INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 6, p. 116-129, 2017

RESEARCH PAPER OPEN ACCESS

Agro-Ecological areas in Senegal affect the genetic structure of *Callosobruchus maculatus* F. The major pest of cowpea

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Key words: *C. maculatus*, Agroecological areas, Haplotype, Genetic diversity

http://dx.doi.org/10.12692/ijb/11.6.116-129 Article published on December 12, 2017

Abstract

The objective of this study is to characterizethe structure of populations associated with cowpea in several agro-ecological zones of Senegal, using genetic markers (sequencing) associated with Bayesian approaches such as Appoximated bayesian computation. Portions of the *Cytochrome b* gene of *Callosobruchus maculatus* L. were sequenced, using samples from agro-ecological areas in Senegal.Sequences show a rather high degree of polymorphism (hd = 0,920±0,00054; Pi= 0,06±0,00021). Results from genetic diversity analysis reveal a higher value of variable sites, number of mutations, haplotypic diversity, nucleotide diversity and number of nucleotide differences in agro-ecological zones of North Peanut Basin (hd =0.978 ± 0.054 ; Pi=0.13825 ± 0.02291) and Senegal River Valley (hd =1.000 ± 0.052 ; Pi=0.03372 ± 0.00777), areas where cowpea is the most widely cultivated in Senegal.The largest number of mutations (134) is observed in the NBA while the lowest value is obtained from SBA (3). F*st* value reveals that the more genetically differentiated populations are those of the SBA and HCSO with a very high Fst value (0.75) whereas the minimum value is encountered between the individuals of the river valley and those South Peanut Basin. Recent studies increasingly highlight the effect of climate change in agro-ecological zones on the structure and dynamics of phytophagous insect populations.

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Introduction

Among the constraints on cowpea production, insect pests are the biggest problem for cowpea. Cowpeas are attacked by a wide spectrum of pests from germination to harvest. In the very broad range of these insect pests of cowpea, the Bruchidae beetles including *Callosobruchus maculatus* Fabricius are among the most formidable because their attacks begin in the field, then to extend to the warehouse where the population of bruchs can grow quickly. The most worrying consequence of these attacks is the qualitative and quantitative reduction of the harvest in the field or during storage. In fact, the larvae of the cowpea shrub grow exclusively in the seed by feeding on the accumulated reserves in the cotyledons.

In this study, we aim to precisely characterize the structure of populations associated with cowpea in several agro-ecological zones of Senegal, using genetic markers (sequencing) associated with Bayesian approaches such as Appoximat and Bayesian Computation; Baumont *et al.*, 2002. Population genetics is a field that allows, through the analysis of DNA samples resulting from individuals in different regions of the studied system, to define genetically distinct populations in a species and to better understand the evolutionary processes that govern them. The use of genetic markers is a very effective way to reveal differences in the genetic composition of organisms living in an ecosystem and to know the extent of differentiation caused by various evolutionary forces between distinct populations (Leclerc *et al.*, 2006). Delimiting the populations of a species in space and assessing the degree of connectivity between them are essential steps in the development of management scenarios that reflect the reality of the system and answer the real needs of people management. When we aim to develop natural population management plans, it is essential to delineate the populations of the studied system in space and time accurately, as well as to assess the degree of connectivity (i.e. gene flow) between them (Webster *et al.*, 2002). In fact, individuals of a species are often grouped naturally in local populations subjected to different evolutionary processes (natural selection, mutation, drift and migration) and different intensities, which generates distinct genetic compositions for each of the groups. The existence of populations more or less spatially isolated and reproductively independent should be considered in the management scenarios in order to avoid the loss of local genetic luggage allowing adaptation to specific conditions (Olver and Shuter, 1995). It is also important to take into account that several genetically distinct populations can be more or less connected to one another by the movement of individuals and thereby form a metapopulation, in which each population is influenced by others (Hanski and Simberloff, 1997). Interactions must be interpreted both in their ecological context at a given time, and as the result of past evolution between species involved co-evolution. A common feature is the inclusion of spatial-temporal heterogeneity of habitats, because it plays a major role in modulating selection pressures and constraints of all kinds (Futuyma, 1998; Hanski, 1999).

The questions discussed in this study can be organized according to two main axes: A first one, more descriptive, brings together the questions relating to the establishment of a genetic differentiation between weevils living in different agro-ecological zones of Senegal: how can we detect and quantify genetic differentiation? At what spatial scale? How are the different characters involved? A second axis, more mechanisms-centered, concerns the questions relating to the relationship between differentiation and the process of evolution, even of speciation: are patterns of genetic differentiation concordant? Can the patterns of spatial variability be directly related to patterns of temporal variability? What are the flows of genes and migrants between individuals in the five geographical areas? In particular, we will try to test several demographic scenarios (ABC approaches). These will allow us to test the hypothesis of the formation of new geographical races, or even a possible allopatric speciation. Collectively these researches should allow better identification of populations of *C. maculatus* susceptible to attack cowpea.

Materials and methods

Sampling

The individuals of the studied species result all of various localities in Senegal. The collection period corresponds to the dry period after wintering. The harvested cowpea seeds are put in jars and stored at room temperature. Insects that emerge are immediately collected and kept in alcohol (96%). All individuals coming from the same agro-ecological zone constitute the same population. We worked in five agroecological zones.

DNA 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 *Cytochrome b* gene region was PCR-amplified to characterize mitochondrial DNA. 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 of water, 2.5 ml of enzyme buffer supplied by the manufacturer, 1 µl of MgCl2, 0.5µl of dNTP, 0.25 µl of each primer, 0.2 unit of Taq polymerase and 2µl 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 min, annealing at 47 °C for 1 min and elongation of the complementary DNA strand at 72 °C for 1 min, a final elongation at 72 ° C for 10 min ended the PCR. Sequencing was performed by ABI 3730xl sequencer (Applied Biosystems).

Molecular analyses

Sequences cleaning and alignment

The analyses begin with the sequences alignment, which is a procedure to obtain a correct set of data by making a homology (correspondence) of the different sites of all the sequences. Beforehand, a cleaning is first made, namely a verification of correspondence between chromatogram and sequences for each individual, so sequence by sequence. The software used is Bio Edit version 5.0.6 (Hall, 2001) which uses the Clustal W algorithm (Thompson *et al.,* 1994). Each time, the cleaning and the correction are manually done before being submitted for alignment by the software. So that the correction is finalized, a reading of amino acids is carried out in order to check the structure of Cyt B codons under MEGA.

Genetic analyses

The number of polymorphic sites, the number of informative sites in parsimony, the rate of transitions/transversions (R) and the nucleotide frequency were calculated by using the MEGA 6 software (Tamura *et al.*, 2016) and the substitution model test. To determine the genetic variation of *C. maculatus*, the number of mtDNA gene haplotypes and nucleotide diversities were calculated by using the DnaSP software version 5.10.01 (Rozas *et al.*, 2012). The haplotype (genic) diversity index is defined as the probability that two alleles or haplotypes pulled at random in a sample are different (Nei, 1987), while the nucleotide diversity is defined as being the probability that two homologous nucleotide sites chosen at random are different.

The genetic structure of the populations was investigated with a molecular variance analysis (AMOVA: Analysis of Molecular Variance, Excoffier *et al.*, (1992). All AMOVAs as well as genetic differentiation by population pair were calculated using the ARLEQUIN v3.5.1.2 software (Excoffier and Lischer, 2010), by calculating the diff erentiation index, F (Wright, 1969; Weir and Cockerham, 1984) classically used to describe the distribution of genetic variability between and within populations. The more F approaches the value of one, the more the populations are genetically structured between them. A permutation test (bootstraps 1000) to evaluate the level of significance of pairwise locality differentiation was applied following the approach described in (Excoffier *et al.,* 1992). Genetic distance (d) between pair of populations was calculated under MEGA, by using the model Kimura (1980) 2-parameter (K2P). Distance isolation (IPD) was examined by performing a Mantel test with the XLSTAT 2012 software (Addinsoft, Paris, France), testing the correlation between the genetic distance matrix (d) and the

matrix of the Euclidean geographical distance (in km) generated in Franson Coord Trans 2.3 (Gps Gate AB, Johanneshov, Sweden) from the geographical coordinates of each locality of collection. The Kendall correlation coefficient was used and the level of significance was tested based on 50,000 random permutations.

Phylogenetic relationships between populations of *C. maculatus* were estimated by the Bayesian inference method with the software Mr. Bayes v. 3.1 (Huelsenbeck and Ronquist, 2001). We used the Akaike Information Criterion (AIC) to estimate the best evolution model for each sequence game and selected in Paup and Mr. Modeltest v2.2 (Nylander, 2004). The Templeton algorithm *et al.* (1992) was used to estimate haplotypic or allelic networks relationships. The networks were built using TCS software version 1.21 (Clement *et al.*, 2000).

The demographic history of *C. maculatus* was investigated by calculating the indices of Tajima's D (Tajima, 1989) and Fu's *Fs* test (Fu, 1997) and by analyzing the distribution disparity (mismatch distribution). The indices of Tajima's D and Fu's Fs are known to be sensitive to departures balance mutation-drift due to the changes in population sizes (eg expansion, bottleneck) and selection (Ramirez-Soriano *et al.*, 2008). These statistical parameters were calculated using DnaSP, and the level of significance was evaluated after 10,000 coalescing simulations.

Under constant population size conditions, Tajima's D and Fu's Fs are expected to approach zero, whereas significantly negative or positive values suggest sudden population expansions or bottlenecks respectively. Significantly negative Fs values and non-negative D values suggest recent demographic expansion while the opposite suggests selection. Distribution disparity analyses (mismatch distribution) compare the observed distribution with that expected from the number of nucleotide mismatches between pairs of sequences. The expected values were built by supposing a constant population size: a recent fast growth of the population is characterized by an unimodal distribution while a multimodal distribution characterizes a population with a demographic balance (Rogers andHarpending, 1992). The sum of squares of the deviations (SSD) between the observed and expected distributions as well as the irregularity index (rg) of the observed distribution of the non-concordant classes was calculated as a statistical test under the assumption of a population in expansion using the ARLEQUIN software.

Results and discussion

This study aims to characterize genetically *Callosobruchus maculatus* ecotypes subservient to different agroecological zones of Senegal. Insect pests are the biggest constraint for the production and preservation of cowpeas. Cowpea, one of the most parasitized crops in Senegal, is attacked by a wide spectrum of pests from germination to harvest.

Table 1. Global Parameters of Sequenced *Cytochrome b* Polymorphism in *C. maculatus* Populations.

n	N	S	Ss	Si	H	Eta	Hd	Pi	k
58	407	149	35	114	30	171	0.920±0.00054	0.06±0.00021	

n: number of sequences ; N: number of sites; S: segregation site; Ss : singleton sites; Si : informative sites; H: number of haplotypes; Hd: Haplotype Diversity; Pi: nucleotide diversity.

The most dreadful and the most fatal of these pests is certainly *Callosobruchus maculatus*, cowpea weevil. Many studies on its bioecology have been conducted on this pest (Huignard, 1976); few have focused on the characterization of infesting populations likely to adapt ecologically and genetically to environmental constraints.

Genetic polymorphism and variability

The values of the different parameters of genetic diversity are given in Table 1.

Cytochrome b (Cytb) is a region located between the positions 14747 and 15887 of the mitochondrial genome, a length of 1140 bases in *C. maculatus*. As required the encoding of proteins, no insertion, deletion or stop codon are present in the 58 analyzed sequences allowing concluding that the sequences represent mitochondrial DNA and not nuclear pseudogens.

The length of the *C. Maculatus* Cyt.b sequences analyzed in this study is 407 base pairs (bp). Sequences show a rather high degree of polymorphism: 149 variable sites among which 35 singleton sites, 114 which are informative in parsimony. Haplotype diversity (0.920 ± 0.00054), as well as the nucleotide diversity (0.06007 ± 0.0002113) are positive.

Table 2. Genetic diversity of *C. maculatus* populations for each agro-ecological zone.

	NBA	SBA	SP	VFS	HCSO
n	10	10	19	9	10
H	9	3	10	9	3
S	124	3	16	45	6
Eta	134	3	17	46	6
R	56.26	0.6	3.57	13.72	1.36
Hd	0.978 ± 0.054	0.378 ± 0.181	0.906 ± 0.040	1.000 ± 0.052	0.511 ± 0.164
pi	0.13825 ± 0.02291	0.00147 ± 0.00081	0.00879 ± 0.00190	0.03372 ± 0.00777	0.00333 ± 0.00187
K	56.267	0.6	3.579	13.72	1.36

n: number of individuals; H: number of haplotypes; Hd: Haplotype Diversity; Pi: Nucleotide diversity; S: segregation site; Eta : Total number of mutations.

Table 3. Genetic differentiation *(Fst)* of *C. maculatus* populations between agro-ecological zones.

	NBA	SBA	VFS	SP	HCSO
NBA	0.00000				
SBA	0.55698**	0.00000			
VFS	0.47479**	0.11783**	0.00000		
SP	0.54577**	0.53961**	0.12196*	0.00000	
HCSO	0.56662**	0.75677**	0.18342**	0.12754	0.00000

Non-significant values (p> 0.05) are not followed by asterisk; Significant values (p <0.05) are marked with *, very significant (p <0.01) with **.

The 58 sequences analyzed in this study therefore represent mitochondrial DNA and not nuclear pseudogenes. A high level of genetic diversity in *C. maculatus* populations is observed with 30 haplotypes found in 58 sequences analyzed. Both haplotypic diversity and nucleotide diversity are positive and high in the global population indicating a large effective stable population signal or an admixture signal from populations that have been isolated from each other (Kébé *et al.*, 2017). This observation has already been made in other Coleoptera populations subservient to stored and marketed foodstuffs (Dia *et al.*, 2014).

Table 4. Genetic distance (D) between and within agroecological zones of *C. maculatus*.

	NBA	SBA	SP	VFS	HCSO
NBA	0				
SBA	0.188	0			
SP	0.2	0.02	0		
VFS	0.196	0.01	0,023	0	
HCSO	0.195	0,011	0,024	0,007	0

When the results are analyzed according to the sampling zones, there is a higher value of variable sites in the agro-ecological zones where cowpea is the most cultivated in Senegal with respectively 124 in the North Peanut Basin and 45 in the valley of the Senegal River (Table 2). The largest number of mutations (134) is observed in the NBA while the lowest value is obtained from SBA (3). The haplotypic diversity of different agro-ecological areas studied vary between 0.378 ± 0.181 and 0.052 ± 1. It is more important in the valley of the Senegal River (VFS); the second largest value of haplotypic diversity (0.978 ± 0.054) is observed in the NBA.

Table 5. Results of the molecular variance test (AMOVA) for populational analysis of *C. maculatus* between agroecological zones.

Source of variation	Variance	% variation
Between agro-ecological zones	7.21381	52.66
Between population of a same agro-ecological zone	-0.12416	-0.91
Inside a population	6.60897	48.25

Table 6. Demographic parameters of the total population of *C. maculatus*.

D Tajima = -0.09888	Fu's Fs = 0.38068	R2 = 0.10355
p-value = 0.54500	p-value = 0.41800	p-value = 0.00000

The nucleotidic diversity gives values fluctuating between 0.00147 ± 0.00081 and 0.13825 ± 0.02291. Its greatest value is obtained in the NBA and its lowest value in the SBA. The average number of nucleotidic difference k is very high in the NBA (56.27) and very low in the SBA (0.6). It is 13.72 in the VFS, 3.58 in the SP zone and 1.36 in the HCSO. Overall the NBA has a greater genetic diversity, followed by VFS. The South Peanut Basin (SBA), where cowpea is not a priority, has the lowest level of polymorphism.

Table 7. Neutrality indices for agro-ecological zones.

	NBA	SBA	SP	VFS	HCSO
D Tajima	-0.05932	-0.08644	-0.08164	-0.06251	-0.07088
P-value	0.52900	0.54700	0.54200	0.53100	0.55300
Fu'sFs	0.27277	0.32846	0.34658	0.26250	0.38331
P-value	0.46200	0.45500	0.44100	0.46100	0.45700
R2	0.16320	0.16190	0.16388	0.16038	0.16193
P-value	0.00000	0.00000	0.00000	0.00000	0.00000

The analysis of results based on the zone of sampling reveals a higher value of variable sites, number of mutations, haplotypic diversity, nucleotide diversity and number of nucleotide differences in agro-ecological zones of North Peanut Basin and Senegal River Valley, areas where cowpea is the most widely cultivated in Senegal. These values are often low to moderate in the other sampled areas. Overall the NBA has a greater genetic diversity, followed by VFS. South Peanut Basin (SBA), where cowpea is not a priority, has the lowest level of polymorphism. The importance of a genetic diversity of a pest correlated with the importance of the cultivation of its host plant has been demonstrated in several studies (Sembène *et al.*, 2010; Sembène *et al.*, 2008; Kergoat *et al.*, 2005). This could be explained by the close relationship between the pest and its plant host which constitutes its substrate reproduction.

Table 8. Demographic parameters for each agro-ecological zone of *C. maculatus*.

	NBA	SBA	SP	VFS	HCSO
SSD	0.0503 4	0.01116	0.01570	0.04009	0.04381
p-value	0.27000	0.51000	0.91000	0.21000	0.51000
Fay and Wu's	-0.05164	0.08747	-0.45298	-0.41760	0.19609
P-value	0.34700	0.34500	0.33700	0.35400	0.33600
Raggedness	0.08684	0.08829	0.08916	0.08969	0.08749
P-value	0.00000	0.00000	0.00000	0.00000	0.00000

The 58 sequences present a total of 30 haplotypes distributed in the 5 agro-ecological zones. H4 and H17 consist of a large number of individuals. H4 is predominantly made up of individuals from the SBA area while H17 is predominantly comprised of individuals from Upper Casamance (HCSO) and Sylvo Pastoral Zone (SP). The results are represented by Fig. 1. Each circle represents a haplotype and has a dimension which is proportional to the number of individuals it contains.

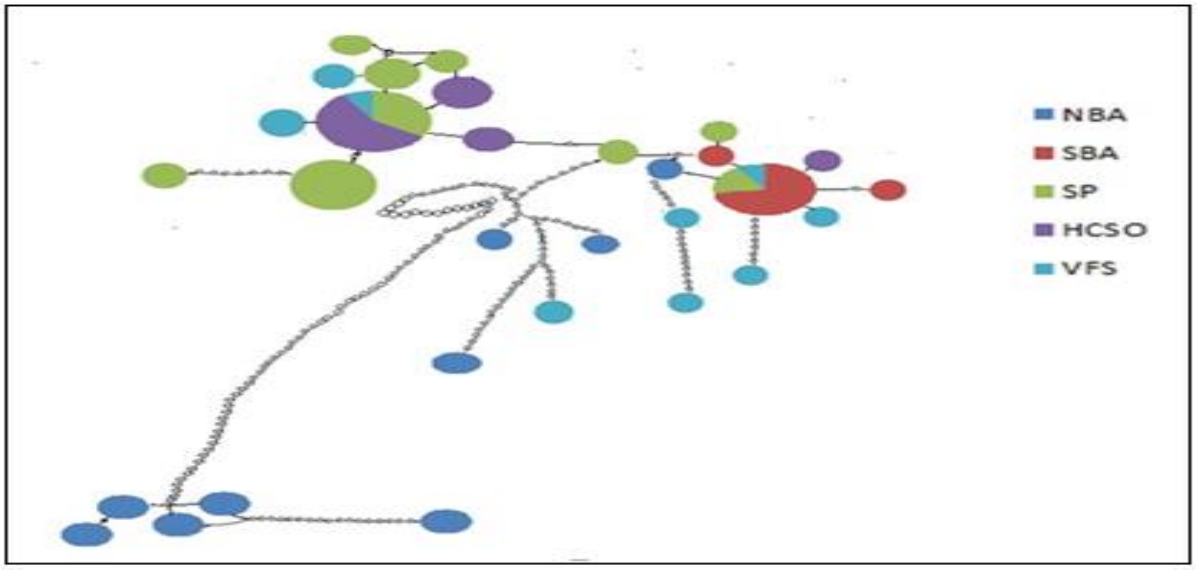

Fig. 1. Network of cytochrome b haplotypes of *C. maculatus* populations found in agro-ecological zones showing relationships between different haplotypes. The branches represent the number of mutation steps between two haplotypes. The sizes of the ellipses are proportional to the observed frequency of each haplotype.

The most representative haplotype (H17= 12 individuals) is located in three agro-ecological zones: HCSO which constitutes more than 50% of the haplotype, SP and VFS. The second haplotype (H4) in terms of number of individuals also crosses 3 areas: SBA which is widely the most representative, SP and VFS. The third haplotype is found in 4 individuals all from the SP area. 6 haplotypes are at most constituted by 3 individuals of the same provenance, and the 21 remaining haplotypes are unique haplotypes. The network shows an isolation of haplotypes formed only of NBA individuals by a long chain of mutational steps.

VFS haplotypes are found at the ends of the network. The distribution of haplotypes within the agro-ecological zones is presented in Fig. 2. Two agro-ecological zones have highhaplotypic diversity: NBA and VFS. The first contains 9 haplotypes in total out of the 10 individuals they contain and records the highest diversity followed by VFS with 8 haplotypes out of 9 individuals. The SP zone contains 9 haplotypes out of 19 individuals. SBA and HCSO show the lowest haplotypic diversity with only 3 haplotypes out of 10 individuals.

The 24 haplotypes on the whole constitute individual haplotypes suggesting in some areas low haplotypic diversity. H4 and H17 which consist of a large number of individuals are located in areas of the South Peanut Basin, Upper Casamance, Sylvo-pastoral zone where genetic diversity is not important. This testifies to these populations a signal of a severe and prolonged bottleneck.

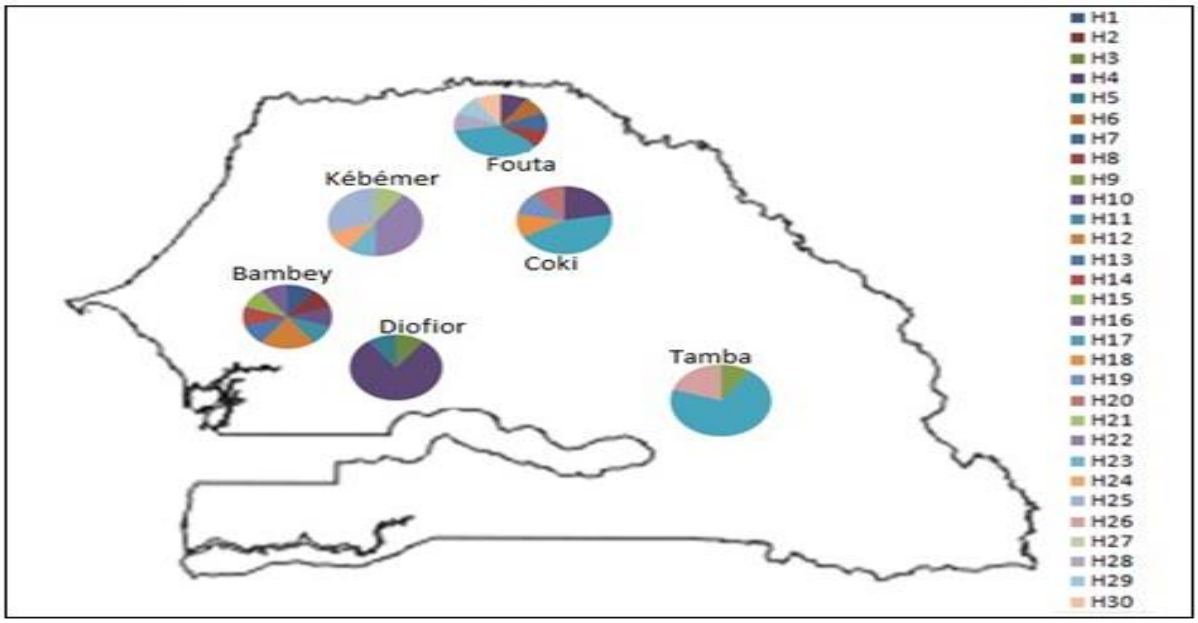

Fig. 2. Spatial distribution of *Cytochrome b* mitochondrial gene haplotypes in sampled agro-ecological zones. The parts of the pie charts correspond to the frequency of each haplotype and the colors referring to the haplotypes in the network.

These results are already seen in Diome *et al.* (2013), working in *Tribolium castaneum* populations in storage infrastructures. Moreover, the network shows an isolation of the haplotypes formed only of the NBA individuals by a long chain of mutational steps indicating that this zone is a zone of permanent mixing of the individuals of *C. maculatus*but whose first infestations would come from the Sylvo Pastoral Zone. With the advance of drought correlated with the intensification of cowpea cultivation, the genetic diversity of the pest has rapidly increased through multiple mutations but not always beneficial to evolution. These statements are supported by the numerous forking mutations and not generating new haplotypes along this mutational chain. The presence of haplotypes of the Senegal River Valley at the extremities of the network could testify to a recent colonization of the valley area by *C. maculatus*. One could even think that this colonization was made recently from individuals who would come from the North Groundnut Basin and the Sylvo Pastoral Zone.

Genetic structuration and phylogenetic evolution

The FST values are presented in Table 3. The value of the global differentiation (Fst) of the populations studied is (0.51).

The comparison of the FST per pair of population reveals that the more genetically differentiated populations are those of the SBA and HCSO with a very high Fst value (0.75) whereas the minimum value is encountered between the individuals of the river valley and those South Peanut Basin.

The highest genetic distance is observed between the NBA and the SP zone (Table 4), while the populations of the HCSO and VFS zones have a low genetic distance. The analysis of the values of the genetic distance within the agro-ecological zone shows that the population of the NBA is constituted, with a value of 0.164, of individuals presenting a significant genetic diversity. This established fact is also noticed to a lesser extent in the VFS (d = 0.035).

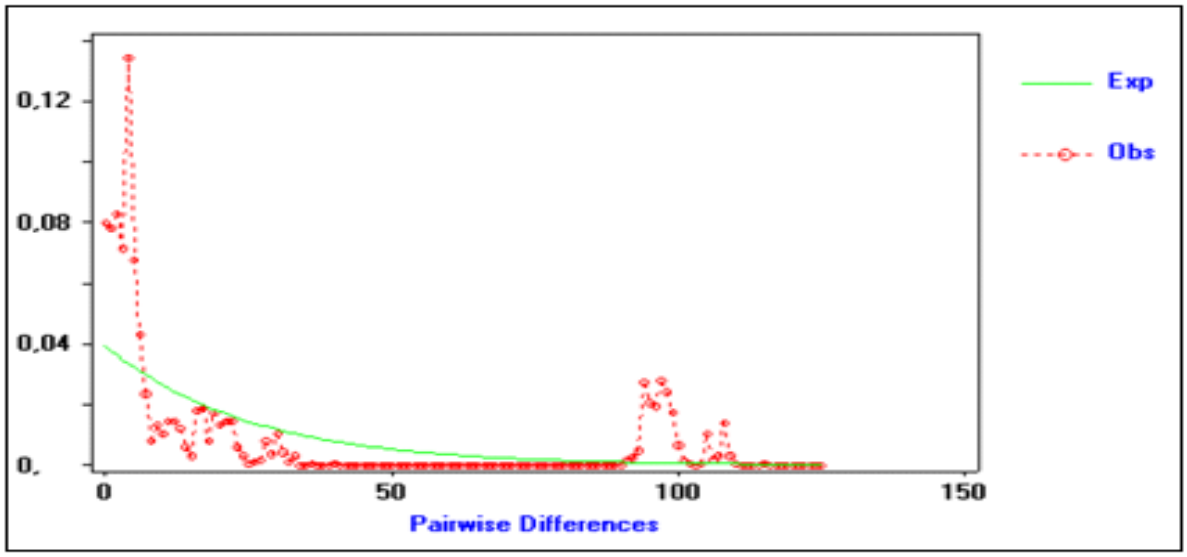

Fig. 3. Mismatch curve of the overall population.

The other zones have a genetic homogeneity of the individuals that compose them with intra-zone distances ranging from 0.001 (SBA) to 0.009 (SP). The intra-HCSO genetic distance is 0.003. The results of Molecular Variance analyzes are presented in Table 5. Although the percentage change between agro-ecological zones is large in absolute terms (52.66) the value of the probability (p> 0.05) reveal that the genetic structure of the populations studied is not significantly different from one agro-ecological zone to another.

The genetic variation (48.25%) within the populations is revealed however significant.

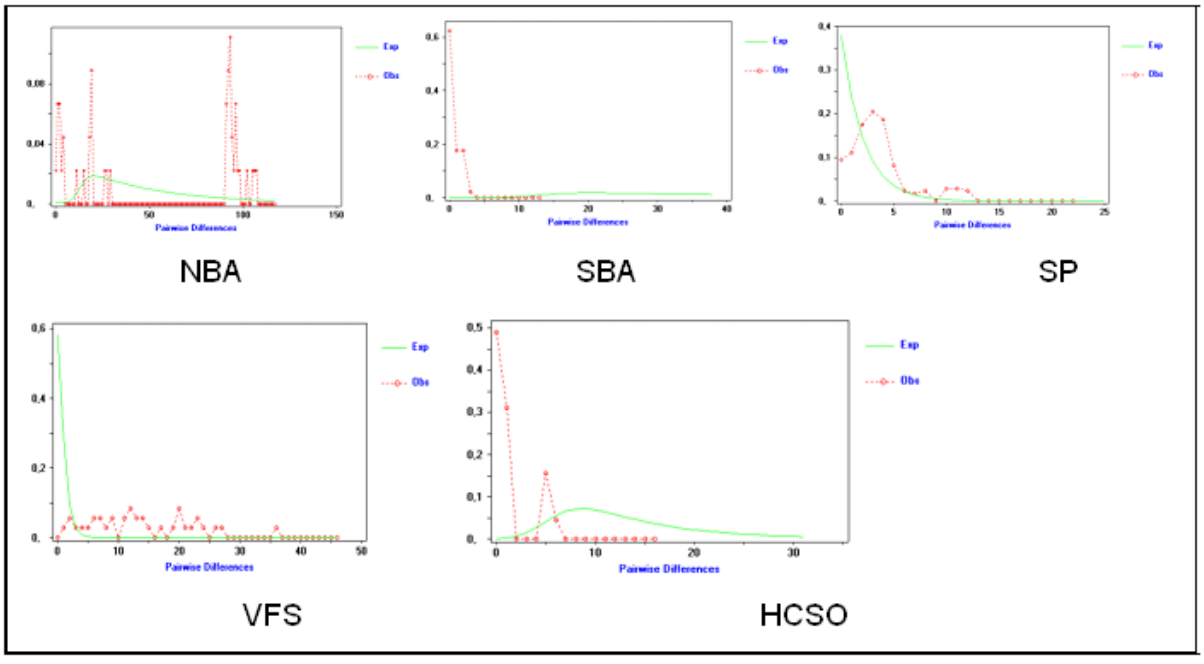

Fig. 4. Mistchmach curve of the population of each agro-ecological zones.

Taken as a whole, populations of *C. maculatus* have a negative Tajima's D and a positive Fu's Fs but both are insignificant (p> 0.05). The R2 of Ramos is however significant. Regardless of the agro-ecological zone, the indices (DT and Fs) are insignificant (P> 0.05). For all agro-ecological zones, DT is all negative whereas FS are all positive. R2, however, has positive values that are all significant (Table 7).

For all the localities, the SSD and the r of Raggedness are all positive but not significant (Table 8). Mismatch analysis reveals a multimodal distribution for all populations studied (Fig. 3 and Fig. 4).

With r = -0.266 and a p-value> 0.05, the mantel test makes it possible to maintain the hypothesis of no correlation between the matrix A and matrix B, i.e. the geographical distance is not correlated to genetic differentiation (Fig. 5).

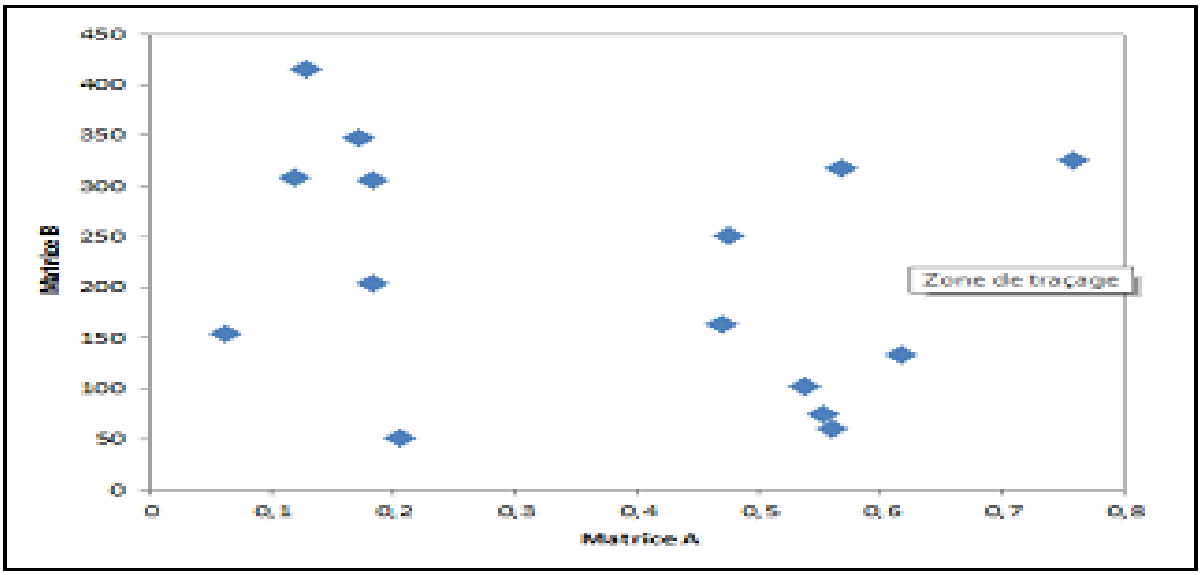

Fig. 5. Mantel correlation test on *C. maculatus* populations.

From external nodes to the internal nodes, we notice a progressive and gradual structuring of the individuals of each of the agro-ecological zones. The first two clades consist of a group of individuals all belonging to the Sylvopastoral zone (clade 1) and another consisting of the remaining individuals (clade 2). The second level of dichotomization isolates, from clade 2, two subclades. The first is heterogeneous but consists of all HCSO individuals (except T6) and a set from all other areas except the NBA and SBA. This subclade is divided into two badly resolved groups with null bootstrap values (Fig. 6). Genetic differentiation values at different hierarchical levels and phylogenetic reconstructions from genetic distances show that genetic structuring is linked to the fact that cowpea weevil develops over several agro-ecological zones that differs as well by rainfall and landscape characteristics determining conditions of different environmental constraints. Most individuals in the Sylvo pastoral zone cluster in a basal clade. The second level of dichotomization isolates, from clade 2, a heterogeneous group including all individuals of HCSO (except T6) and a set of individuals from all other areas except that of the NBA and SBA. It is evident that there is some genetic structuring dividing the population of *C. maculatus* into ecotypes partially adapted to different agro-ecological zones. Still, the review of the results of Molecular Variance analyses shows that the percentage of variation between agro-ecological zones (52.66) is important and demonstrates the establishment of a certain isolation of different agro-ecological populations .The background noise which prevents clear demonstration of this structuration is the genetic variation (48.25%) within populations which turns out by against significant and could be explained by the existence of homogenization factors linked by for example, cowpea trade in Loumas or seed transfer; this also would justify the lack of correlation between geographical distance and genetic differentiation.

This type of genetic isolation mechanism observed today in *C. maculatus* has been demonstrated by (Sembène *et al.*, 2010) at the peanut weevil which is actually made up of several biotypes.

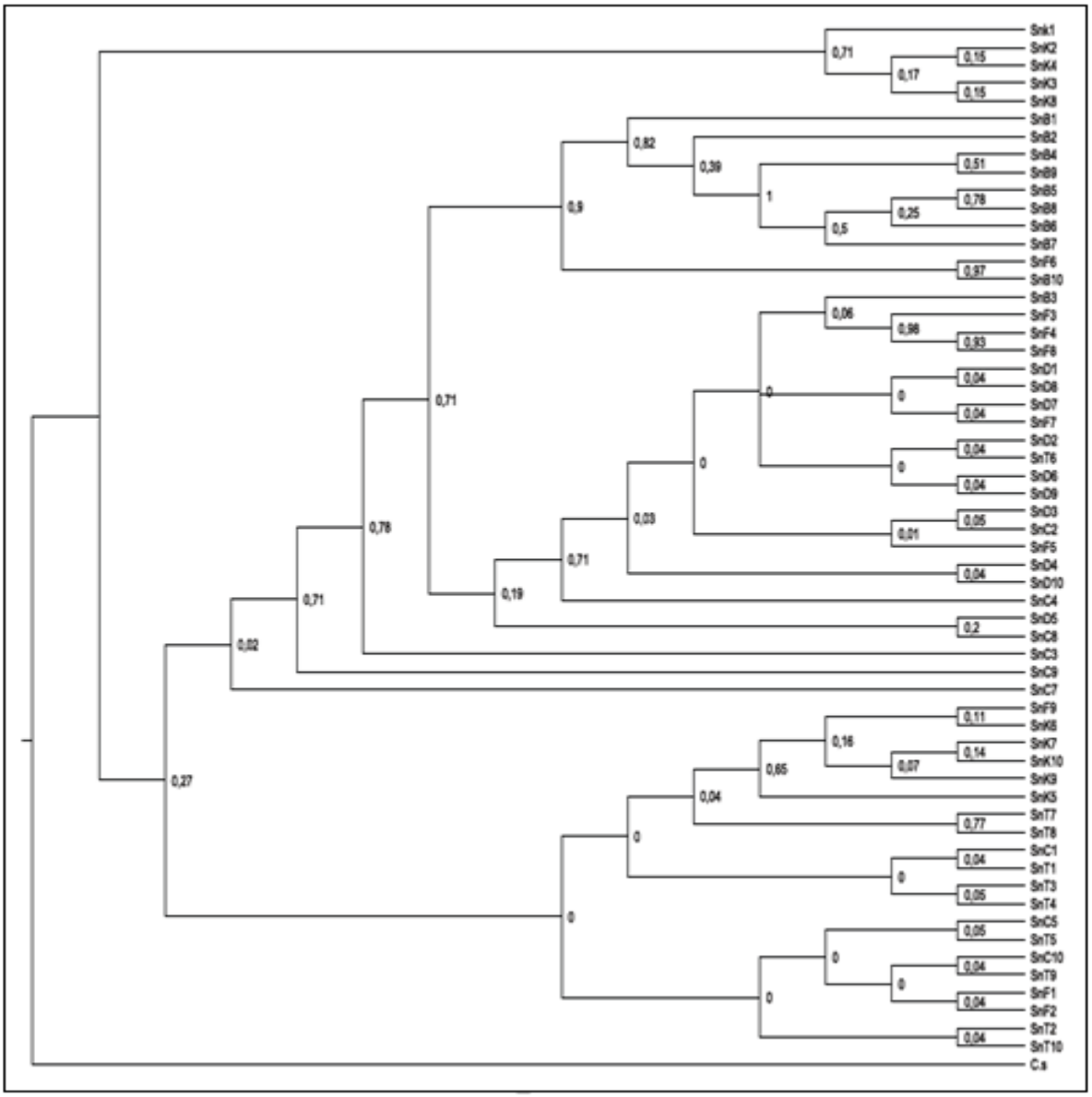

Fig. 6. Phylogram of individuals of Senegal specimen *C. maculatus* using the Bayesian inference method obtained under the optimal model GTR + G 5 with the MEGA software. *C. serratus* is the out-group.

This genetic adaptation to environmental, ecological and dietary factors may result from the existence of a high degree of individual variability in the selection of the nesting site (Delobel *et al.*, 1995). However, the demonstration of all the pre and post-zygotic mechanisms that have favored the formation and maintenance of biotypes or ecotypes among beetles (phytophagous seminivore) requires a fieldwork of around 20 years or more (see the work of Feder (1998) on *Rhagoletis pomonella*), especially since the evolutionary history of phytophagous insects is linked to the vegetal groups that serve them as food. It is important to know how phytophages have responded to the diversification of plants or varieties and whether they have been able to act on them. Ehrlich and Raven (1969) think that the associations between plants and insects observed today have been shaped by a process of coevolution step by step in which plants have developed defenses against their natural enemies which, in turn, have developed mechanisms to circumvent these defenses. This famous hypothesis is opposed by authors who consider the adaptive radiation of a line of phytophagous insects could only be done long after the morphological and chemical diversification of its host plants; it is the idea of "sequential evolution" found in Futuymaand Mc Cafferty (1990).

According to Bush and Diehl (1982), the formation of host races or biotypes in a phytophagous insect involves changes in food preferences and/or nesting sites, physiological adaptations to the new host, preferential crosses between partners associated with each guests. Interactions must be interpreted both in their ecological framework at a given moment, and as the result of a past evolution between the species involved (co-evolution). A common feature is that spatial and temporal heterogeneity of habitats is taken into account because it plays a major role in modulating the selection pressures and constraints of all kinds (Futuyma, 1998, Hanski, 1999).

The questions that we therefore can ask are the following: are cultivated varieties are function of agro-ecological zones? What is the influence of cowpea varieties in the setting up of genetic structuring of *C. maculatus*? However, it must be added the constant pressure of chemicals on *C. maculatus* to protect crops and types of storage infrastructure that may vary from one area to another.

In all cases, the results of the neutrality test with the D of Tajima confirm the hypothesis of a demographic expansion of populations in all the agro-ecological zones. This is confirmed by the irregularity indices of Raggedness and SSD. We thus obtain a multimodal distribution which confirms the hypothesis of a population in demographic expansion. These results confirm those of Kébé (2013) who indicate a rapid demographic expansion of local populations of *C. maculatus* after working on two genes taken separately.

Conclusion

At the level of evolutionary biology, it is obvious that differentiation into ecotypes or host races is a phenomenon widely discussed in phytophagous insects. Indeed in many cases, it is observed after the introduction of a new plant. Recent studies, however, increasingly highlight the effect of climate change and the characteristics of agro-ecological zones on the structure and dynamics of phytophagous insect populations.

Acknowledgements

The authors thank Dr. Fatimata MBAYE for assisting in the realization of molecular manipulation and Mame Diarra GUEYE (Master Degree) for the translation of the document.

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