Genetic diversity of *Callosobruchus maculatus* Fabricus (Cowpea weevil) populations in various agro-ecological areas of five countries in West African sub-region

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**ABSTRACT**

The cowpea (*Vigna unguiculata* (L) Walp) is a legume of African origin. It is a source of very important nutritional needs like protein and vitamins for the developing countries especially countries of West Africa. The attack by the Bruchinae seed-beetle *Callosobruchus maculatus* (Fab) whose larvae develop in seeds causes losses ranging from 30 to over 80% of the harvest between 6-7 months of storage. The objectives of this study were to identify the different haplotypes of the weevils’ *C. maculatus* populations, to study genetic diversity in different agro-ecological zones and then highlight the phylogenetic affinities between the weevils from these different areas. In this study, we have analysed the sequences of the mitochondrial gene cytochrome b and 28S ribosomal gene in 75 individuals. 42 haplotypes for cytochrome b have been identified in three clades against 30 for the 28S divided into two clades. Individual haplotypes were mainly from the Guinean zone. Genetic distance and nucleotide diversity showed a trend of population structure of weevils between the different agro-ecological zones. This work is corroborated by low values of diversity within the zones and highly significant between them. Although Sudanian’ zone seemed not to confirm this view because of a relatively small percentage of alignment for individuals from Tenkodogo (Burkina Faso). The phylogenetic reconstructions have thus shown that gene flow was maintained even if it remained within narrow zones between agro-ecological zones for the populations of West African *C. maculatus*.

1. **Introduction**

The cowpea (*Vigna unguiculata* L. Walp), plant of African origin, is after the peanut (*Arachis hypogaea* L.), the most cultivated leguminous in Africa and Asia. It presents an important source of proteins and vitamins for the populations in developing countries especially in West Africa (Ndiaye et al., 2011). However due to a number of diseases and pests which attacks the cowpea, its yields are low despite the attempts to varietal improvements.
brought on the plant (Diaw, 1999). Of these pests, the most harmful to cowpea is the Bruchinae Beetle Callosobruchus maculatus Fabricius (Ndoye et al., 2011). The larvae feed on the cowpea’s seeds especially in the fields and during storage (Seck, 1994). In Senegal, Seck (1994) estimated that 50% of the seeds are destroyed within six months storage. Approximately 30% of the annual cowpea production in Niger and 80% in Togo and Burkina Faso is destroyed by C. maculatus (Alzouma, 1995; Ngamo and Hance, 2007).

Climate change has conditioned an increase in rainfall variability in the equatorial regions (Dore, 2005). West Africa offers three principal agro-ecological zones: the Sahelian dry zone, the Sudanian semi-arid zone and Guinean humid zone. Cowpea is cultivated in all these zones because it is adapted to many types of agro-climates because of the multiplicity of these cultivars and the varietal improvement technology (Brink and Balay, 2006). The variability of rainfall and therefore of vegetation in these various zones would impact on biodiversity induced by changes in the natural environment (Saidou, 2011). Thus the current gene flow between populations is likely to be interrupted as adaptability to the agro-climatic conditions would change the operating mode and the morphology of individuals subject to the laws of natural selection. Differences between these zones may lead to a restructuring of C. maculatus populations’ in the West African sub-region.

While analyzing’s DNA samples from individuals of C. maculatus infesting cowpea in various agro-ecological zones in West Africa, our particular objectives were: i) to evaluate the number of different haplotypes found in the sub-region, ii) to evaluate their genetic diversity from the parameters of genetic distances and nucleotide diversity and phylogenetic affinities between populations. This will allow us to see if these bruchids are structured or not depending on the agro-ecological zones considered. To reach those objectives we performed the PCR-sequencing of the mitochondrial cytochrome b gene and 28S ribosomal gene.

2. Materials and Methods

2.1. Sampling

Fourteen samples of cowpea seeds were collected during the dry period for two years (2009 and 2010) in five countries in West Africa as mentioned in Table 1. Sampling consisted to take a certain amount of cowpea seeds infested in several attics or storage areas for the same locality in order to gather as much information as possible for the given locality. The samples were then transported to the laboratory for mass rearing. Adults of C. maculatus were obtained by rearing larvae infesting these seeds and were fixed and stored in 96% ethanol until used for genetic studies. Samples were distinguished by two criteria: the geographical origin and agro-ecological zone sampled. Each sample was coded using the first two letters of the country (Mali and Niger) or initials of the country (Burkina Faso and Senegal) and the first letter of the place of harvest. For Togo we used the first letter of the country followed the first two letters of the place of harvest.

2.2. Genetic study

2.2.1. Genotyping of Cytochrome b and 28S in C. maculatus

DNA extraction of C. maculatus was made using the Qiagen DNeasy Tissue Kit. The insects were dissected. Only the chest and legs were used to avoid contamination by fungi or bacteria that are usually in the abdomen and elytra of the insect. PCR was performed on the mitochondrial gene coding: cytochrome b. This gene was amplified using the primers CB1 (5’-TATGTACTACATGAGGACAAATATC-3’) and CB2 (5’-ATTACACCTCCTAATTTATAGGAAT-3’). The Cytochrome b gene was revealed polymorphic and discriminative in other insects in previous studies (Sembène, 2000). The 28S (ribosomal gene) was also amplified by another primers D2CDF45F (5’-TACCGTGAGGAAAGTGGAAA-3’) and D2CRD45R (5’-AGACTCCTTGGTCCGTGTTT-3’). Amplification was performed in a reaction volume of 25µl containing 18.3µl of water, 2.5µl of buffer 10 X, 1µl of additional MgCl2, 0.5µl of dNTP, 0.25µl of each primer, 0.2µl of Taq polymerase and 2µl of DNA extract. PCR were performed for 35 cycles on Eppendorf Thermocycler with the amplification conditions as indicated in Table 2. DNA fragments were visualized on a 1.5% agarose gel. Samples that amplified were purified and sent to Macrogen Korea for sequencing.

2.3. Statistical analysis

Alignment was done by ClustalW (Thompson et al., 1994) as implemented in BioEdit 7.0.8 software. The alignments were manually checked and corrected. Haplotypes were identified and verified by the software v. DNASP4.10 (Rozas and Rozas, 2005)
and Arlequin 3.0. (Excoffier et al., 2005). Correlation tests of the genetic distance and the geographical distance between populations (Mantel test) were conducted by the Pearson bilateral correlation in the software XLSTAT 2011. The resulting data were subsequently used to calculate the standard clues of genetic variations such as the position and the nature of the mutations, the genetic distance intra/inter groups, the number of polymorphic sites, conserved sites, singleton sites, segregating sites and parsimony informative sites. The transitions/transversions rate bias (R) and the frequency of nucleotides are also calculated with the very software MEGA4 by the substitution Pattern test. Phylogenetic relationships were reconstructed with MEGA4 software v.4.0.0.162 using the Neighbor-Joining method (N-J), (Saitou and Nei, 1987) based on ecotypes’ matrix of genetic distance (the Kimura’s distance 2-parameter) was taken two by two in order to model the evolutionary processes. The Maximum Parsimony method (MP), (Ficht, 1971) was carried out with the heuristic search option with random stepwise taxon addition replicates, using the branch swapping tree bisection-reconnection option. A bootstrap procedure (500 iterations with the same option of heuristic search) was used to establish the score of each node (Felsenstein, 1981) by retaining groups compatible with the 50% majority rule consensus. A strict consensus tree was computed whenever multiple equally parsimonious trees were obtained. The Maximum Likelihood method (ML, Felsenstein, 1981) was used to test all the stories that may have led to the current data set analysed. This analysis was performed by MEGA version 5.0. Node stability was evaluated using 100 bootstrapping replications, and the majority-rule consensus trees were conducted. In all phylogenetic analyses Caryedon serratus was considered as out-group.

3. Results

3.1. Nucleotide variability

Of the 110 starting sequences, only 75 sequences were chosen, because after alignment, there were individuals which were in the sequences of cytochrome b and they were not sequenced in 28S gene and vice versa. Thus, we found it necessary to work on the sequences obtained in both portions of the two genes. Allowing to have the 75 individuals sampled, three from Burkina Faso, 16 from Mali, 6 from Niger, 26 from Senegal and 24 from Togo. Cytochrome b with 451 base pairs has 280 conserved sites, 171 variable sites, 107 singletons and 64 parsimony informative sites (Figure 1). The

<table>
<thead>
<tr>
<th>Country</th>
<th>Locality</th>
<th>Number of Individuals</th>
<th>Code of Individuals</th>
<th>Agro-ecological zone</th>
<th>Year of sampling</th>
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<tr>
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<td>4</td>
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<td>Guinean</td>
<td>2010</td>
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Table 1: indicates the countries, localities, number of scored individuals, and sample code of individuals, area’s climate and year of the populations analyzed.

<table>
<thead>
<tr>
<th>Genes</th>
<th>PCR conditions</th>
<th>Preliminary denaturation</th>
<th>Number of cycles</th>
<th>Initial denaturation</th>
<th>Hybridization</th>
<th>Elongation</th>
<th>Final elongation</th>
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<td>Time</td>
<td>T°C</td>
<td>Time</td>
<td>T°C</td>
<td>Time</td>
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<tr>
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<td>94</td>
<td>3mn</td>
<td>35</td>
<td>94</td>
<td>1mn</td>
<td>47</td>
<td>1mn</td>
</tr>
</tbody>
</table>

Table 2: Comparison of amplification conditions of studied genes.
nucleotide frequency was 0.362 for adenine, 0.299 for thymine, 0.227 for guanine and 0.112 for cytosine. The mutation rate of transition type was the order of 78.15% while 21.85% are transversions. The ratio (R) transition / transversion are 2.04 (Figure 2 and figure 3). The fraction of 404 base pairs of the 28S conserved 260 sites for 144 polymorphic sites, 94 singleton and 31 parsimony informative sites (Figure 1). The nucleotide frequency is 0.153 for adenine, 0.244 for thymine, 0.321 for guanine and 0.282 for cytosine. 65.28% of mutations are transition-type, 21.53% were transversion type and 13.19 were deletions. The ratio (R) transition / transversion was 4.16 (Figure 2 and figure 3).

3.2. Diversity and genetic distances

3.2.1. Diversity and genetic distance of Cytochrome b

Comparison of genetic diversity parameters of C. maculatus in different agro-ecological zones (Figure 4) shown that the Guinean area, with 24 individuals presented the most haplotypes (19 haplotypes) while the Sudanian zone for 20 individuals had 11 haplotypes. The Sahelian zone had 16 haplotypes for a total of 31 individuals. The genetic distance between agro-ecological zones was the largest in Sudanian zone with a value of 0.038. The value of nucleotide diversity (π) was 0.035162 which confirmed the variability that was expressed in this zone. Individuals in the Sahelian zone had the lowest values of genetic diversity. Their genetic distance and nucleotide diversity (π) took the values of 0.019 and 0.018168. Guinean was the intermediate zone with distances and diversity values equal to 0.022 and 0.021530.

Comparison of genetic distances between different areas showed that the couple formed by the Sudanian and the Guinean zones has the highest value: 0.031. The shortest distance was found between the Guinean area and the Sahelian zone which was 0.022. Between it and the Sudanian zone genetic distance was 0.029 (Figure 5).

3.2.2. Diversity and genetic distance of 28S ribosomal gene

The calculation of genetic distances with the sequences of 28S (Figure 6) were in agreement with the results obtained with cytochrome b. The Sahelian zone presented less diversity than other areas with a distance equal to 0.013 and (π) equal to 0.010872. Sudanian zone had the highest genetic distance with a value of 0.016. This was confirmed by nucleotide diversity (π) which was 0.014453. The high genetic distances are associated with high nucleotide diversity. Individuals of Guinean zone had distances equal to 0.013 and a nucleotide diversity of 0.012360.

Genetic distances of the 28S inter-area showed the torque between the Sahelian and Guinean areas with the lowest distance value (0.013). The greatest value was found between the Sahelian and Sudanian zone and between it and Guinean zone and was of the order of 0.014 (Figure 7). Thus the calculations showed that 28S sequences were less variable than those of cytochrome b.

3.2.3. Diversity and genetic distance of 28S + Cytochrome b genes

Genetic diversity by the splicing of two genes (Figure 8) also showed that the Sudanian zone remained the most diversified with a genetic distance of 0.027 against 0.018 for Guinean zone and 0.016 for the Sahelian zone. The nucleotide diversity of the cytochrome b and 28S gave rise the Sudanian zone the highest variability with a value of 0.025612, followed by the Guinean area to 0.017313 and finally the Sahelian zone to 0.014752.

Genetic distances between the various agro-ecological zones gave the same results as those calculated with the cytochrome b and 28S taken separately. The couple formed by the Sudanian and Guinean zones had the highest value: 0.023 followed the couple Sudanianzone/Sahelian zone: 0.022 and finally the shortest distance found between the Sahelian and Guinean zones (0.018) (Figure 9).

3.3. The Mantel correlation test

Since the P-value calculated (=0.138) is greater than the significance level Alpha = 0.05, we cannot reject the hypothesis H0 (the matrices are not correlated) that there is no correlation between geographic distance and the genetic distance. The risk of rejecting the null hypothesis H0 is true then it is 13.81% (Figure 10).

3.4. Phylogenetic trees

3.4.1. Phylogenetic tress of cytochrome b

The topology obtained with the Maximum Parsimony is associated with high bootstrap values and includes individuals in three clades (Figure 11a). Clade 1 is the largest and contains 40 individuals from 16 haplotypes of which 9 individual haplo-
Figure 1: Characteristic relating of genes 28S and Cytochrome b diversity according to the number of variable, conserved, singleton, parsimony informative sites and the number of nucleotide and sequences.

Figure 2: Frequency of mutations of the genes according to the rate of transitions, transversions, deletions and the number of haplotypes.

Figure 3: Nucleotide frequency of 28S and cytochrome b.

Figure 4: Genetic diversity according the number of haplotypes and the nucleotide diversity for the different agro-ecological zones for the Cytochrome b.

Figure 5: Genetic distance intra and between agro-ecological zones for the Cytochrome b.

Figure 6: Genetic diversity according the number of haplotypes and the nucleotide diversity for the different agro-ecological zones for the 28S.
types. This clade is composed mainly of individuals from the Sahelian zone (19) and the Sudanian zone (14). Individuals of Guinean zone were 7 in number. The clade had 7 sub-clades. The most important were the sub-clade 5 that consisted of individuals from Kébémer and belonging to the Sahelian zone. The sub-clade 6 contained all individuals of Tambacounda except two individuals. The sub-clade 7 consisted of two individuals of the Sudanian zone that was MaS1 and MaS10, and 4 individuals of Guinean zone. This clade contained 21 of the 26 individuals from Senegal. Clade 2 consisted of 24 individuals with 16 haplotypes. All agro-ecological zones were represented with relatively equal proportions. This clade consisted of two sub-clades. In percentage terms, the Guinean area was the most represented with 46% of the total of its individuals. While the Sahelian and Sudanian zones were respectively 29 and 20% of their total individuals. Clade 3 contained only eight individuals, including 3 Sahelians, 1 Sudanian and 4 Guineans. This clade contained neither of individuals from the Burkina Faso or Niger. The phylogenetic tree had three individuals belonging to none of the three groups identified (Bf1, Bf7 and Tad3) from Sudanian and Guinean areas).

The tree reconstructed by the neighbor-joining method had very low bootstrap values (Figure 11b). It contained three major clades as the MP. But had irregularities with respect to clade formed by the method of MP because clades did not contain the same individuals. Clade 1 of NJ contained all individuals of clade 1, clade 3 and sub-clade 1 of clade 2 of the MP tree. Clade 2 consisted of some individuals of sub-clade 2 of MP clade 2 that was NiN8, NiN10, MaS2, MaS8, TAS5, TMa1 and TMa2. The remaining individuals namely Bf8, Tad1, SnF3, SnF4 and SnF8 are classified in clade 3 of NJ.

3.4.2. Phylogenetic trees of 28S

The Neighbor-Joining tree assigns the presence of two clades (Figure 12a). Clade 1 had 67 individuals belonging to 22 haplotypes. It consisted of all individuals from the Sahelian and Sudanian zone (except Bf1 and Bf7) plus 75% of individuals’ Gui-
Figure 11A: Phylogenetic tree obtained by the Maximum Parsimony’s method of Cytochrom b gene rooted with Caryedon serratus.

Figure 11B: Phylogenetic tree obtained by the Neighbor Joining’s method of Cytochrom b gene rooted with Caryedon serratus.
ean zone. This clade contained two sub-clades: the sub-clade 1 and the sub-clade 2 consisting of individuals of the Guinean zone from Togo, and another sub-clade including individuals from Mali but not sharing the same agro-ecological area. Clade 2 consisted of six individuals for many haplotypes from Sudanian and Guinean zone. Individuals TMa4 and TMa5 were classified as either clade 1 or clade 2 and in each represent a single haplotype.

The maximum parsimony (Figure 12b) had the same topology as the NJ as well as for the clade 1 for the clade 2. The only difference is that the sub-clades, for the MP, are 4 in number of bootstrap values with much more significant (100%). The sub-clades 1 and 4 were composed of individuals from Togo and Mali whereas other sub-clades of Mali but did not share the same agro-ecological zones.

3.4.3. Phylogenetic trees of 28S+Cytochrome b

The dendrogram of the two portions of 28S and cytochrome b genes had the same topology as the shaft consisting of the cytochrome b to the MP bootstrap values with relatively equal (Figure 13a). The only differences observed between these trees were in the clade 1 with the presence of SnT3 in the sub-clade formed by individuals of Tambacounda in the Sudanian zone. The sub-clade 7 of clade 1 consisted of MaS1and MaS10. Other individuals namely TDa5, TGb1, TGb3 and TTs4 are classified in sub-clade 1 of clade 2. Individuals MaB4, MaBS and TDa2 earlier in the cytochrome b clade 3, form the sub-clade 3 of clade 2. Thus the clade 1 had 37 individuals from the Sahelian zone, 14 from Sudanian zone and 4 individuals from Guinean zone. Clade 2 comprised 31 individuals with 11 individuals in the Sahelian zone, 4 individuals of the Sudanian zone and 16 individuals from Guinean zone including 11 individuals in sub-clade 1. Finally the clade 3 had 4 individuals (SnC9, TMa3, TTs1 and TTs3).

The maximum likelihood (Figure 13b) showed the same characteristics as the NJ tree of the cytochrome b except here there are two clades instead of three. Clade 2 of NJ cytochrome b is now included in the clade 1 of ML. All agro-ecological zones are represented in different clades of the cytochrome b NJ and the ML two genes.

4. Discussion and conclusion

The objective of the work was to study the genetic structure of populations of the cowpea weevil (C. maculatus Fab.) in various agro-ecological zones of the West African sub-region and assess their phylogenetic affinities with the technique of PCR-Sequencing of mitochondrial cytochrome b and ribosomal 28S genes. The cytochrome b gene amplification revealed 42 haplotypes including 30 individual haplotypes. However the ribosomal 28S gene showed for the 75 sequences obtained, 30 haplotypes including 29 individual haplotypes. This prompted (Ndong et al., 2011) to question whether the gene is used to characterize strains Bruchinae. The polymorphism of these genes in addition to environmental factors to which C. maculatus populations' evolve, was perhaps due to the use of insecticides for cowpea storage. Since insecticides act on the airways by inhibiting the activity of monooxygenases (Ketoh et al., 1998), polymorphism of cytochrome b becomes much stronger because this gene plays a role in breathing. Plus the fact that insecticides affect the biological balance of the ecosystem cowpea beetles commodities have developed resistance phenomena due to the multiplicity, nature, dose and processing technique (Delobel & Tran., 1993). According to FAO studies in 2003, wetland farmers would use more insecticides on crops because of the variety of pests that semi-arid to arid zones. This would explain the large number of haplotypes found in Sudanian and Guinean zones. On the other hand, there is the significant presence of other host plants of the natural weevil. These plants allow the beetle to survive better in the fields during the dry seasons. The Sahelian zone offers these special features. Formerly, this area was suitable for growing cowpeas but with the fall of precipitations, insects trying to adapt to new climatic conditions of the area and the introduction of improved varieties of cowpea. Millet is the only crop that matches the conditions of the Sahel (Guèye, 2011). Therefore, only the haplotypes that are better adapted to their changing environment are likely to survive. The sequence of bases in genes are therefore a function of environment and conditions where live weevils. This is because several individuals’ haplotypes essentially consisted of individuals from the same agro-ecological zone. The splicing of the two portions of genes 28S and cytochrome b also shows that there are mutations of nucleotide sequences for all from the three agro-ecological zones sampled.

Sequence analysis of two genes shows that haplotype diversity is significant. Genetic distances within the areas are relatively lower than those between the areas except the Sudanian zone which constitutes the upper limit. The 28S with low ge-
Figure 12A: Phylogenetic tree obtained by the Neighbor Joining’s method of 28S gene rooted with Caryedon serratus.
Figure 12B: Phylogenetic tree obtained by the Maximum Parsimony’s method of 28S gene rooted with Caryedon serratus.
Figure 13A: Phylogenetic tree obtained by the Maximum Parsimony’s method of 28S and cytochrome b genes rooted with Caryedon serratus.

Figure 13B: Phylogenetic tree obtained by the Maximum likelihood’s method of 28S and cytochrome b genes rooted with Caryedon serratus.
netic distances do not structure weevil populations. However, with a value of 0.031 between the Guinean and the Sudanian area and 0.029 between the last and the Sahelian zone, cytochrome b gives more information on structuring bruchids according to the areas in which they originate. These genetic distances are nearly equal to the distance found by Sembène (2006) which is of the order of 0.035. This distance structures biotypes population of Caryedon serratus subervient to Pilostigma reticulatum, Bauhinia rufescens and Arachis hypogaea from Senegal. The splicing of genes can confirm this structuring by its high values of genetic distances between the various areas sampled. All of which leads that geographical distance would have no effect on the Bruchina’s structural but different haplotypes can be met within a biotype thus constituting different ecotypes (Dione et al., 2011). This is confirmed by the Mantel test indicating that the risk to reject the hypothesis that there is no correlation between genetic distance and geographic distance is 13.81%. Indeed, individuals of a species are often grouped naturally subject to different local evolutionary processes (natural selection, mutation, drift and migration) of different intensities, resulting in distinct genetic compositions for each group. Thus, the variation in flowering time is evolving strategies related to crop adaptation to different environmental conditions and therefore agro-ecological (Roux et al., 2006). Therefore, to ensure that the pest offspring adjust their life cycles in harmony with that of the host plant. This change in cycle induced by the genome gives each individual the ability to survive in the environment to which it evolves. It’s named co-evolution. Thus approaching the individuals with whom he lives while away from other individuals who do not share the same agro climate. This is what makes the genetic diversity within the zones is small compared to the diversity between areas. However, Burkina Faso has a high variability possibly due to the multiplicity of varieties grown and the fact that the authors suggest that this is the starting point of infestation by the cowpea weevil in Africa.

The cytochrome b phylogenetic reconstructions by parsimony showed two major clades. The same number of clades has been revealed on the groundnut bruchid (Caryedon serratus) by Ndiaye and Sembène (2011). The distribution of bruchid does not fully explain the structure revealed by the genetic diversity parameters because although different ecotypes are found in every country, it is clear that there’s a significant gene flow between them. In this, is the fact that several genetically distinct populations may be more or less bound by the movement of individuals and thus form a meta-population in which each population is influenced by other (Hanski and Simberloff, 1997), especially between the CILSS (Comité permanent Inter-États de Lutte contre la Sécheresse dans le Sahel) member country such as Burkina Faso, Mali, Niger and Senegal linked by trade. This is affirmed by the distribution of Niger individuals' in the cladograms that have an affinity with individuals from Mali and Togo. Niger, a major producer of cowpea serves as a crossroads between West, East and North Africa by trade, is none the less significant importer of cowpea from Benin which is a country also practicing trade with Togo (Adigoun, 2002). However, within a clade, it there’s an internal structure that can highlight the central role of agro-ecological zone. This is true of the population of Tambacounda, which is encountered in a clade. This is explained by the fact cowpea in the eastern part of Senegal is mainly food and this place is very isolated. This is more likely in the Guinean area where physical barriers (trees, rivers, etc.) play a role in the migration of the weevil. The country maintains trade links with Ivory Coast and Benin, which is a major producer of cowpea.

Weevils from different agro-ecological zones of West Africa were genetically characterized by the technique of PCR-Sequencing of mitochondrial cytochrome b gene and ribosomal 28S gene. This helped get the weevils are divided into three clades for a total of 42 haplotypes for cytochrome b and 30 haplotypes for 28S. Individual haplotypes are more numerous in Guinean area.

Genetic diversity is much more pronounced in Sudanian zone especially in Burkina Faso. This genetic diversity tends to give a structure of different populations of weevils in ecotypes because the genetic distances separating the various areas are higher than those in areas and have very significant values.

The current study, verified by phylogenetic reconstructions did stink be fully confirmed, but nevertheless it remains valid because the ecotypes-groups are much more accentuate in the cladograms.

Diversity between populations of cowpea weevils from the same country and same locality worth studied for a better strategy of protection the
stocks of cowpea in the sub-region:

- By studying the genetic diversity of the *C. maculatus* populations according cowpea varieties grown in West Africa especially in Burkina Faso and Niger.
- By studying the genetic diversity of *C. maculatus* populations indentured to different host plants.
- Estimating differentiation, the genetic structure of populations, genes flow at different special scales by selectively neutral markers, and variables that are informative microsatellite loci.
- In determining populations of *C. maculatus* to design control methods other than the chemical method which is dangerous and expensive.

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**References**


