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Impact of Agro-Ecological Zones on Genetic Structuration of *Callosobruchus maculatus* F (Coleoptera: Bruchidae) the Major Pest Weevil in West Africa



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ABSTRACT

In West Africa, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is one of the most dangerous insect pests of cowpea seeds (*Vigna unguiculata* L. Walp). The infestation begins in the field of crops and continues in the stocks where the damage can be considerable in the absence of any measure of protection. This study focused on measuring the impact of agro-ecological zones on the variability, differentiation and genetic structuring of *Callosobruchus maculatus* in the sub-region. However, there is low variability and high polymorphism for populations in the Sahelian zone and the Guinean zone, but high variability and low polymorphism for populations in the Sahelo-Sudanian zone and the Sudanian zone. Indeed, the indices of genetic diversity suggest a high haplotypic diversity H_d and a low nucleotide diversity P_i for the four populations revealing a moderate demographic expansion. This result is supported by the differentiation index (FST), which indicates a lack of differentiation and genetic structuring between geographically distant populations such as the Sahelian population of Senegal and the Guinean population of southern Togo. This reflects an average structuring of the populations according to the agro-ecological zones and this result was confirmed by the analysis of the molecular variance or AMOVA. Indeed, genetic divergence is more important within each population than between different populations. Thus, the agro-ecological zones have a small influence on the variability, differentiation and genetic structuring of *Callosobruchus maculatus* because of a strong circulation of haplotypes in the subregion by human mediation favoring a large gene flow.

INTRODUCTION

To meet the food demand of the populations living in developing countries where meat consumption is still a luxury, pulses are an indispensable source of protein. Among these legumes, cowpea: *Vigna unguiculata* L. (Walp), is one of the staples of populations. Because of its role in restoring soil fertility and its compatibility with several crop combinations, this plant is an essential component of cropping systems in savanna areas in tropical Africa (Mulongoy *et al.*, 1992). Cowpea seeds are rich in protein, carbohydrates and vitamins. They contain more than 25% protein and are rich in lysine and tryptophan. Cowpea balances cereal and tuber-based diets that are generally low in protein and high in carbohydrate (Singh *et al.*, 1992). Cowpea leaves are eaten as vegetables in Africa while residues are a good source of animal fodder in the rural world.

Despite its importance, cowpea yields are below average in certain parts of Africa at 700 kg / ha (FAO, 1998). 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 (Delobel and Tran 1993; Alzouma, 1995). 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 bruchids 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 (Doumma *et al.*, 2011). In fact, the larvae of the cowpea shrub grow exclusively in the seed by feeding on the accumulated reserves in the cotyledons (Kébé and Sembène, 2011).

For this present work, the general objective is to determine the genetic structuring of *Callosobruchus maculatus* according to some agro-ecological zones of West Africa to develop effective control methods against this insect pest of cowpea seeds. 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 (Raymond, 1996). 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). To achieve this general objective, the following specific objectives have been defined: (i) To evaluate the diversity and genetic structuring of populations according to agro-ecological zones and (ii) To determine the demographic evolution of *Callosobruchus maculatus* populations and infer the phylogenetic relationships between haplotypes of different agro-ecological zones.

MATERIALS AND METHODS

Sampling and DNA extraction

For this work, the sampling was conducted in five (5) countries covering approximately fourteen (14) localities and sixty-eight (68) individuals harvested from the four agro-ecological zones studied (see Table I). These countries are among the biggest producers of cowpea in the world (FAO stat, 2008). Specimens of *Callosobruchus maculatus* that were the subject of this study result from cowpea seed collected during the dry period (after wintering) over two years (2009 and 2010). Insects are collected from their emergence and kept in 100% ethanol before use. Each population corresponds to a group of individuals from the same site see Figure 1.

Table I: Distribution of individuals by country and locality sampled.

Country	locality	Number of individuals	Code of individual	the Agro-ecological zones
Burkina faso	Tenkodogo	2	bf	Sudanese
Mali	Bougoudere	8	MAB	Sahelo-Sudanian
	Sikasso	8	MaS	Sudanese
Niger	Niamey	6	NiN	Sahelo-Sudanian
	Coki	4	SnC	Sahelo-Sudanian
Senegal	Kebemer	2	SnK	Sahelo-Sudanian
	Fouta	5	SnF	Sahelian
	Tambacounda	8	SnT	Sudanese
Togo	Adidogome	3	dBP	Guinean
	Assigame	4	TAs	Guinean
	Dapaong	4	TDa	Sudanese
	Gbossine	5	TGb	Sudanese
	Mango	5	TMa	Sudanese
	Tsevie	4	TTs	Guinean
total	14	68		4

The first step in laboratory manipulations was to extract the genomic DNA of *C. maculatus* individuals according to the DNeasy Tissue kit (QIAGEN Inc) extraction kit from the head, thorax and legs of the insect.

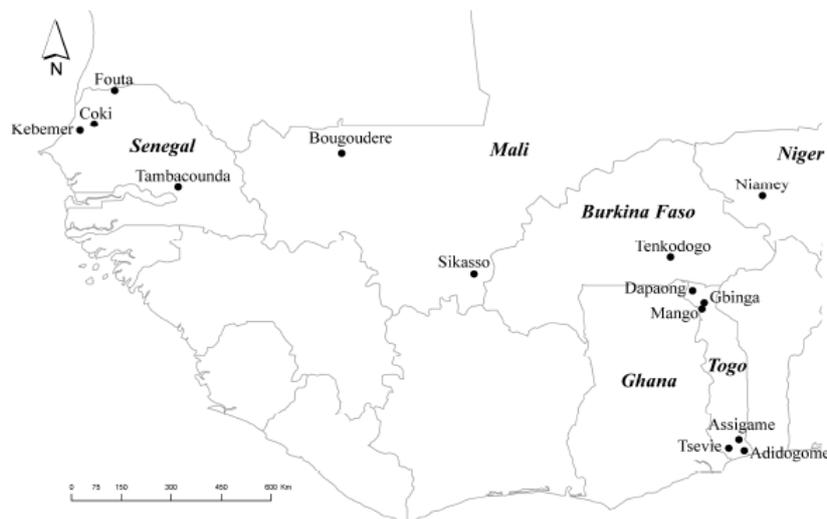


Figure 1: Geographical distribution of localities of origin of *C. maculatus* populations.

Tenkodogo (Bf), Bougoudere (MaB), Sikasso (MaS), Niamey (NiN), Coki (SnC), Fouta (SnF), Kébémér (SnK), Tambacounda (SnT), Adidogomé (TAd), Assigame (TAs), Dapaon (TDa), Gbinga (TGB), Mango (TMA), Tsevie (TTs), (Kebe, 2017).

Molecular markers: CytB and 28S

Two molecular markers have been studied in this paper to investigate the probable influence of agro-ecological zones on the structure of *C. maculatus*.

The mitochondrial gene cytochrome B is a polymorphic gene with maternal transmission and is of great interest.

The 28S gene codes for 28S ribosomal RNAs of the large ribosome subunit and its transcription gives rRNA to accomplish catalytic functions in protein synthesis. The 28S gene has the advantage of being a biparental transmission gene unlike cytochrome B.

After PCR, sequencing was performed in South Korea according to standardized MacroGen assay protocols. On the other hand, we provide the primers ourselves. The tubes containing the PCR products are sent with two other 1.5 ml tubes each containing a 5 μ M primer solution, from MacroGen for purification and sequencing.

The cytochrome b primers are mtD26 (5'-TATGTACTACCATGAGGACAAATATC-3') and mtD28 (5'-ATTACACCTCCTAATTTATTAGGAAT-3') and the 28S primers are D4-5F (5'-CCCGTCTTGAAACACGGACCAAGG-3') and D4-5R (5'-GTTACACACTCCTTAGCGGA-3').

Genetic analyses

The molecular markers used are a part of the cytochrome B mitochondrial gene with 448 nucleotides and the 28S nuclear gene for 404 nucleotide sites of *C. maculatus*.

But these two genes were conjugated for more reliability in the results of analyses in the order Cytochrome b-28S for a length of 852 nucleotide sites.

Genetic variability

With the use of software such as BioEdit, MEGA6 version 6.06 (Tamura *et al.*, 2013) or DnaSP version 5.10.01 (Rojas *et al.*, 2012); a study of genetic variability can be initiated for the estimation of polymorphism. Molecular analysis of the nucleotide sequences allows to find the number of conserved sites, the number of variable or polymorphic sites and the number of informative site in parsimony between sequences on the one hand and on the other hand to determine the mutation rates R according to the report transitions on transversions related to the different mutations possible by MEGA6 version 6.06.

The mean number of nucleotide differences (k) was calculated and then the number of haplotypes (h) as well as the haplotype (Hd) and nucleotide (Pi) differences for each population of the four agro-ecological zones were by means of DnaSP software version 5.10.01.

Differentiation and genetic structuring

The differentiation index (FST) was determined by the software Arlequin version 3.5.1.3 (Excoffier and Lischer, 2010). According to Wright (1931), the closer FST is to 1, the more genetically structured the populations are. On the other hand, populations do not show allelic differences or differentiation if the FST is zero. The negative FST values are equivalent to 0. For each value of the FST, the P value allows to accept or reject it according to whether it is respectively significant or insignificant.

The genetic distance (D) of Nei (1987) within and between populations of the four agro-ecological zones was calculated using MEGA6, using the Kimura (1980) 2-parameter model (K2P). These parameters, such as fixation indices, account for genetic differentiation within and between agro-ecological zones.

To determine the genetic structuring of populations, molecular variance analysis (AMOVA) is used using the Arlequin version 3.5.1.3 program. AMOVA estimates indices of genetic structuring using information from the allelic content of haplotypes such as allelic frequencies. Information on the differences in allelic content between haplotypes is considered as a Euclidean matrix of squares of distances. The significance of the covariance components associated with the different possible levels of genetic structuring (intra and inter-populations) is tested using non-parametric permutation procedures.

Demographic evolution

The demographic evolution of *C. maculatus* in West Africa will be studied by calculating the D indices of Tajima and F_s of F_u with the software Arlequin. Then we analyze the disparity of distribution or mismatch distribution (Roger and Harpending, 1992). The "Mismatch distribution" graph is the graphical representation of the distribution of genetic distances between individuals in a population taken two by two. The analysis of Mismatch distribution is accompanied by the analysis of two indices that test the quality of adjustment of the distribution as the SSD (Sum of Squares Deviations) and the Rag (index of irregularity). The Mismatch distribution graphs are built with the DnaSP software and the SSD and Rag indices are obtained by the Arlequin software. Molecular evolution modeling allows to reconstitute the phylogeny of a population. The haplotype network was built using the Network version 4.6.1.3 software (Bandelt *et al.*, 1999). The haplotypic network is indeed a phylogenetic representation of the tree in the form of a star expressing the close kinship between haplotypes and their distribution in the different localities.

RESULTS

Genetic variability

68 sequences were analyzed and we found for the global data set on average 73.59% of conserved sites, invariable or monomorphic and 26.40% of the variable sites among which 9.74% of informative sites in parsimony for the total population. The mutation rate R is

5.062. By analyzing the populations by agro-ecological zone, we notice a considerable increase in the rate of conserved sites from 87.79% to 96.47% and a sharp decrease in the rate of variable sites from 12.20% to 3.52% compared to the average (Table II). This allows supposing a low genetic variability of the species according to agro-ecological zones.

Table II: Distribution of genetic variability according to the agro-ecological zones.

populations	Total population	Sahelian population	Sahelo-Sudanian population	Sudanese population	Guinean population
Settings					
Conserved sites	73.59%	96.47%	90.84%	87.79%	89.43%
Polymorphic variable sites	26.40%	3.52%	9.15%	12.20%	10.56%
Sites in informative parcimony	9.74%	1.99%	1.05%	6.10%	1.87%

By considering agro-ecological zones, the mutation rate R varies between 3,351 and 11,559 depending on the population. But it is weak for the Sahelian population coming from Fouta and for the Guinean population coming from the South of Togo. However, the mutation rate R is high for the Sahelo-Sudanian population. Indeed, in the total population; 83.82% of the mutations are transitions that rarely result in amino acid changes while 17.18% of the mutations are transversions favoring amino acid change and protein structure. This can lead to variability and genetic polymorphism. We also notice a decrease in the transition rate and an increase in the transversion rate for the Sahelian and Guinean populations. On the other hand, the Sahelo-Sudanese and Sudanese populations show an increase in the transition rate and a decrease in the rate of transversion compared to the total population, indicating a decrease in variability and genetic polymorphism in these different populations of *Callosobruchus maculatus* (see Table III).

Table III: Nature and mutation rates by agro-ecological zones

populations	Total population	Sahelian population	Sahelo-Sudanian population	Sudanese population	Guinean population
Settings					
Transition rate (Ts)	83.82	78.02	91.86	87.78	80.31
Transversion rate (Tv)	17,18	21.98	8.14	12.22	19.69
R = Ts / Tv mutation rate	5,062	3,351	11,559	6,764	4,041

The haplotypes h and the average number of nucleotide differences between sequences k are considered relatively high. The indices of nucleotide diversity Pi and haplotypic Hd are determined within each population and for all individuals in its totality (see Table IV). The results reveal the same trend with high haplotypic diversity close to 1 and a very low nucleotide diversity in the different populations of agro-ecological zones compared to the total population.

Table IV: indices of genetic diversity.

Settings populations	h	Hd	pi	k
Sahelian zone	5	1.000 ± 0.1600	0.01808 ± 0.00000	15,400
Sahelo-Sudanian zone	14	0.958 ± 0.0008	0.01229 ± 0.00003	10.384
Sudanese zone	23	0.962 ± 0.0005	0.01476 ± 0.00001	12,161
Guinean zone	11	1.000 ± 0.0015	0.02207 ± 0.00004	18.491
Total population	49	0.978 ± 0.0090	0.01622 ± 0.02940	13.202

Differentiation and genetic structuring

Differentiation indices (F_{st}) are between 0.031 and 0.120 (see Table V). They are relatively weak for the populations of the different agro-ecological zones but they are for the most part insignificant (p -value > 0.05) except three values taken in pairs. One of the most significantly elevated F_{ST} values showing moderate genetic differentiation is noted between the Sahelo-Sudanian and Guinean populations ($F_{st} = 0.066$ and p -value = 0.000) despite the geographical distance of their localities. The other two significantly low F_{ST} revealing a low genetic differentiation are noted between the Sahelo-Sudanese and Sudanese populations ($F_{st} = 0.031$ and p -value = 0.036) on the one hand and between the Sahelian and Sudanian populations ($F_{st} = 0.081$ and p -value = 0.054) which are located in geographically close localities. Contrariwise, non-significant F_{st} recorded between the Sahelian population and the Sahelo-Sudanese population but also the Guinean population suggest a very weak differentiation despite the geographical distance that separates them. This same result is recorded between the Sudanese and Guinean populations. Indeed, geographic distance does not necessarily induce a genetic distance, (Table V). Thus, agro-ecological zones have little influence on the differentiation of *C. maculatus* populations in West Africa.

Table V: Fst values between population pairs.

populations	1	2	3	4
1- Sahelian zone	---			
2- Sahelo-Sudanian zone	0,120	---		
3- Sudanian zone	0.081 *	0,031 *	---	
4- Guinean zone	0,051	0.066 **	0,039	---

* significant p-value ($p < 0.05$) ; ** p-value very significant ($p < 0.01$).

Intra and inter-population genetic distances (D) for the four populations were calculated under MEGA6, using the Kimura (1980) 2-parameter model (K2P). Intra-population genetic distances are apparently low in each of the four populations, ranging from 0.013 to 0.018. They are very low between different populations of 0.001 to 0.002 (see Table VI). The highest genetic distance is 0.018 and it is recorded in the population of the Sudanian zone, while the lowest genetic distance 0.001 is recorded between two geographically distant populations: the Sahelian and Guinean populations. So there is a negative correlation between genetic distance and geographical distance. Nevertheless, there is a high rate of mutation in individuals of the Sudanese, Sahelo-Sudanese and Guinean population, probably related to a large gene flow.

Table VI: Intra and inter-population genetic distances.

populations	Genetic distance				
	Intra agro-ecological zone	Inter agro-ecological zones			
		Sahelian zone	Sahelo-Sudanian zone	Sudanese zone	Guinean zone
Sahelian zone	0,013	-----			
Sahelo-Sudanian zone	0.016	0,000	-----		
Sudanese zone	0,018	0,002	0,002	-----	
Guinean zone	0.016	0,001	0,001	0,002	-----

The analysis of the molecular variance or AMOVA (Table VII) reveals a percentage of insignificant and weak variance (4.93%) between the populations of the different agro-ecological zones. In parallel, the AMOVA result provides a low overall F_{ST} of 0.049 that is 4.93% of variation among populations in agro-ecological zones, but it is highly significant ($p < 0.05$). Thus, the differentiation is generally weak resulting in a weak structuring of the total population of West African *C. maculatus*. This result is confirmed by the low values of the sum of the squares, the degree of freedom, and the components of variance and the percentage of variation between the different populations. On the other hand, within a population, we find very high values of the degree of freedom according to the number of samples, the sum of the squares, the component of variance and the percentage of variation.

Table VII: Analysis of molecular variance

Source of variation	degree of freedom (df)	Sum of squares	of Components of variance	Percentage of variation
Between populations	3	42.319	Va = 0.4411	4.93%
Intra-populations	64	508.401	Vb = 7.943	95.07%
Total	67	550.721	8.355	100%
F_{ST}		0.049 *		
p-value		0.0107		

* significant p-value ($p < 0.05$)

Demographic evolution

Demo-genetic tests reveal positive values of Tajima D and Fu's F_s but not significant ($p > 0.05$) for the population of the Sahelian zone. On the other side, the Tajima's D values are significantly negative ($p < 0.05$) in the other three populations. The values of Fu's F_s are quite negative and are not significant in these three Sahelo-Sudanian, Sudanese and Guinean populations (Table VIII).

Table VIII: Indices of demographic evolution.

populations	Total population	Sahelian zone	Sahelo-Sudanian zone	Sudanese zone	Guinean zone
Tajima's D	-1.3149	0.51922	-2.15510 *	-1.85470 *	-1.76936 *
p-value	0.1830	0.6900	0.0060	0.0100	0.0170
Fu's Fs	-1.5217	0.20943	-1.40359	-2.91811	-1.97460
p- value	0.2255	0.3310	0.2500	0.1560	0.0950

* significant p-value ($p < 0.05$).

The two distribution mismatch curves represent the allelic frequencies observed (in red) and expected (in black which represents the graphical model of an expanding population) as a function of the nucleotide differences between pairs of sequences. The non-superimposition of the two curves suggests an unimodal distribution thus revealing a recent fast growth of different populations of *C. maculatus* and a demographic expansion according to the agro-ecological zones (Figures 2 and 3). But the analysis of the mismatch distribution is always accompanied by the analysis of two evolution indices that test the quality of adjustment of the distribution. These indices are the SSD and the index of Raggedness (index of irregularity). The Irregularity Index (R) and the SSD are all positive and insignificant for each population in the four agro-ecological zones (Table IX). Then there is no difference between the observed values and the simulated values. This confirms the analysis of the mismatch distribution that bears the signature of a moderately expanding population in each population of the four agro-ecological zones.

Table IX: SSD and Raggedness index values.

	Total population	1-Sahelian	2-Sahelo-Sudanian	3-Sudanian	4-Guineenne
Rag index (R)	0.04730	0.14000	0.02044	0.00843	0.02050
p-value	0.81000	0.77000	0.80000	0.89000	0.96000
SSD	0.02030	0.05988	0.00706	0.00548	0.00884
p-value	0.77500	0.64000	0.73000	0.92000	0.97000

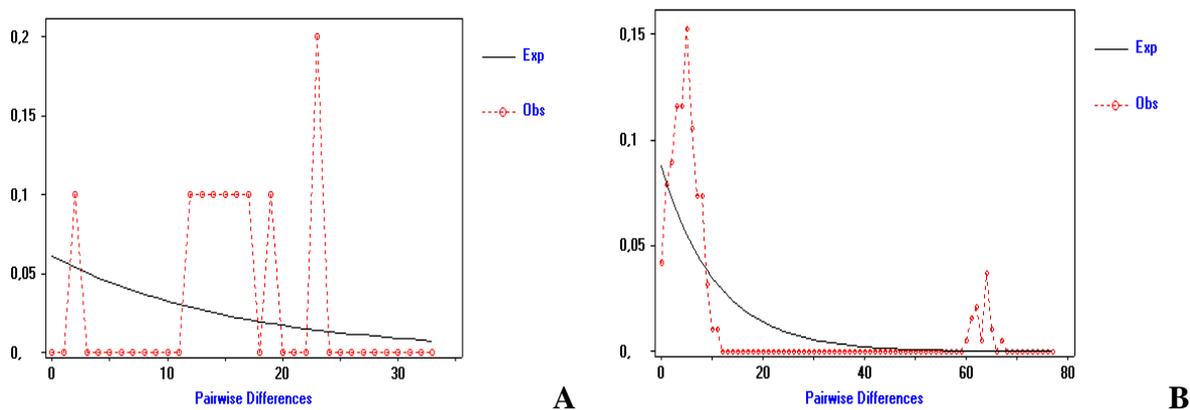


Figure 2: Distribution of the number of nucleotide differences between haplotypes taken two by two of the Sahelian (A) and Sahelo-Sudanian (B) populations of *C. maculatus*.

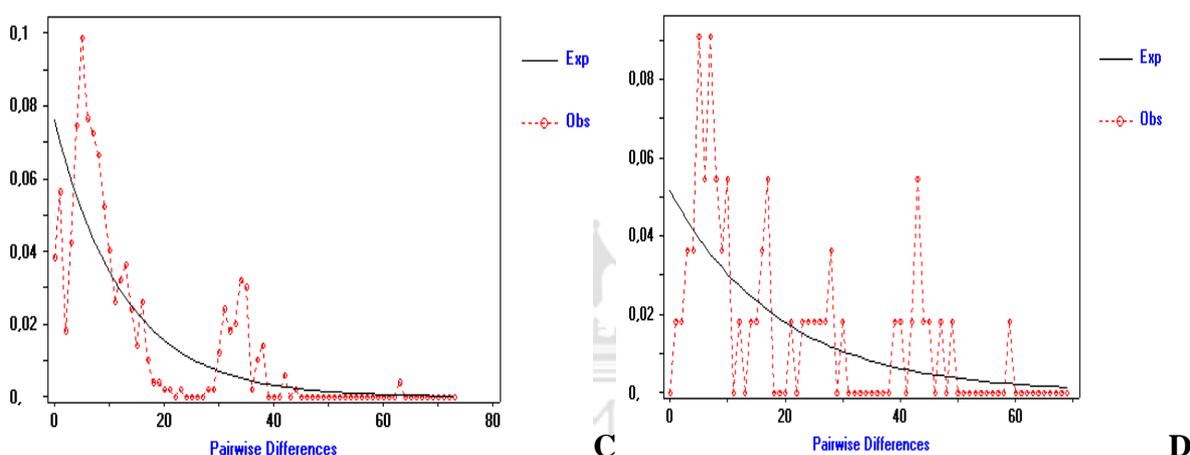


Figure 3: Distribution of the number of nucleotide differences between haplotypes taken two by two of the Sudanian (C) and Guinean (D) populations of *C. maculatus*.

The minimum network of haplotypes reveals the phylogenetic affinities of the haplotypes inferred with the sequences of the contiguous genes by grouping the individuals into 50 haplotypes or alleles out of a total of 68 sequences. Each circle represents a haplotype grouping one or several individuals and the lines indicate the number of mutational steps. The topology of the haplotype network (Figure 4) reveals a significant number of individual haplotypes (43 haplotypes), 5 private haplotypes and an H3 ancestral majority haplotype (MaB3) found in 3 agro-ecological zones. It contains 6 individuals identified in the localities of Bougouni, Sikasso, Niamey, Assigame, Mango and Tsevie belonging to three agro-ecological zones: Sahelo-Sudanese, Sudanese and Guinean. Another major haplotype H19 (SnT2) with 6 individuals is found in the Sudanian zone, more exactly in Tambacounda. The third most representative haplotype H10 (MaS6) has 5 individuals found in Sikasso, Niamey

and Coki, it is present in 2 agro-ecological zones. The frequency and proportion of nodes are connected to the mutation rate and the number of mutational steps that favored the differentiation of individuals from the ancestral haplotype. The resulting haplotype or alleles network is characterized by a star shape with all alleles derived from ancestral haplotype (MaB3) of Mali which represents 10.29% of the dataset. The connections between the central allele and the less frequent alleles vary from 1 to 9 mutational steps (Figure 4). The second most common allele (SnT2) is found in 6 individuals from Tambacounda (Senegal) and indirectly derives from the basic allele after 4 no mutation. The third major haplotype (MaS6) groups 5 individuals divided into two agro-ecological zones and is located at 5 mutational steps. All other haplotypes are shared at most by two individuals from one and the same agro-ecological zone.

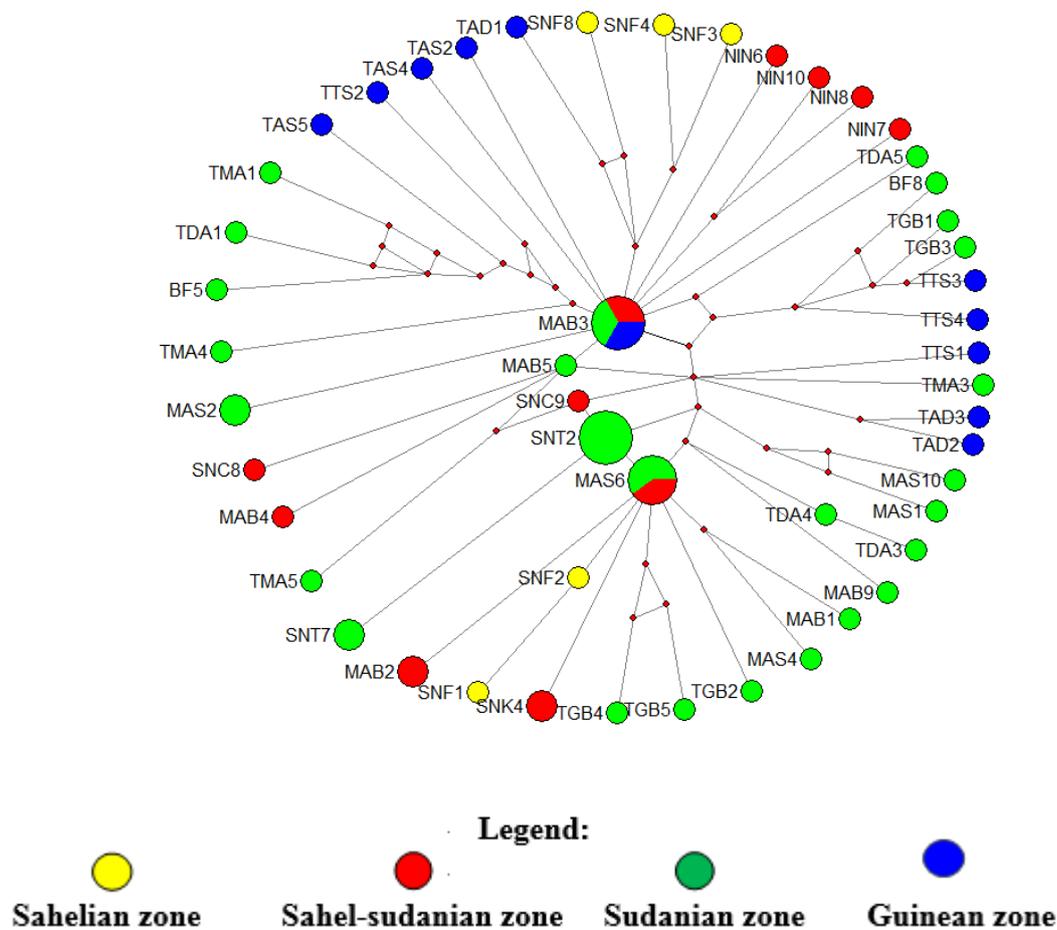


Figure 4: Haplotypic network of the main haplotypes of *Callosobruchus maculatus* according to the agro-ecological zones of West Africa.

DISCUSSION

In the total population of *Callosobruchus maculatus*, the study of variability shows a high rate of conserved sites and a low rate of variable sites of DNA sequences. This low genetic variability nevertheless leads to low polymorphism since most of the mutations are transitions (74%) that do not often result in amino acid changes and protein structure. According to the agro-ecological zones, this evolutionary trend continues in each population with an increase in the rate of transitions and a decrease in the transversions rate, which favors an increase R mutation rate from 6.764 to 11.559 for the Sahelo-Sudanese and Sudanese populations. On the other hand, the Sahelian and Guinean populations show a decrease in the transition rate and an increase in the rate of transversion, thus leading to a decrease in the mutation rate R from 4,041 to 3,351. In fact, the Sahelian and Guinean populations have undergone more genetic variability than the Sahelo-Sudanian and Sudanian populations. This is explained by a low gene flow in their localities on the one hand and on the other hand the low production and circulation (trade) of cowpea seeds in localities of the Sahelian and Guinean zones such as Fouta (Senegal), Adidogome, Assigame and Tsevie (Togo) because of the predominance of cereal crops (Senegal) and tubers tubers (Togo).

The analysis of the indices of genetic diversity confirms the previous results because it appears low values of the number of haplotypes h (5 and 11) then leading to an increase in the average number of nucleotide differences k between sequences (15,400 and 18,491) for both Sahelian and Guinean populations where variability is higher. On the other hand, high values of h (14 and 23) correlated with a decrease of k (10,384 and 12,161) for the Sahelo-Sudanian and Sudanian populations lead to a decrease in genetic variability. This also suggests significant gene flow in the subregion and lack of reproductive isolation, so individuals from different populations can reproduce without any genetic barrier. This situation is observed in *Sitophilus zeamais* populations within West African zones (Bambou *et al.*, 2015) and in ecotypes of *Callosobruchus maculatus* by Tine *et al.*, (2013).

The analysis of differentiation indices or F_{ST} showed a moderate differentiation between populations of *C. maculatus* geographically close and very little differentiation between populations of *C. maculatus* geographically distant. This suggests a negative correlation between geographic distance and genetic distance on the evolution of *C. maculatus*. These results are really confirmed by the calculation of intra and inter-population genetic distances. Such results have already been obtained by Kafom *et al.* (2017) by working on populations of

C. maculatus subservient to several agro-ecological zones of Senegal. The same is true of *Tribolium castaneum* (Diome *et al.*, 2013, Dia *et al.*, 2014) and *Sitophilus zeamais* (Bambou *et al.*, 2015).

Genetic distance indicates the genetic divergence within and between populations, that is to say the distances separating two groups. Between the different populations, it appears a very small genetic distance of 0.000 to 0.002 that is at the most 0.2% of nucleotide replacements. Thus genetic differentiation is rather weak that it causes almost no variability and polymorphism between populations from different and distant agro-ecological zones. On the other hand within each population, there appears a rather important genetic distance of 0.013 for the Sahelian population to 0.018 or about 1.8% of mutations on the nucleotide sequence of the individuals of the Sudanese population. This confirms a weak differentiation of *C. maculatus* according to the agro-ecological zones, especially as there are more mutations by transition between bases of the same nature as mutation by transversion between bases of different nature. Thus, agro-ecological zones have a weak influence on differentiation and probably on the structuring of *Callosobruchus maculatus* populations (Kafom *et al.* 2017).

The analysis of the molecular variance or AMOVA reveals a significantly low overall F_{ST} of 0.049 corresponding to the percentage of variation between populations of different agro-ecological zones (4.93%). On the other hand, a very high percentage of variability is recorded between individuals in a population (95.07%). This globally reflects a moderate differentiation and weak genetic structuring of populations of *C. maculatus* following agro-ecological zones in West Africa due to a large gene flow.

This weak to moderate differentiation may be due to the fact that the subregion is home to three international organizations such as the ECOWAS, UEMOA and CILSS that promote the free movement of people and goods on the one and on the other hand the development of strategies to fight against poverty. However, it is necessary to add the constant pressure of chemicals on *C. maculatus* to protect crops and the types of storage infrastructure can vary from one area to another. Then trade and movement of foodstuffs, including cowpea seeds in West Africa, favor the dispersal of *C. maculatus* haplotypes in the subregion through human mediation. These results are in agreement with those of Kébé (2017) with only the mitochondrial gene of Cytochrome B while the 28S nuclear gene revealed no differentiation. Consequently, the absence of reproductive isolation favors the meeting of haplotypes and the reproduction between individuals of different populations causing an important gene flow.

This promotes a moderate differentiation and a low genetic structuring like Ndong *et al.*, (2014).

Neutrality test results with Tajima's D and Fu's Fs confirm the hypothesis of a demographic expansion of the Sahelo-Sudanian, Sudanian and Guinean populations of *C. maculatus* in West Africa while the Sahelian the population is expanding moderately or steadily despite the low differentiation. This result is due in part to the use of a mixed gene (contiguous) with a mitochondrial part and a nuclear part sometimes with gaps. These results are confirmed by the analysis of the distribution curves of allelic frequencies compared to the nucleotide differences taken by pairs of sequences or mismatch distribution. There is an unimodal distribution for the global population and in each of the four populations, suggesting a demographic expansion. But with the mismatch graph, it is necessary to use the indices of irregularity or Raggedness and of SSD or sum of deviation squares to validate the quality or fitness of the frequency distribution in pairs observed in relation with their p-values. It is noted that, in the four populations, p-values are not significant. This suggests that there is no gap between the simulated or expected values and the observed values because the two curves follow each other on all the distance pairs. An unimodal distribution is thus obtained which confirms the hypothesis of a moderate expanding population due to a large gene flow and movement of individuals of *C. maculatus* facilitating their reproduction and the increase of new alleles and characters within populations in the absence of reproductive isolation. These results confirm those of Kébé (2017) who indicate a rapid demographic expansion of local populations of *C. maculatus* for both genes taken separately.

Haplotypic network analysis reveals the phylogenetic relationships between different alleles or haplotypes circulating in the subregion. There are about three major and ancestral haplotypes from which emanate the four intermediate haplotypes that will be responsible for the 43 peripheral eccentric haplotypes including 4 double haplotypes (with 2 individuals) and 39 individual haplotypes. The network topology suggests that the ancestral Haplotype H3 represented by MaB3 is found in three agro-ecological zones: Sahelo-Sudanian, Sudanian and Guinean following a wide dispersal of *C. maculatus* individuals following the trade of cowpea seeds. All other haplotypes or alleles probably come from Bougoudere (Mali) to spread to other West African countries. Network topology suggests that the original home of expansion of *Callosobruchus maculatus* remains Africa, precisely in Mali and more exactly in Bougoudere where we find the ancestral and widespread haplotype. Indeed, West Africa

was a single market under the era of colonialism with the free movement of foodstuffs including cowpea seeds. Probably an event of great dispersion of cowpea seeds with famine cycles has also favored the distribution of *C. maculatus* haplotypes in the subregion to Togo. This new approach of contiguous genes reveals a weak genetic divergence between populations according to the agro-ecological zones. In fact, moderate genetic differentiation is due to the reproduction between individuals of different populations. However, chromosomal mixing during cross-over favor the accumulation of responsible micro mutations during the mean structuring time of *C. maculatus* depending on the agro-ecological zones. In fact, the analysis of phylogenetic results improves the knowledge of the bioecological behavior of insect pests of legumes such as *Callosobruchus maculatus* for cowpea and *Caryedon serratus* for peanuts (Sembène *et al.*, 2010). These studies contribute to the development of biological or integrated control strategies against insect pests of crops such as Coleoptera (Bruchidae) pests of food legumes taking into account the genetic aspect.

CONCLUSION

The present work was mainly based on the study of the impact of agro-ecological zones on the differentiation and structuring of *C. maculatus* populations in West Africa. It has been observed that agro-ecological zones have played a minor role in the differentiation and structuring of this insect pest of cowpea seeds in stock. However, cowpea is a cheaper source of complementary protein for people in developing countries, and it would be important to improve the means of production on the one hand and to reduce the losses caused on the other by its main insect pest. Traditionally, one or the other of the two genes is used in phylogenetic analyzes with sometimes mixed results depending on the advantages and disadvantages of each of the two genes. The advantage of this combined approach including mitochondrial and nuclear genome is to obtain more reliable solid results in phylogeny. Our results revealed sometimes a very weak genetic differentiation between geographically distant populations, sometimes a moderate differentiation between nearby populations favoring an average structuring of the populations of *Callosobruchus maculatus* according to the agro-ecological zones. At the end of this study, it appears that the highly polymorphic mitochondrial gene with maternal and clonal transmission is more suitable for demonstrating genetic variability and differentiation, whereas the polymorphic nuclear gene with two-way transmission is more adapted in the demographic evolution and the phylogenetic relations, from where the interest of combining the two genes. For more reliability in the results of

analyzes, it seems now more judicious to adopt this new combined approach of both genes, which offers new perspectives to the phylogeny and the better future to the population genetics and its applications.

But these results are hypothetical and they deserve to be explored to consider a better strategy to protect leguminous seeds in stock, especially cowpeas:

- By increasing the sampled localities especially in the largest cowpea producing countries including Niger and Nigeria to see better the gene flow.
- Taking into account the variety of cowpea to evaluate the adaptability of this insect.
- Taking into account the presence or absence of its parasitoid in sampled localities to assess the impact of coevolution.
- Using other more suitable molecular markers such as microsatellites in estimating genetic diversity between populations.

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