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Genetic diversity and structure of *Callosobruchus maculatus* populations in the different agro-ecological zones of Senegal

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ABSTRACT

The objective of this study is to determine the diversity and genetic structure of cowpea weevil populations in the different agro-ecological zones of Senegal. Thus, to achieve this objective, individuals of *Callosobruchus maculatus* from localities in each zone were used, after sampling and massive breeding. The sequenced gene is Cyt-B. The results showed high haplotypic diversity (0.901 ±0.00033) and low nucleotide diversity (0.010 ±0.0000003). These diversities studied by agro-ecological zone show that the Casamance zone has the greatest haplotypic diversity (0.901; P>0.05) while the eastern Senegal zone has the lowest value (0.439; P>0.05). The nucleotide diversity is lower in the Sylvo-pastoral zone (0.00112; P<0.05) and higher in the Casamance zone (0.01052; P<0.05). The genetic structuring shows that the Senegal River Valley-eastern Senegal zone couple is more differentiated [FST(Fixation index)]=0.70687; P<0.05) and the Casamance-Groundnut Basin zone couple is less differentiated (FST=0.21248; P<0.05). The greatest genetic distance was found between the Sylvo-pastoral zone and the River Valley and the smallest between the eastern Senegal zone and the River Valley. However, a significant genetic variation within the populations was noted (64.93%). Overall we have a low level of diversity and weakly structured populations except those of Tamba and Fouta. A slight influence of the zones was also noted.

Contribution/ Originality: This study revealed the influence of agro-ecological zones on the structure and diversity of the populations of *C. maculatus* which develop there. Indeed, the agro-ecological zones, despite having different climatic conditions, have a weak influence on the structuring of the populations of *C. maculatus* even if they present a high genetic diversity.

1. INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp. (Fabaceae) is a legume that plays an important role in the food balance of populations in tropical regions. These seeds are the cheapest source of protein for most African populations [1]. Indeed, the seeds constitute an important source of proteins which can fill the insufficiencies in animal proteins of the food rations in the sub-Saharan countries. Cowpea also represents a significant source of income for farmers in West Africa. Despite its importance, cowpea agro-systems harbor enough pests and beneficials. Several insect species can significantly reduce cowpea yields and productivity [2]. During storage, insects and mainly certain families of Coleoptera (Bruchidae and Curculionidae) attack the grains [3]. If no protection is made, after seven months of storage, the loss of foodstuffs can be total [4]. *Callosobruchus maculatus* (*C. maculatus*) is a cosmopolitan

species almost found in all ecosystems. Because of its high reproductive potential, it is the most formidable predator in cowpea stocks where it does enormous damage. In Senegal, the cowpea weevil is known to all farmers on all sides. In the different agro-ecological zones of the country, the observation is the same: a species very harmful to cowpea stocks; those areas where agriculture is the main subsistence activity and where cowpea is a valuable commodity: food, economic and agronomic values. This means that several cowpea protection methods are invented, but until then the problem of cowpea storage arises, difficult to control this species. Due to the strong pressure of pests in vegetation and in stock, cowpea cultivation is faced with multiple problems in the peasant environment: low yields, difficult storage, etc. It is therefore urgent to find better strategies to control these pests and those that deteriorate stocks.

Of all the proposed strategies, the one concerning the biology of the *C. maculatus* bruchid is the least known and least mastered; even though the populations of this insect may be genetically different and environmental factors could be a cause. So doing genetic studies on bruchid is more than necessary. There is a wide range of molecular markers available today that can be used to accomplish this type of research. A genetic marker is potentially useful for inferring whether populations are open or closed to exchange of individuals, it must have a known mode of inheritance and be variable within the species [5]. This justifies our choice to use mitochondrial DNA (desoxyribonucleic acid) and specifically the cytochrome B gene (Cyt-B). Thus, by analyzing mitochondrial DNA extracts from individuals of *C. maculatus* from different localities in the areas studied, we can define whether populations are genetically distinct or not. We have thus put forward the hypothesis that the agro-ecological zones of Senegal have an impact on the structuring of the diversity of *C. maculatus* populations. The objective of this work is to determine the genetic diversity and the structure of *C. maculatus* populations encountered in the different agro-ecological zones of Senegal as well as their phylogenetic relationships in order to see how to fight against this formidable pest.

2. MATERIALS AND METHODS

2.1. Sampling

Samples were taken almost everywhere in Senegal, which allowed us to cover all the agro-ecological zones except that of the niayes where cowpea is rarely grown. In fact, for each zone, samples from different localities were collected. Samples were collected during the post-harvest period from farmers or in the market of each locality. They have been clearly identified by inscribing the name of the locality of origin. These samples, weighing a minimum of 250g, were sent to the breeding laboratory where they were transferred to well-ventilated jars that did not allow the insects to escape from emergence. Rearing was done in the laboratory under ambient conditions until the emergence of adults of *C. maculatus* with regular monitoring. Following emergence, the insects were collected and placed in 96° alcohol. The tubes containing the specimens of *C. maculatus* were placed in the refrigerator for good conservation.

These specimens were used for genetic studies. Before the work for the genetic studies, the identified samples were coded according to an alphanumeric code defined by the 1st letters of the binomial name of the species (C for *Callosobruchus* and m for *maculatus*), followed by the 1st letter of the name of the locality sampled after comes a second letter indicating the second name of the locality which is finally followed by the serial number indicating the individual (Example: Code CmCd1; *Callosobruchus* (C) *maculatus* (m) Carrefour (C) diaroumé (d) individual (1). For the other samples, we simply took the name of the locality (see Table 1).

Individuals from samples from the same area form a population. This corresponds to a total of 7 populations. The size of the populations studied is a function of the number of individuals taken per sample; this number varies according to locality.

Table 1. Sampling summary.

Agro-ecological zones	Sampled localities	Sample codes	Number of individuals	Geographical coordinates	
				Latitude	Longitude
Groundnut basin (ZBA)	Fatick	Cmfatick	10	14°20'22"N	16°24'40"W
Casamance zone (ZC)	Diaroumé crossroads	CmCd	07	13°03'31"N	15°38'20"W
	Diannah malary	CmDm	07	12°50'15"N	15°15'07"W
Eastern Senegal zone (ZSO)	Tamba	Tamba	12	13°46'14" N	13°40'2"W
Senegal river valley zone (ZVFS)	Fouta	Fouta	09	16° 32'01"N	16°08'19"W
Sylvo-Pastoral zone (ZSP)	Barkédji	CmBb	09	15°16'40"N	14°52'02"W
	Koki	Koki	10	15°30'20"N	15°59'30"W
Total			64		

2.2. DNA Analysis

2.2.1. Cytochrome B (Cyt-B)

One of the genes most regularly used in studies on the molecular evolution and structuring of insect pest species is the mitochondrial Cyt-B gene [6, 7] mtDNA is haploid (N), non-recombinant [8]. It is transmitted almost exclusively by the maternal organism mtDNA is more suitable for demonstrating specific variability than nuclear DNA. The gene that encodes Cyt-B is widely used in molecular phylogeny [9, 10] and also in population genetics [11].

Although Cyt-B is subject to strong evolutionary constraints, some of its internal regions are more or less conserved than others due to their functional restrictions [12].

2.2.2. Extraction of DNA from C. Maculatus

The extraction is done in four steps: tissue digestion, cell lysis, DNA purification and elution.

In our study, we extracted DNA from the tissues of the insect *C. maculatus* with the zymo research kit following the standard protocol. For each individual, the head, thorax and legs were removed and placed in a 1.5 ml tube.

2.2.3. Amplification of DNA by the Polymerase Chain Reaction (PCR)

The forword and reverse primers used are respectively mtD26 and mtD28

(5'-TATGTACTACCATGAGGACAAATATC-3')

(5'-ATTACACCTCCTAATTTATTAGGAAT-3')

The PCR was carried out with the One Taq Quick-Load 2X Master Mix kit in a reaction volume of 25 µl containing 12.5 µl of Master Mix, 08.5 µl of pure water, 1 µl of the primers (i.e. a volume of 0. 5 µl per primer) and 1 µl of MgCl2 as catalyst.

The amplification conditions are: (i) a polymerase activation step (hot start) and initial denaturation of 03 minutes at 94°C, (ii) 35 cycles of denaturation at 94°C for 01 minute followed by a time of one minute at 47°C for annealing and extension of the primers or elongation for a time of one minute at 72°C and (iii) a final extension at 72°C for 10 minutes.

2.2.4. Sequencing

DNA sequencing consists of determining the sequence or sequence of nucleotides in a given DNA fragment. In our study, the sequencing was done by a South Korean company called Macrogen. The sequenced gene is cytochrome B, which is a mitochondrial gene of great interest.

2.3. Genetic Analysis

2.3.1. Alignment, Cleaning and Correction of Sequences

According to Swofford, et al. [13], sequence alignment is important in determining whether or not sites are similar. Sequences were aligned as a whole using Bio Edit software ver. 7.2.5 [14]. Errors within the sequences have been identified and corrected manually using the same software. As the correction was made, the alignment was renewed using the crustal w algorithm [15] which is part of the so-called global alignment methods.

2.3.2. Analysis of Genetic Diversity

Since the gene studied is Cyt-B; a mitochondrial gene, we first checked the codon structure of the gene sequences by transforming them into amino acids using MEGA (Molecular Evolutionary Genetics Analysis) 7.0.14 software [16]. This operation revealed no evidence of putative nuclear pseudo-genes. Then the parameters such as the number of polymorphic sites, the number of informative sites in parsimony, the nucleotide frequency were also determined using the MEGA7.0.14 software [16]. Finally, the number of haplotypes as well as the haplotypic and nucleotide diversities were determined using the software DNASp version 5.10.01 [17].

The haplotypic diversity index is defined as the probability that two randomly selected alleles or haplotypes in a sample are different [18], while nucleotide diversity is defined as the probability that two randomly selected homologous nucleotide sites be different. These parameters made it possible to determine the genetic variation of *C. maculatus*.

2.3.3. Structure and Genetic Distance

The genetic differentiation between pairs of populations was calculated using the Arlequin v3.1 software [19], by calculating the differentiation index F [20, 21]. This indix were 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.

The genetic distance (d) between pairs of populations was calculated under MEGA, using the Kimura [22] 2-parameter (K2P) model.

A permutation test (1000 boostraps) was applied following the approach described by Excoffier and Heckel [19] to assess the level of significance of the differentiation by pair of localities.

2.3.4. Molecular Analysis of Variance (AMOVA)

With an analysis of molecular variance (AMOVA: Analysis of Molecular Variance [19]), we carried out an investigation of the genetic structure of populations. AMOVA is an analysis of variance/covariance of haplotypic (or allelic) frequencies. information from these frequencies, it uses molecular data taking into account the number of substitutions between haplotypes (or alleles).This is an analysis of the variance measured at each level of the structure (intra- population, inter-population, intra-group and inter-group, where here, a group represents an agro-ecological zone and the populations represent the sampling localities).The Arlequin V3.1 software [19] was used to perform the test of molecular variance (AMOVA) between agro-ecological zones.

2.3.5. Phylogenetic Analyzes

The maximum parsimony and Neighbor-Joining phylogenetic trees were constructed by the MEGA7.0.14 software [16].

The Neighbor-Joining method [23], is based on the principle of minimum evolution which makes the assumption that the true tree is the tree of the shortest length such that the lengths of its branches describe as faithfully as possible the evolutionary distances between the taxa considered.is based on a matrix of genetic distances of haplotypes (the distance of kimira2parameter) taken two by two to model the evolutionary process.

The maximum parsimony method [24], considers that a tree is optimal when the total length (number of steps necessary to explain the analyzed dataset) is minimal. A consensus of all the selected trees is then achieved.

We used the Akaike Information Criterion (AIC) to estimate the best evolutionary model as selected in Paup and MrModeltest version 2.2 [25]. The model selected here for the Cyt-B is the HKY(Hasegawa-Kishino-Yano) +G(Gamma distribution) +I(Invariable). Knot robustness was assessed for 1000 bootstrap repetitions. Boostrap is a method of resampling nucleotide sites by random drawing with replacement, which corresponds to a random weighting of sites. The boostraps value of a node corresponds to the frequency of this node in the set of trees inferred from pseudo sequence matrices thus obtained. The reconstructions were rooted with a homologous sequence from the species *Callosobruchus chinensis*.

3. RESULTS

3.1. Genetic Diversity: Polymorphism and Variability

Cyt-B is a region located between positions 14747 and 15887 of the mitochondrial genome of *C. maculatus*, i.e. a total length of 1140 base pairs.

In our study, the length of the Cyt-B sequences studied is 418 base pairs (bp). A total of 64 sequences were studied. They have no insertion, deletion or stop codon. These sequences represent those of mitochondrial DNA. The values of the different parameters of genetic diversity are recorded in Table 2.

These sequences show a rather low degree of polymorphism; they are not very polymorphic with 14 variable sites including 01 singleton site and 13 which are informative in parsimony. This means that we have 404 preserved sites. Haplotypic diversity (0.901 ± 0.00033) is high while nucleotide diversity (0.010 ± 0.0000003) is low.

Table 2. Global parameters of Cyt. B polymorphism of C. maculatus populations.

N	Ns	Sc	Sv	Ss	Si	H	Dh(Hd)	Dn(Pi)
64	418	404	14	01	13	20	0.901±0.00033	0.010± 0.0000003

Note: N: Number of sequences; Ns: Number of sites; Sc: Conserved sites; Sv: Variable sites; Ss: Singleton sites; If: Informative sites; H: Number of haplotypes; Dh(Hd): Haplotypic diversity; Dn(Pi): Nucleotide diversity.

The sequences taken by sampled locality show a haplotypic diversity between 0.200±0.154 and 0.889±0.075. The locality of Fatick has the highest haplotypic diversity while Koki presents the lowest. The nucleotide diversity is between 0.00048±0.00037 and 0.01050±0.00166 with the locality of Koki having the smallest value of this diversity while Barkédji has the greatest value. The Koki population is the least diverse compared to all populations: it showed the lowest haplotypic and nucleotide diversity. The p-values of the haplotypic diversity of the populations are not significant whereas for the nucleotide diversity, they are all significant Table 3.

Table 3. Genetic diversity of *C. maculatus* populations by sampled locality.

Localities / Settings	Barkédji	Carrefour diaroumé	Diannah malary	Fatick	Koki	Fouta	Tamba
Number of individuals	09	07	07	10	10	09	12
Name of haplotypes	04	03	05	06	02	04	03
Sites variables	11	06	07	11	01	04	02
Haplotypic diversity	0.806	0.714	0.857	0.889	0.200	0.583	0.439
P-value	0.089	0.127	0.137	0.075	0.154	0.183	0.158
Nucleotide diversity	0.01050	0.00752	0.00797	0.00973	0.00048	0.00213	0.00112
P-value	0.002	0.001	0.002	0.002	0.000	0.001	0.000

The haplotypic diversity per agro-ecological zone is between 0.901 and 0.439. The Casamance agro-ecological zone (ZC) has the highest value while the eastern Senegal zone (ZSO) has the lowest diversity value. The p-values reveal no significance. For nucleotide diversity, it is lower in the sylvo-pastoral zone (0.00112) while the highest

value (0.01052) is found in the Casamance zone (ZC). The p-values of all areas are significant (Table 4). The Casamance agro-ecological zone is the most diversified.

Table 4. Genetic diversity of *C. maculatus* populations according to agro-ecological zones.

Ago-ecological zones / Sittings	ZBA	ZC	ZSO	ZSP	ZVFS
Number of individuals	10	14	12	19	09
Name of haplotypes	06	08	03	05	04
Sites variables	11	11	02	11	04
Haplotypic diversity	0.889	0.901	0.439	0.696	0.583
P-value	0.075	0.052	0.158	0.095	0.183
Nucléotide diversity	0.010	0.012	0.001	0.010	0.002
P-value	0.002	0.001	0.000	0.001	0.001

3.2. Genetic Structure of Populations

3.2.1. Genetic Differentiation

The values of genetic differentiation (F_{ST}) are calculated by considering different levels (total population, between localities and between agro zone) and recorded in Tables 5 and 6.

Overall the value of genetic differentiation (F_{ST}) of the studied populations is 0.54266 with a P-value of 0.00000±0.00000; therefore a significant F_{ST}. The genetic differentiations taken by population pairs show that the Koki-Fouta and Koki-Tamba populations are the most genetically differentiated with very high and significant F_{ST} values of 0.93498 and 0.94524 respectively (P-values=0, 0000). The least differentiated populations are those of Barkédji-Carrefour diaroumé which have a negative (-0.05009) and insignificant F_{ST}. The P-values reveal four F_{STs} that are not significant: Barkédji-Carrefour diaroumé and Barkédji-Fatick F_{STs} are not significant at all. These populations did not show significant genetic differentiation (P-values significantly greater than 0.05). The F_{STs} of Carrefour-Fatick and Diannah-Koki are not significant but their P-values are very close to 0.05 (Table 5).

Table 5. F_{ST} between the different localities sampled.

Localities	Barkédji	Carrefour diaroumé	Diannah malary	Fatick	Koki	Fouta	Tamba
Barkédji							
C. diaroumé	-0.050						
P-value	0.622						
D. malary	0.268	0.399					
P-value	0.018	0.009					
Fatick	0.007	0.186	0.406				
P-value	0.315	0.054	0.000				
Koki	0.647	0.788	0.340	0.709			
P-value	0.000	0.000	0.054	0.000			
Fouta	0.273	0.419	0.726	0.243	0.935		
P-value	0.000	0.000	0.000	0.018	0.000		
Tamba	0.266	0.404	0.716	0.451	0.945	0.707	
P-value	0.000	0.000	0.000	0.000	0.000	0.000	

Depending on the zones, the F_{ST} of the whole is 0.35074. Taken two areas, the F_{STs} show that the ZVFS and ZSO areas have the highest value (0.70687); these two areas are genetically the most differentiated. Areas with lower F_{ST} (0.21248) are ZC and ZBA; they are the least differentiated.

P-values reveal non-significance only between ZSP and ZC zones; these two agro-ecological zones are not significantly differentiated (P-value > 0.05); see Table 6.

Table 6. F_{ST} between different agro-ecological zones.

Agro-ecological zones	ZSP	ZC	ZBA	ZVFS	ZSO
ZSP					
ZC	0.043				
P-value	0.135				
ZBA	0.289	0.2125			
P-value	0.000	0.000			
ZVFS	0.505	0.453	0.243		
P-value	0.000	0.000	0.009		
ZSO	0.459	0.389	0.451	0.707	
P-value	0.000	0.000	0.000	0.000	

Table 7. Genetic distances (D) between and within agro-ecological zones of *C. maculatus* (bottom) and standard errors (top).

Agro-écological zones	ZSP	ZC	ZBA	ZVFS	ZSO
ZSP		0.003	0.004	0.004	0.004
ZC	0.011		0.004	0.004	0.003
ZBA	0.014	0.013		0.003	0.003
ZVFS	0.015	0.013	0.008		0.003
ZSO	0.012	0.010	0.010	0.005	

3.2.2. Genetic Structure

3.2.2.1. Genetic Distance

Table 7 shows the genetic distances (D) between and within agro-ecological zones of *C. maculatus* (bottom) and standard errors (top). The genetic distances between agro zones vary from 0.005 to 0.015. The greatest distance is obtained between the Sylvo-pastoral zone (ZSP) and that of the Senegal River valley (ZVFS). On the other hand, the smallest distance is observed between the zone of eastern Senegal (ZSO) and the zone of the Senegal River valley.

3.2.2.2. Molecular Variance (AMOVA)

Table 8 shows the results of the molecular analyzes of variance on the populations of *C. maculatus* grouped according to agro-ecological zones. Molecular analysis shows that the percentage of variation is higher at the level of populations forming the same group (64.93) as well as the sum of the squares (91.43). Between groups, the percentage variation is 35.07 and is 45.73 within populations.

Table 8. Results of the molecular variance test (AMOVA).

Source of variation	Sum of squares	Percentage change	D.f
Between groups (9zones agro)	48.241	35.07	04
Between population within groups	91.431	64.93	59
Within population	62.323	45.73	57

3.3. Analysis of Phylogenetic Trees

From external nodes to internal nodes, the grouping by agro-ecological zone appears clearer. The phylogenetic trees obtained (Neighbor Joining and Maximum Parsimony) show an identical topology from the outset. Each tree presents an individual that is related to all the others that remain: this is for example the individual Koki 10 for the methods of Neighbor Joining and maximum parsimony. Maximum parsimony exhibits six clades. The C1 clade, identical to the C1 clade also of the Neighbor Joining, is formed by only two individuals of Diannah malary (CmDm2 and CmDm5).

The maximum parsimony and the Neighbor Joining show that the individuals from Tamba form an almost homogeneous clade (respectively C8 and C6) and that those from Koki are also in a single clade, that of C3 for these

Figures 1 and 2. However, the Neighbor Joining method presents the highest number of clade (eight in total) and which are more homogeneous. Note that at the level of this tree, the clades C2, C4, C5, C6 and C7 immediately groups together the same individuals having constituted the clades C2, C4 and C5 of the maximum parsimony method.

For all the trees, we found low values of boostraps, therefore weak nodes.

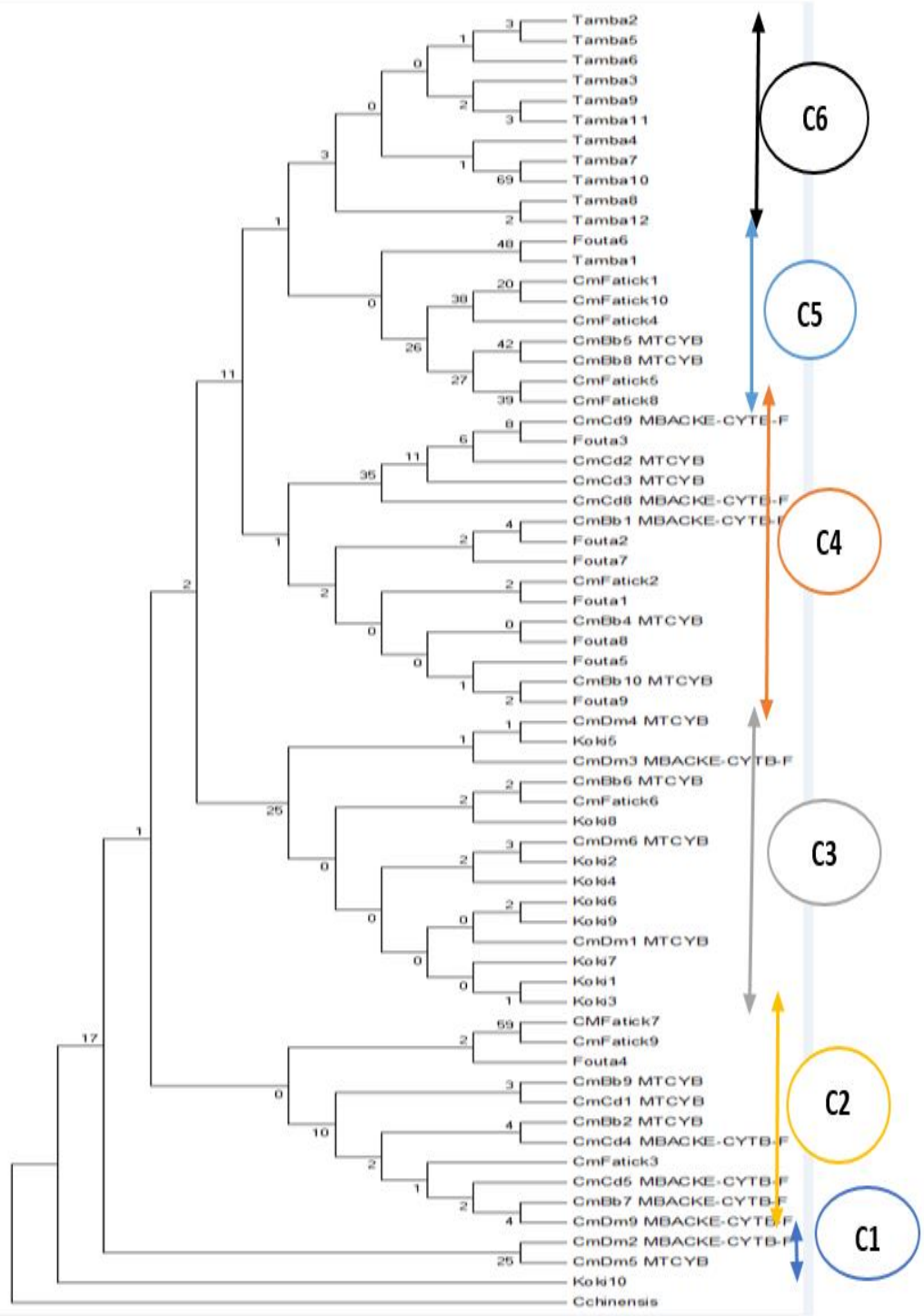

Figure 1. Consensus maximum parsimony tree.

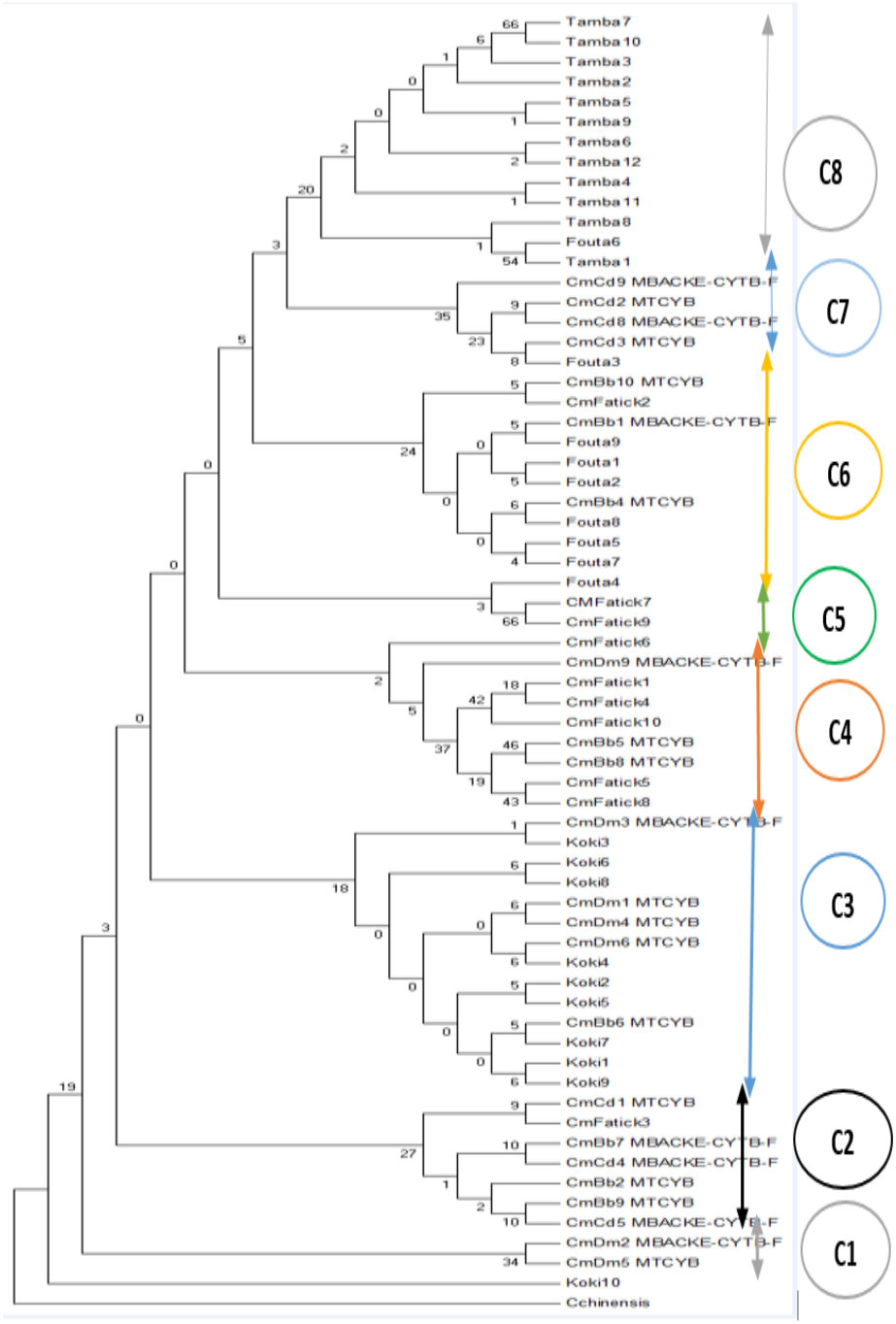

Figure 2. Neighbor-Joining tree

4. DISCUSSION

Insect pests of cowpea stocks, populations of *Callosobruchus maculatus* have been the subject of various studies, few of which are devoted to the genetic aspect.

The objective of this work is to characterize the insect populations of different agro-ecological zones using the mitochondrial gene of Cyt-B.

Strong haplotypic diversities were observed in all the populations studied except that of Koki and that of Tamba which are more or less diversified. The behavior of the latter could be due to the fact that this commodity (cowpea) does not circulate in the locality of Koki and is one-way traffic for Tamba. It may also be due to the dominance of the cultivation of a single variety. According to Salducci, et al. [26], a low haplotypic and nucleotide

diversity on mitochondrial DNA can be the signal of a severe and prolonged bottleneck. The locality of Fatick is an area where several varieties of cowpea are grown, favoring the circulation of weevil, which may explain the diversity obtained. The localities of Diannah and Carrefour are commercial meeting areas with products coming from everywhere, which would explain the diversity of these populations of *C. maculatus*. As for the locality of Barkédji, it is a breeding area where cattle feed would facilitate the flow of *C. maculatus*. That of Fouta, which is booming in the cultivation of cowpea with seasonal and flood recession crops, this also facilitates the exchange of insects via the seeds used and sometimes taken from the markets. In these localities where there are high haplotypic diversities and low nucleotide diversities, this could correspond to a rapid multiplication of the population. According to Sinama [27], the high haplotypic diversity and the low nucleotide diversity noted (on the mtDNA) may be the result of rapid population growth from an ancestral population with low numbers and for which there is no did not have enough time to recover a high diversity between haplotypes. However, we noted a nucleotide diversity which is low. This reflects a level of genetic and nucleotide diversity that is within the standard ranges of the genetic diversity of insect pests [6]. The relationship between haplotypic diversity and nucleotide diversity provides information on the demographic history of a population.

According to the agro-ecological zones, there is a strong haplotypic diversity in all the zones with the zones (ZC and ZBA) which are respectively the most diversified (0.901 and 0.889) while the eastern Senegal zone remains the least diversified (0.439). This can be explained by the fact that the ZBA is a cowpea growing area and with several varieties at the same time, combined with trade which is much more marked in the ZC. By observing the results, we find that the populations of the ZC do not share any individual between them, which makes this area more diversified. This result would result from conservation measures that can give rise to closed or isolated populations despite sharing the same agro-ecological zone knowing that in this zone cowpea comes from everywhere.

The F_{ST}s between the populations of the different localities vary from -0.05009 to 0.94524. This reflects a very low level of genetic differentiation between the populations of Barkédji and Carrefour but very high between those of Koki and Tamba, as well as between Koki and Fouta. The differentiation is rather moderate between the other pairs. According to the zones, the zone of the Senegal River valley and the zone of eastern Senegal are the most genetically differentiated. This could be due to isolation by distance or arising from the characteristics of each agro-ecological zone. The Casamance zone and the Sylvo-pastoral zone, which are less differentiated, would form a single population. This observed structuring, which is generalist, could be explained by the fact that *C. maculatus* behaves like a panmictic unit. This is in line with the work of Kébé [28]. This way of meeting is accentuated by commercial exchanges. However, it should also be noted that the geographical distribution of the food resource can also limit the dispersal of certain individuals and contribute to the under-structuring barely observed for the areas of eastern Senegal and the river valley.

Phylogenetic trees show groups or clades each containing different individuals. These groups are not defined by locality or zone, they are made up of individuals coming from different localities or zones, even if they are far from each other. It should be noted that the individuals from Tamba (ZSO), those from Koki (ZSP) and those from Fouta (ZVFS) seem to form homogeneous groups. The AMOVA carried out shows that the molecular variations observed are more present within the populations themselves than between populations. More than 64% was attributed to intra-population genetic variation. This result is close to that of Kébé [28], who obtained 73% following the combination of two genes (28S and Cyt-B).

5. CONCLUSION

Ultimately, we can say that the populations studied present a high level of diversity. Their structuring is also not well done; there is no population that has clearly distinguished itself even if those of Tamba and Fouta which have a high F_{ST} seem to be isolated. However, the effect of agro-ecological zones is felt in the distribution of

populations, but this does not turn out to be very decisive. This agrees with the conclusions of Kébé [28]. Knowing the structure of *C. maculatus* populations according to agro-ecological zones is a first and essential step in order to be able to control this insect that is harmful to cowpea. However, more extensive studies are needed.

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