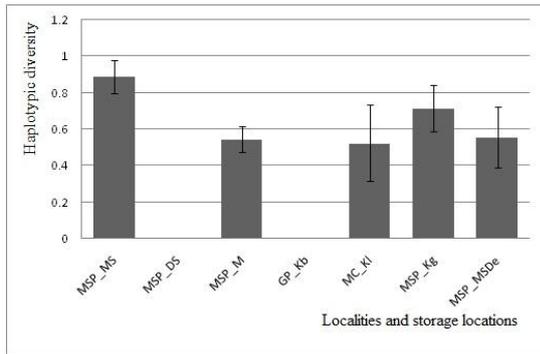


Fig. 1. Diversity haplotypique populations of *T. castaneum* in the storage infrastructure.

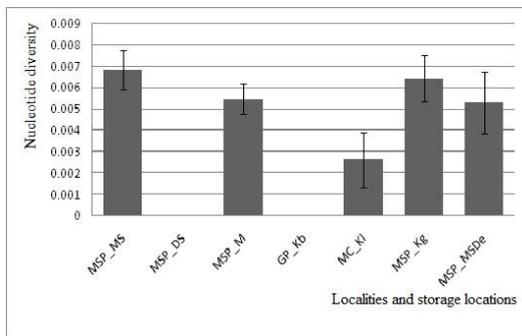


Fig. 2. The nucleotide diversity of populations of *T. castaneum* in the storage infrastructure.

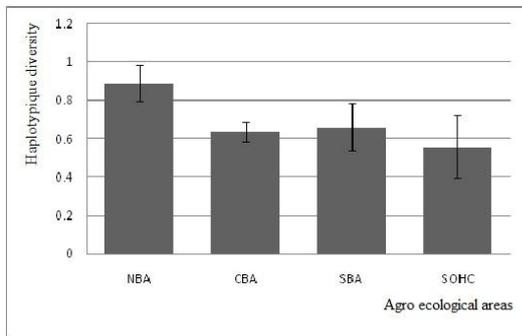


Fig. 3. Diversity haplotypique populations of *T. castaneum* in agroecological areas.

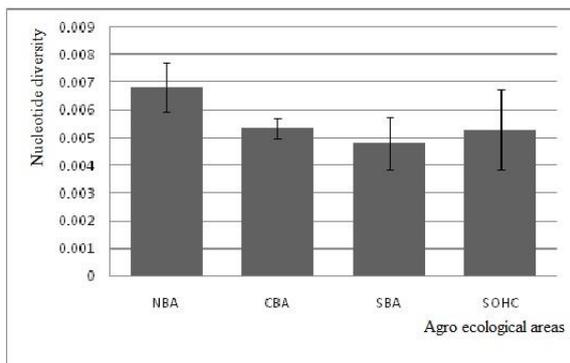


Fig. 4. nucleotide diversity of populations of *T. castaneum* in agroecological areas.

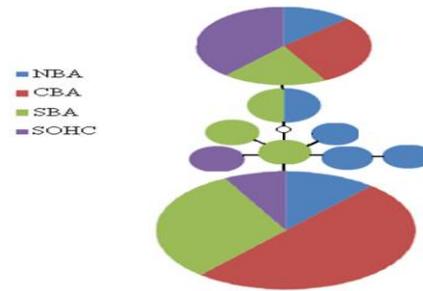


Fig. 5. network of haplotype of cytochrome b of populations of *T. castaneum* encountered in areas agro.

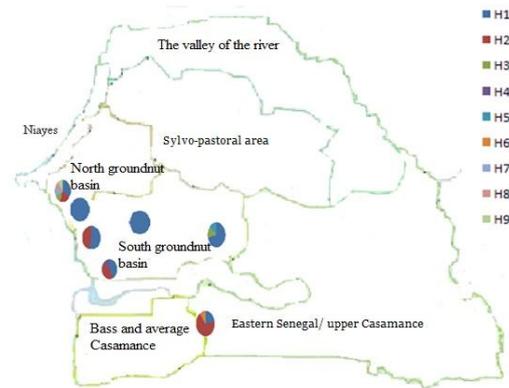


Fig. 6. The distribution of haplotypes of cytochrome b in the localities and zones agroecological sampled.

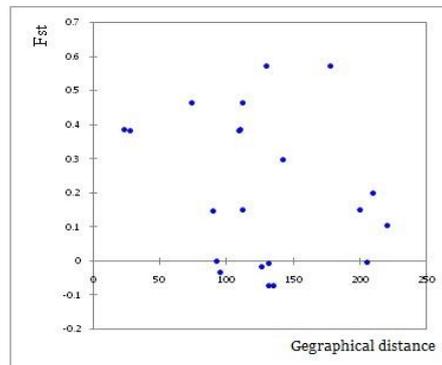


Fig. 7. Test of Mantel on the populations of *T. castaneum*.

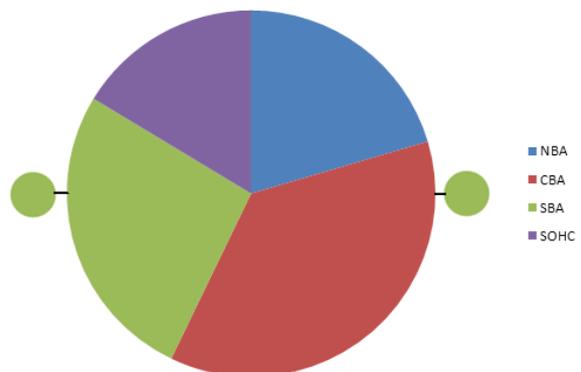


Fig. 8. Network of haplotype map of the 28S.

The genetic diversity of the population of T. castaneum in the stocking infrastructures

Upon the 50 sequences of *T. castaneum*, we have got 9 haplotypes dispatched into agroecological areas. 6 haplotypes in Sandiara, 1 in Djilas, 2 in Mbam and 3 respectively in Koungheul, Karang and Diaroume. Four haplotypes have respectively 63, 41 (H5) 63, 66 (H7) 63, 91 (H8) and 63, 66 (H9) (Table 2). Some of the individual mutations have led to an appearance of AT in their sequence. We can see accurately the stocking store of (0,889) of Karang (0,714) and of Diaroume (0,556) present the stronger haplotypique diversities (Figure 1). Sandiara and Diaroume are places where weekly market takes place and Karang is located in frontierebetween Senegal and gambia. The

stronger nucleotide diversities are also encountered in Sandiara and Karang (Figure 2). The haplotypique and nucleotidique diversity are no in the stocking store of a producer of the locality of Djilas (Table 3). The stronger values of *fst* are obtained between the populations of staking store of the producers of Djilas and Diaroumé (0,58573) and between between, the stoking store of producers from Diaroumé and the marketing store of Koungheul (0,35712). These three values of *fst* are significatives. The genetic differentiation between the populations of Sandiara and Karang an one hand and between the population of Diaroumé and karang on the other one is too weak (Table 4). The values of *F_s* and that of *D* of Tajima are not significative (Table 5).

Table 3. The genetic diversity of populations of *T. castaneum* in the storage infrastructure.

	MSP_MS	MSP_Ds	MSP_Mm	GP_Kb	MC_Kl	MSP_Kg	MSP_MDe
N	9	6	11	1	7	6	9
H	6	1	2	1	3	3	3
S	7	0	4	-	3	5	5
Hd	0.889 ± 0,091	0	0.545 ± 0.072	-	0.523 ± 0.209	0.714 ± 0.127	0.556 ± 0.165
Pi	0.00682 ± 0.00090	0	0.00547 ± 0.00072	-	0.00263 ± 0.00129	0.00644 ± 0.00108	0.00529 ± 0.00145

N: number of individuals H: number of haplotype

Hd : diversity haplotypique Pi: nucleotide diversity

S: site of segregation.

Table 4. The *F_{st}* populations of *T. castaneum* in function of storage infrastructures. The significant indices ($p < 0.05$) are marked with asterisk (*).

	MSP_MS	MSP_Ds	MSP_Mm	GP_Kb	MC_Kl	MSP_Kg
MSP_Ds	0.26343 *	-				
MSP_Mm	0.00522	0.30526	-			
GP_Kb	-0.33333	0.00000	-0.20000	-		
MC_Kl	0.04371	0.05618	0.12271	-0.83333	-	
MSP_Kg	-0.05529	0.34477	-0.09691	-0.25000	0.10784	-
MSP_MDe	0.07143	0.58573 *	0.04408	0.28571	0.3571 *	-0.02158

MSP_MS : Store of Stock of a Producer_Sandiara with presence of a market

MSP_Ds : Store of Stock of a Producer_Djilas

MSP_Mm : Store of Stock of a producer_mbam

GP_Kb : attic of a Producer_Keur Bouky

MC_Kl : Store of Commerce_Koungheul

MSP_Kg : Store of Stock of a Producer_Karang

MSP_MDe : Store of stock of a producer_diaroume with presence of a market.

The genetic diversity of the populations of T. castaneum in the agroecological area

The haplotypic diversity is more important in the North area of the groundnut basin with a value of

0,889, followed by the Southern area 0,659 that of the East of Senegal upper casamance (0,556) and finally the centre of groundnut basin (0,636) (Table 6). The nucleotidique diversity is more importante also in the first 3 areas but the nucleotidique diversity in the area of Eastern Senegal upper Casamance is

more important to that of the South of the groundnut basin.

The strongest haplotypique diversities and nucleotidique are notice in the Northern area of the groundnut basin and the weakest diversity at the center of the groundnut basin (Figure 3 and 4).

Table 5. Indices of neutrality of populations of *T. castaneum* encountered in the infrastructure for different storage.

	MSP_MS	MSP_Ds	MSP_Mm	GP_Kb	MC_Kl	MSP_Kg	MSP_De
DT	0.25402	0.00000	2.18825	-	-0.65405	1.28799	0.62496
Fs	-1.39457	-	4.46464	-	0.10980	1.85327	1.91896

Table 6. The genetic diversity of populations of *T. castaneum* for each zone agro-ecological.

	NBA	CBA	SBA	SOHC
N	9	17	15	9
H	6	2	5	3
Hd	0.889±0.091	0.636±0.052	0.659±0.123	0.556±0.165
Pi	0.00682±0.0009	0.00426 ±0.00038	0.00458±0.00096	0.00529 ±0.00145

N: number of individuals H: number of haplotype

Hd : diversity haplotypique

Pi: nucleotide diversity

NBA: North groundnut basin CBA: Center groundnut basin

SBA: South groundnut basin SOHC: Eastern Senegal/Casamance high.

The neutrality test achieved for each ecological area

The value of Tajima's D and that of Fu's Fs are higher at the center of the groundnut basin respectively 1,5041 and 4,54061. The value of the first one is positive upon the four agroecological area. On the other hand the value of Fs is negative in the air the Northern and southern area of the value of the

groundnut basin (Table 7). The genetic differentiation between the agroecological areas located in the groundnut basin are too weak and vary from 0,05069 to 0,0207 (Table 8). However we obtain higher values between those areas and that of Eastern Senegal upper Casamance. The values vary from 0,09375 to 0,21103.

Table 7. Test of neutrality achieved for each agroecologicals areas.

	DT	Fs
NBA	0.25402	-1.39457
CBA	1.5041	4.54061
SBA	0.63804	-0.02554
SOHC	0.62496	1.91896

Table 8. Genetic differentiation between zones agroecologicals areas (fst).

Areas	NBA	CBA	SBA
CBA	0.0207		
SBA	-0.01528	-0.05069	
SOHC	0.09375	0.21103	0.21393 *

*: Indicates that the value is significant.

The haplotype network of T. castaneum population

Every disc tally with an haplotype, and their size is proportional to the individual number correspondent to the haplotype. The white circle tally with a mutational step between haplotypes. In the first one it is the centre of the groundnut basin which is more represented in the eastern area of Senegal upper Casamance in the 2nd haplotype (Figure 5). The third one contains 2 individuals. The one originate from the North of groundnut basin and the other in the South of the groundnut basin. The six haplotypes left are individuals 3 of them come from the North, 2 from South of the groundnut basin and one from the East of Senegal upper Casamance. The strongest haplotypique diversity is encountered in the North of the groundnut basin with 6 haplotypes met at the

same locality (Sandiara) (Figure 6). There is also strong haplotypique diversity in the Southern area of the groundnut basin 3 in Koungeul and in Karang. At the centre of the groundnut basin despite the important number of individuals and localities (3localities) taken (17 individuals). In this area we have only 2 haplotypes. At length the area of Eastern Senegal upper Casamance (locality taken) presents 3 haplotypes. Haplotype 1 and 2 are present in the three agroecological areas. The haplotype number 6 is only present in Eastern Senegal upper Casamance, the haplotype number 4 is only present on the border of Senegal and Gambia and the haplotype number 5 in Koungeul. The haplotype number 2 is also encountered in Chicago.

Table 9. Test of molecular variance between areas agro by creating an intermediate zone between the North and the South of the groundnut basin. The significant indices ($p < 0.05$) are marked with star (*).

Source of the variation	Indices of fixing	Variance	Percentage Of the variance
Between zones agroecological	0.00161	0.00052	0.16
Mêmezone Agroecological	0.12151	0.03930	12.13
Within a population	0.12292 *	0.28417	87.71

The analysis of Molecular Variance (AMOVA)

The variance at the interior of a population (0,28417) with a pourcentage of 87,71% is more important than that between population of a same agroecological area (0,03930) with a percentage of 12,13% which is more important in its turn than the variance between agroecological areas (0.00052) with 0,16% (Table 9).

The Mantel test

The p-value (0,769) calculated is superior to the meaning level alpha (0,05). We cannot deny the nonexistent hypothesis H_0 . Then there is no correlation between the geographical and genetic differentiation (Figure 7).

The nuclear Marker 28S

In the haplotype net work of the ribosomal gene 28S, we have three haplotypes on the 51 sequences (Figure 8). In fact it is only the locality of Karang which has only presented more than one haplotype (3 of 6

individuals). The majoritary haplotype is present in the whole agroecological areas. The two other haplotypes are only present in the locality of Karang and different from the haplotype number 1 by only one mutational step. The haplotypique diversity is more important in the localite of Karang. The haplotype number 1 is fixed in all the agroecological areas and the localities apart from the Southern area of the groundnut basin and the localities of Karang.

Discussion

The objective of the job is to identify the haplotypes of the *T. castaneum* species in the stocking places of the millet and to see the effect of the agroecological areas of Senegal about the genetic structuration of this insect. The mitochondrial gene of cytochrome b show that the population of *T. castaneum* encountered in Senegal contain different haplotypes. These contain some percentage relatwely equivalent to AT. According to Friedrich and Muquin (in press), the At

are introduced by mutation into the mitochondrial genome of *T. castaneum* as that has been noticed for other mitochondrial genomes (Foster *et al.*, 1997). Important haplotypic diversity has been encountered in all the stocking places apart from the stocking store of Djilas. However, the haplotype richness in the localities of Sandiara and Diaroume could be due to the marketing of cereals at the market of these villages which can lead to an importation of haplotypes coming from other areas. The important haplotypic diversity in the locality of Karang might also be due to the fact that this latter can be found on the border between Senegal and Gambia countries which have marketing relations with other countries and may impact from Gambia products infested by *T. castaneum* species. The grains taken from Koungeul come from a store of commerce where many cereals and leguminous plants have been preserved. In fact, *T. castaneum* is a polyphagous insect and there would be individual passages which have genetically recycled in the other cereals such as the maize or other leguminous plants as peanut, in millet. The locality of Mbam located at the head of the groundnut basin presents the weakest haplotypic diversities. In fact the samples of that locality come from the stocking store and there is not much importation of cereals or leguminous plants in that locality in view of the fact that the crop is abundant. At the stocking places (store of Sandiara, Mbam, Karang, Diaroume and the commerce store of Koungeul), we can notice an important haplotypic diversity and the weak nucleotide diversity. According to Sinama (2009), the important haplotypic diversity and the weak nucleotide diversity can be the result of the fast growth of the population from an ancestral population with a weak effective size and for that there hasn't been enough time passed to find again an important diversity between haplotypes on the other hand in our study. The store of Djilas presents a nonexistent haplotypic and nucleotide diversity. This shows that the store of Djilas and also that of Mbam are locally supplied. A weak haplotypic and nucleotide diversity on the mitochondrial DNA can be the signal of a strict and extended bottleneck (Salducci *et al.*, 2004). The values of F_{st} between

stocking places vary from -0,83333 to the store of commerce of Koungeul and the granary of Keur Bouky about 0,58573 between the stocking store of peasant from Diaroume and that of Djilas. The F_{st} found between the stocking store of Diaroume and that of Djilas (0,58573) is superior to that found by Semeao *et al.* (2010) which vary from 0,018 to 0,143. The values of Tajima's D are positive in the stocking store of a producer of Djilas pointing out probably a random evolution of the population in this place of stock. In the store of commerce of Koungeul, the value of Tajima's D is negative which could correspond to a demographic expansion (Excoffier *et al.*, 2005). Regarding the agroecological areas one can notice that the more important haplotypic diversity is noted at the north of the groundnut basin and in the area of Eastern Senegal upper Casamance. These areas present also negative values of F_{st} which show that at the North of the groundnut basin and in the area of Eastern Senegal upper Casamance, the population of *T. castaneum* have known a demographic expansion. In fact, the area of the Northern of the groundnut basin presents the most important haplotypic diversity with the presence of six haplotypes H1, H2, H3, H7, H8 and H9. The three first haplotypes are present in the other agroecological areas but the haplotypes H7, H8 and H9 are present only in the North of the basin. The haplotype H1 has an important geographical distribution because we have encountered it in all localities and agroecological areas and would be a national haplotype. The haplotype H2 would have also an important geographical distribution in the world because it tallies with the haplotype encountered in Chicago. According to Drury *et al.* (2009), *T. castaneum* in spite of being good colonizers are supposed to be founded by coleoptera genetically related from an only source. The test of AMOVA shows 0,16% of variance between the different taken agroecological areas. However the variability genetic at the interior of a population is more important to that between the populations. This could result taking into account that the cytochrome *b* is a gene with a rapid evolution. According to Drury *et al.* (2009) the regional genetic variation between

populations of *T. castaneum* is more important to that observed at the drosophile (*Drosophila melanogaster*). Despite the different varieties cultivated between the everall groundnut basin and the area of Eastern Senegal upper Casamance, one can notice that there is no significant genetic differentiation between population of *T. castaneum* encountered in these two areas. The comparison between different population of *T. castaneum* with the help of the test of AMOVA has not found significant levels of genetic differentiation. These results are similar to those of Semeao *et al.* (2010). It has been shown that the anthropic transport of stored products such as the plan which played an important role in the mixed population (Ryne et Bensch, 2008). The most important genetic differentiation is noticed between the South OF THE Groudnut basin and the area of Eastern Senegal upper Casamance despite the proximity of thee latter. The Mantel test shows that these are no correlation between the genetic and geographical distance. These results are in harmony with the results of Semeao *et al.* (2012). The absence of isolation by the distance suggest higher levels of flow of genes at the *T. castaneum*. In fact, there is transfer of cereals between surplus area and deficit one carried out by dralers especially in the groundnut basin. The anthropic dispatching would then prevent a structuration of *T. castaneum* especially from the interior of the country where exchanges of cereals grain or flow are done in all the country. The ribosomal gene of 28S presents 3 haplotypes out of 50 sequences. According by Pai and Yang (2003), the polyandre's matron behiour at *T. castaneum* species can evalve by an increasing of genetic diversity. These polyandre mating would have no influence on the mitochondrial gene of the cytochrome b on uniparental and maternal transmission and the ribosomal gene 28S of two parental transmission. The weak variability observed upon the ribosomal gene 28S show that this gene is not discriminating among population of *T. castaneum* subseriew to the same host plant.

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