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Genetic characterization of populations of *Bruchidius atrolineatus* Pic. (Coleoptera-Chrysomelidae-Bruchinae) from the different agroclimatic area of Niger

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Key words: *Bruchidius atrolineatus*; genetic characterization; cowpea; agro-climatic zone; Niger

1 ABSTRACT

***Bruchidius atrolineatus* Pic is one of the two main insect pests of cowpea seeds in the Sahelian zone. Attacks begin in the field and continue during storage where the damage can be considerable if no control measures are taken. During this study several aspects concerning the genetics of *B.atrolineatus* were examined. Sampling was done in Niger and genetic analysis at the BIOPASS laboratory of IRD Bel Air of Dakar. The mitochondrial marker (cytochrome b) was used. Based on the comparison of the genetic differentiation by pair (Fst by pair),the results showed that all the populations of the pest are close to each other. About 90% of the genetic variation observed is due to the variation within the constituted groups (agroclimatic zones). There is therefore no significant difference between the climatic zones of Niger. The consequence is that the population of *B. atrolineatus* appears to form a homogenous genetic unit.**

2 INTRODUCTION

The genus *Bruchidius* includes nearly 300 described species to date (Kergoat Silvanus 2004). It is a highly economically important group with several species that cause significant damage to stored foodstuffs (Alzouma 1987, Delobel and Tran 1993). Some of these species are widely distributed due to the introductions of infested seeds by transport means (Kergoat *et al.,* 2004). *Bruchidius atrolineatus*, a major pest of cowpea in West Africa, is one of these species. Attacks begin in the crop fields and continue in stocks where the damage can be very important without any adequate control measure. The female of this species has opportunistic behavior by depositing its eggs on the most abundant phonological stage in the crops (Doumma, 1998, Doumma *et al.,* 2006, Doumma, 2012). The description of the genetic variability within natural populations and the understanding of its evolution over time are essential for the knowledge of the functioning of populations of species of economic interest. Thus, population genetics of this species are still poorly studied in Africa despite its economic importance. However, understanding the genetic aspects of the geographic variation

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and population structure of this species can provide important biological information for the deployment of control strategies against this pest. For example, an investigation of the genetic diversity of *Callosobruchus chinensis* (L.) populations from pre- and post-harvest natural sites showed that those from postharvest hosts were probably the refuge of a common haplotype, while host populations of natural habitats far from agricultural areas were characterized by local haplotypes (Tuda *et al.,* 2004). In *B. atrolineatus*, the literature provides little information on populations that infest pods and cowpea seeds in Niger. Are these populations genetically different and/or homogeneous? It is only known that Niger has four agro-ecological zones that can affect the insect populations dynamics. The present study tried to explore the genetic structure of the Nigerian *Bruchidius atrolineatus* populations.

3 MATERIAL AND METHODS

3.1 Collection cowpea pods and seeds: Infested cowpea pods and seeds were collected from five sites from tree agroclimatic zones of Niger (Tableau 1).

Table 1: Summary table of sampling.

Localities	Country	Areas agro-climatic	Geographic coordinates	Observations
Makalondi	Tillabéry	Sahelian	12° 49' 59"N 01° 41' 33"E	Niger-Burkina border
Niamey	Niamey	Sahelian	13° 21' 40"N 02° 06' 23"E	
Doutchi	Dosso	Sahelian	13° 38' 21"N 04° 01' 43"E	
Gaya	Dosso	Sudano-Sahelian	11° 53' 03"N 03° 26' 57"E	Niger-Bénin border
Bermo	Maradi	Sahel-Saharan	14° 42' 26"N 6° 37' 06"E	Pastoral area

The adults of *B. atrolineatus* emerging from the pods or seeds were considered as the primary strain. These adults were used directly for the genetic study

3.2 Choice of adults for each locality: For each of the localities, except Bermo), 10 individuals from each of the two varieties of cowpea (KVX30-309-6G and TN5-78) were used . Individuals from Bermo were taken on only one TN5 / 78.. For each population, individuals were numbered from 1 to 10

3.3 Genetic analysis

3.3.1 Choice of code for each individual: A code corresponding to the agroclimatic zone, the locality of collection of the cowpea samples and the resulting species of hog was chosen. This coding has been recorded in Table 2.

3.4 Extraction, PCR-sequencing: Extractions of *B. atrolineatus* DNA were performed using Qiagen DNeasy Kit protocols. The mitochondrial marker (cytochrome b) was retained. The CB1 primers (5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3 ') and CB2 (5'-ATT ACA CCT CCTAAT TTA TTA GGA AT-3') were used for the amplification of the cytochrome gene b. The PCR (Polymerase Chain Reaction) was carried out in three steps: (i) initial denaturation at 94 ° C for three minutes, followed by 35 cycles each comprising a denaturation step at 94 ° C for 1 minute; (ii) hybridization at 47 ° C for 1 minute; (ii) elongation of the complementary DNA strand at 72 ° C for 1 minute and final elongation at 72 ° C for 10 minutes terminates the PCR.

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Table 2: Origins of *Bruchidius atrolineatus* strains and codes used

Agroclimatic zones	Localities	Codes utilisés	
		Individuals fromTN5/78	Individuals from KVX
Sahelian zone	Makalondi	SMBaT1, SMBaT2, SMBaT3, SMBaT4, SMBaT5, SMBaT6, SMBaT7, SMBaT8, SMBaT9 et SMBaT10	SMBaK1, SMBaK2, SMBaK3, SMBaK4, SMBaK5, SMBaK6, SMBaK7, SMBaK8, SMBaK9 et SMBaK10
	Niamey	SNBaT1, SNBaT2, SNBaT3, SNBaT4, SNBaT5, SNBaT6, SNBaT7, SNBaT8, SNBaT9 et SNBaT10	SNBaK1, SNBaK2, SNBaK3, SNBaK4, SNBaK5, SNBaK6, SNBaK7, SNBaK8, SNBaK9 et SNBaK10
	Doutchi	SDBaT1, SDBaT2, SDBaT3, SDBaT4, SDBaT5, SDBaT6, SDBaT7, SDBaT8, SDBaT9 et SDBaT10	SDBaK1, SDBaK2, SDBaK3, SDBaK4, SDBaK5, SDBaK6, SDBaK7, SDBaK8, SDBaK9 et SDBaK10
Sahelo-Sudanian zone	Gaya	SSGBaT1, SSGBaT2, SSGBaT3, SSGBaT4, SSGBaT5, SSGBaT6, SSGBaT7, SSGBaT8, SSGBaT9 et SSGBaT10	SSGBaK1, SSGBaK2, SSGBaK3, SSGBaK4, SSGBaK5, SSGBaK6, SSGBaK7, SSGBaK8, SSGBaK9 et SSGBaK10
Sahelo-Saharan zone	Bermo	SshBBaT1, SshBBaT2, SshBBaT3, SshBBaT4, SshBBaT5, SshBBaT6, SshBBaT7, SshBBaT8, SshBBaT9, SshBBaT10	×

For examples, SMBaT1 means S: Sahelian zone, M: Makalondi, Ba: *Bruchidius atrolineatus*, T: TN5-78 and 1: number of the individual. SMBaK1 S: Sahelian zone, M: Makalondi, Ba: *Bruchidius atrolineatus*, K: Variety KVX and 1: number of the individual.

3.5 Sequence processing and analysis: The DNA sequences were edited and aligned with BioEdit v. 7.0.5.3 (Hall, 1999) using the Clustal W algorithm version 1.4 (Thompson *et al.,* 1994). The nucleotide sequences of the sequences were checked and corrected minutely with reference to the electropherogram. The standard indices of genetic variation (number of polymorphic sites, number of informative sites total haplotype numbers by localities, intra / inter-haplotype genetic distances) are explained with the MEGA 5 software (Tamura *et al.,* 2011). In order to estimate the genetic diversity in each population, haplotypic diversity (Hd) and nucleotide diversity (π) (Nei and Tajima 1981, Nei, 1987) were calculated under DNAsp version 5.10.01 (Librado & Rozas, 2009) and Arlequin Software Version 3.1 (Excoffier *et al.,* 2005). The genetic structure of the populations was apprehended by a hierarchical analysis, the molecular analysis of variance (AMOVA), using the software Harlequin Version 3.1 (Excoffier *et al.,* 2005). Intra / inter-population genetic distances (localities), within / between agro-climatic or varietal zones were calculated using the MEGA version 5.0 software (Tamura *et al.,* 2011). Neutrality tests, namely Tajima D statistics (Tajima, 1989) and Fs de Fu (Fu, 1997), were calculated to estimate population growth and test the divergence from equilibrium, with DNAsp and the Harlequin software. The phylogenetic affinities of *B. atrolineatus* individuals were estimated with MEGA 5 software (Tamura *et al.,* 2011) and Mrbayes v. 3.1 (Huelsenbeck *et al.,* 2001). The

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haplotype network was built using TCS software version 1.21 (Clement *et al.,* 2000).

4 RESULTS

4.1 Genetic diversity of the global population: After the treatment phase (cleaning and alignment), 77 sequences were selected from the 89 sequenced samples. The other sequences were eliminated either because they are short, or because they are incomplete or irregular. The length of the sequences is 365 sites of which 324 (88.76%) are monomorphic (invariable). The difference between the haplotypes is due to 40 (10.94%) sites of which 28 sites (70%) are non-informative or singletons and 30% of the variable sites (12 sites) are informative in parsimony (Table 3).

Table 3: Parameters of the global genetic diversity of *Bruchidius. atrolineatus* populations

Parameter	**Cytochrome b**
Sample size	77
Number of haplotypes	41
Length of sequences (sites)	365
Total number of variable sites	40 (10,94%)
umber of informative sites in parsimony	12 (30%)
Number of non-informative sites or singleton	28 (70%)
Number of invariable sites	324(88,76%)
Heterotypic diversity (Dh)	0,931±0,02
Nucleotide diversity (Pi)	0,00664±0,00062

Table 4 reveals that the singleton sites are due to two variants for 89.28% which corresponds to 25 sites and for 10.71% to three variants, i.e., 3 sites. While for sparse sites, 58.33% of are due to two variants, 33.33% to three variants and only 8.34% are due to four variants. The haplotypic analysis carried out showed that among the 77 sequences analyzed, 41 have different haplotypes of which 31 are individual and four localized haplotypes (H17, H26, H32, H33) (Appendix 4). The haplotypic diversity (Dh) is 0.931 ± 0.02 while the nucleotide diversity (Pi) is 0.00664 ± 0.001

Table 4: Sites and their components

<table>
<tr><th>Invariable Sites</th><th colspan="6">Number of variable sites</th></tr>
<tr><td rowspan="5">324 (88,76%)</td><td colspan="6">40 (10,94%)</td></tr>
<tr><td colspan="3">Number of non-informative sites or singletons</td><td colspan="3">Number of informative sites sparingly</td></tr>
<tr><td colspan="3">28 (70%)</td><td colspan="3">12 (30%)</td></tr>
<tr><td>2 variant</td><td>3 variant</td><td>4 variant</td><td>2 variant</td><td>3 variant</td><td>4 variant</td></tr>
<tr><td>25 (89,28%)</td><td>3 (10,71%)</td><td>0%</td><td>7 (58,33%)</td><td>4 (33,33%)</td><td>1 (8,34%)</td></tr>
</table>

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4.2 Phylogenetic affinity: The typology of the haplotype tree constructed from *Bruchidius atrolineatus* cytochrome b sequences by the Bayesian inference method shows the existence of two clades (Figure 1). Clade I has a very wide geographical distribution because it contains the haplotypes of individuals from all sampled sites. It contains 17 haplotypes but is supported by a relatively low probability value (16%). Clade II has 12 haplotypes. These haplotypes also come from all sites sampled but much more sustained than clade I (probability value less than 37%). In both cases, no aggregation of agroclimatic zones was identified.

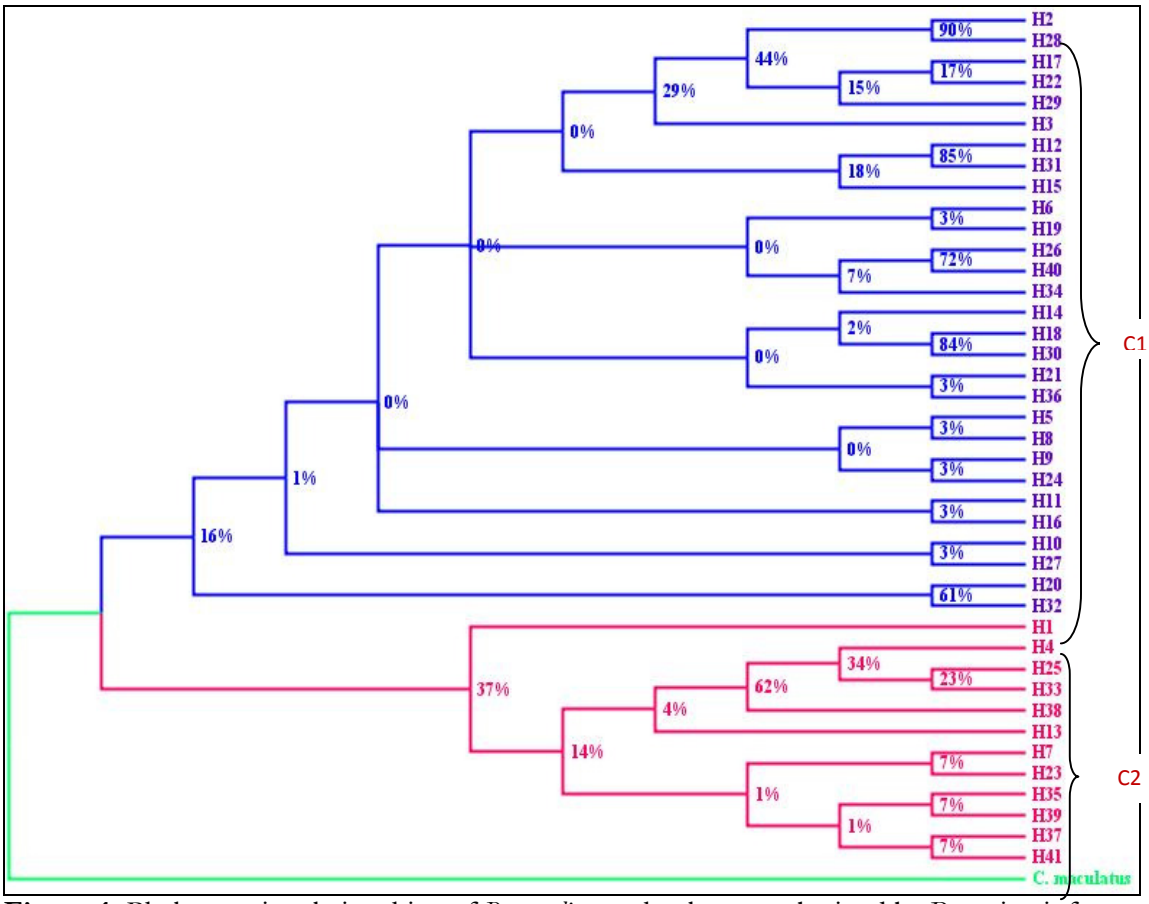

Figure 1: Phylogenetic relationships of *B. atrolineatus* haplotypes obtained by Bayesian inference.

The haplotype network obtained (FIG. 2), in star form, reveals two very frequent haplotypes that are haplotypes 8 and 7 (H8 and H7) found respectively in 17 individuals and 11 individuals. H7 is distributed in all sampled sites while H8 is distributed only in Sahelian and sahelo-sudanian agroclimatic zones.The connection between haplotype 8 and other drifting haplotypes is 0 at a mutation rate. The connection between haplotype 7 and other haplotypes is 0 to three mutational steps. Five localized haplotypes were identified and 31 individual haplotypes obtained.

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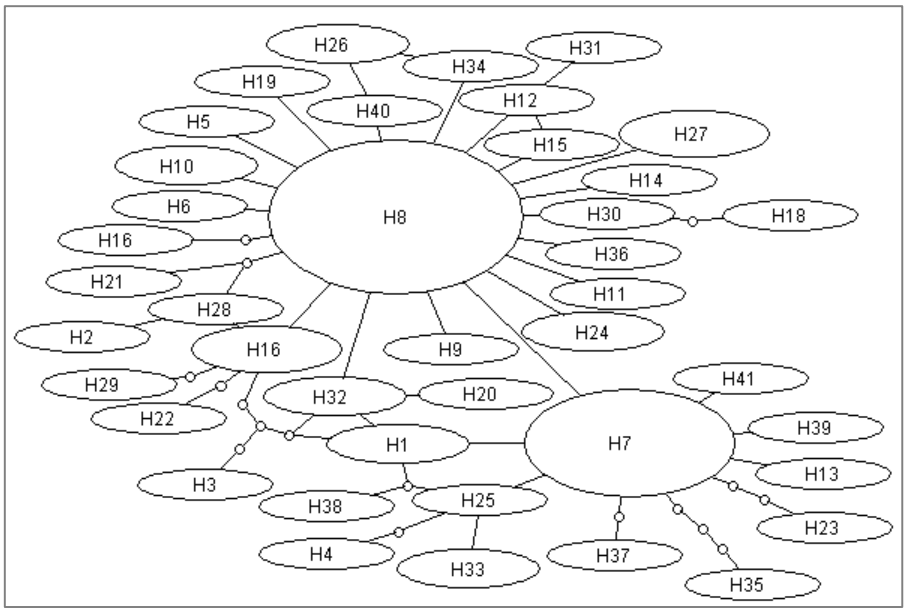

Figure 2: Haplotype network obtained for the population of *Bruchidius atrolineatus* based on the sequences of Cyt b.

4.3 Genetic diversity of *B. atrolineatus* populations according to agroclimatic origin

4.3.1 Variation of genetic diversity: The haplotypic diversity varied slightly from 0.906 ± 0.030 to 0.972 ± 0.064, while the nucleotide diversity varied from 0.0061 ± 0.0038 to 0.0080 ± 0.0052 in the different populations (Table 5). The highest haplotypic diversity was obtained in the population of the Sahelo-Saharan zone while yhe the lowest haplotypic diversity was obtained in the Sahelian zone. The most important nucleotide diversity was obtained in the Sahelo-Saharan zone. The smallest nucleotide diversity was obtained in the Sahelian zone.

Table 5: Genetic diversity of different *Bruchidius atrolineatus* populations according to agroclimatic zones

Agroclimatic zone	Sample size	Alleles number	Haplotype diversity	Nucleotide diversity
Sahelian zone	52	28	0,906±0,030	0,0061±0,0038
Sahelo-Sudanian zone	16	11	0,950±0,036	0,0075±0,0047
Sahelo-Saharan zone	9	8	0,972±0,064	0,0080±0,0052

4.3.2 Genetic structure of *B. atrolineatus* populations: Molecular variance analyzes (AMOVA) on *B. atrolineatus* populations show insignificant genetic variation between different climatic zones (Table 6). Ninety-nine percent (99.29%) of the genetic variation is observed within the agro-climatic zones and less than 1% (0.71) only of the variation is observed between agro-climatic zones.

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Table 6: Results of Molecular Variance Analysis (AMOVA)

Source of variation	Sum of squares	Percentage of variation (%)	F (P value)
Between population within group	2,758	0,71	F_{st}=0.00707 (P= 0.27)
Inside populations	90.009	99.29	

The overall value of the Fst found with this cluster (agroclimatic zones) is 0.00234 (P = 0.0960). The comparison of genetic pairwise differentiation (Fst) shows that all populations are very close to each other (Table VII), the values of genetic differentiation (Fst) being insignificant between all climatic zones. On the other hand, the examination of the inter-population genetic distance (Table VII), revels that the latter is relatively more important between the population of the Sahelo-Sudan zone and that of the Sahelo-Saharan zone (Fst = 0.008). The lowest is noted between the population of the Sahelian zone and that of the Sahel-Saharan zone (Fst = 0.007) or Sahelian and Sahelian zone (Fst = 0.007).

Table 7: The values of the genetic differentiation (Fst) at the bottom and the genetic distance (D) at the top of the diagonal between the three populations of *Bruchidius atrolineatus* defined.

Localities	Sahelian zone	Sahelo-Sudanian zone	Sahelo-Saharan zone
Sahelian zone	-	0,007	0,007
Sahelo-Sudanian zone	0	-	0,008
Sahelo-Saharan zone	0,032	0,001	-

4.3.3 Evolution and demographic history of the population of *B. atrolineatus:* Depending on the climatic zones, the neutrality tests reveal a negative Tajima D on all the populations with a significant P value except those of the Sahelo-Saharan zone (Table VIII). Examination of Fu's Fs values reveals that this index is significantly negative for all the populations. Thus, the populations of the Sahelian and Sahelo-Sudanian zones are characterized by a Tajima D and Fs of Fu significantly negative. The populations of the Sahelo-Saharan zone are characterized by a negatively insignificant Tajima D and a significantly negative Fs of Fu. The Mismatch curves (Figure 4) show a unimodal pace for all the populations that means that these populations are in demographic expansion. This is confirmed by the P values of the sum of deflection squares (SSD) and the Raggedness index (Rag) (Table 8) which are positively insignificant for all the populations and confirms the demographic expansion of the populations of *B. atrolineatus* in Niger.

Tableau 8: Neutrality index (Tajima D, Fs of Fu), Sum of Square Deviation (SSD) and irregularity index of Radggness (Rag) for each population of *Bruchidius atrolineatus*.

Index	Sahelian zone	Sahelo-soudanian zon	Sahelo-sahaian zone
Tajima D	-2.11197 (P=0.004)	-1.68055 (P=0.035)	-1.28067 (P= 0.124)
FS Fu	-26.83777 (P<0,00001)	-5.67156 (P=0.002)	-4.41067 (P=0.003)
SSD	0.00160 (P=0.470)	0.01468 (P=0.210)	0.02278 (P=0.290)
Raggedness (Rag)	0.04520 (P= 0.400)	0.08528 (P=0.160)	0.10262 (P=0.240)

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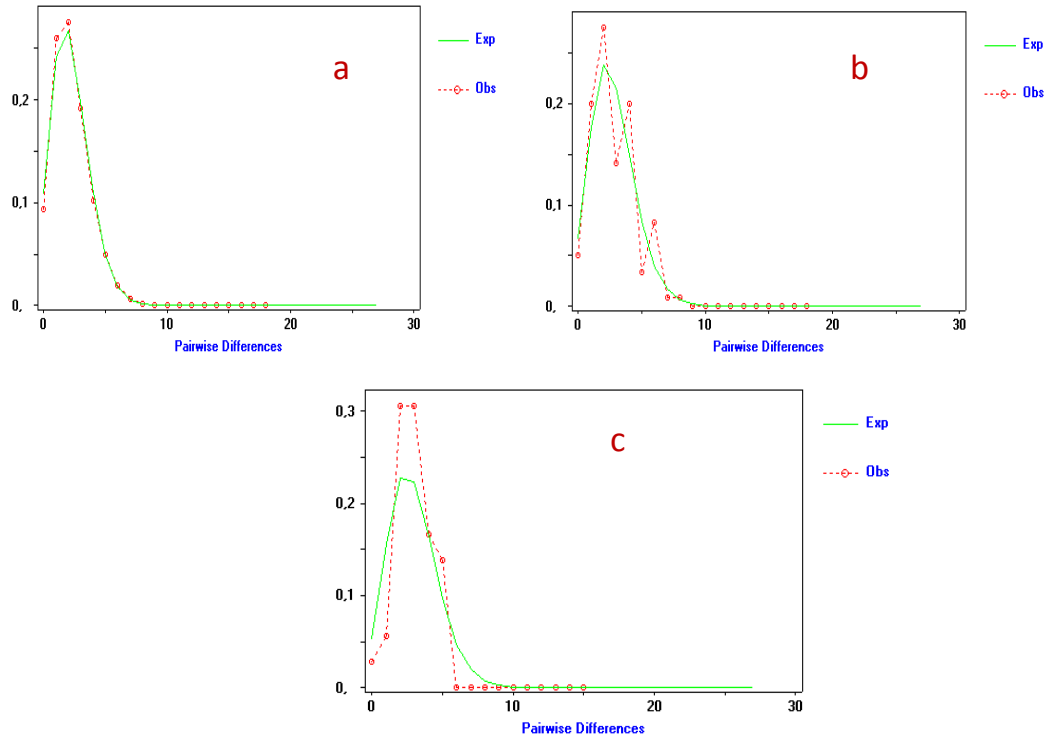

Figure 4: Distribution of differences between haplotypes taken two by two (Mismatch distribution) within the populations of the three agroclimatic zones: (a) Sahelian zone; (b) Sahelo-Sudan zone and (c) Sahelo-Saharan zone.

5 DISCUSSION

Various approaches have been used to study the populations of *Bruchidius atrolineatus* (Alzouma, 1987, Huignard, 1985, Glitho, 1990, Doumma, 1998), all of which pertain to its biology and / or ecology.. This work is the first one carried out on the genetic characterization of *B. atrolineatus* populations in the Sahelian zone. It based on the use of the cytochrome b gene showed high genetic variation with 41 haplotypes out of 77 specimens including 31 individual haplotypes. This is not surprising since cytochrome b is a mitochondrial gene highly mutational that varies rapidly in in the assessment of genetic diversity of insect pests (Sembene *et al.,* 2010, Alvarez *et al.,* 2005, Kébé, 2013, Ndaye 2014, Diome, 2014). It is probably the best known gene, which respects the structure and the function of its protein product (Esposti *et al.,* 1993). Comparable results were obtained by Kébé (2013) who observed 44 haplotypes of the 98 sequences in *Callosobruchus maculatus*, by Ndiaye (2014) who found 37 haplotypes for 46 sequences in *Caryedon serratus* and Diome (2014) who noted 9 haplotypes for 50 sequences. The total number of mutations found with this gene is 49. These mutations induce the appearance of haplotypes and are at the origin of the strong haplotypic variation observed in this study as it was obtained in *Tribolium castaneum* (Diome, 2014; Foster *et al.,* 1997) and in *C. maculatus* Kebe (2013). The number of haplotypes found in this study varies according to the studied agroclimatic zone. This number is 33 in the Sahelian zone, 16 in the Sahelo-Sudan zone and 10 in the Sahelo-Saharan zone. The number of Haplotypes found according to localities or cowpea varieties also varies. It should be pointed out that the sampling imbalance in favor of the Sahelian zones may have played a

significant role in the estimation of both haplotypic and nucleotide diversity. The level of genetic diversity observed in our experimental conditions is similar to that observed in other insect pests by several authors (Sezonlin *et al.,* 2006, Franco *et al.,* 2010, Torres-Leguizamòn *et al.,* 2011, Kébé, 2013, Ndiaye, 2014). The analysis of the molecular variance according to the agroclimatic zones showed that more than 90% of the observed genetic variation is explained by the variation within the populations. These results are confirmed by the values of genetic differentiation (Fst) pairwise and genetic distances that are insignificantly depending on climatic zones. This could indicate that gene flow occurs between different populations of *B. atrolineatus*. Similar results were obtained for other insect pests such as the *aphid (Sitobion avenae F)* in China (Xu *et al.,* 2011), the beetles *(Phyllodecta vulgatissima* L. and *P. vitellinae* L) in the United Kingdom (Batley and *al.* 2004) or *diamond back, Plutela xylostella* (L.) (Endersby and *al.* 2006). The high value of the percentage of variance within the population is due to the fact that cytochrome b is a marker that changes very fast. The commercialization of cowpea seeds between different localities, the exchange of cowpea seeds, the storage conditions, could be at the origin of the strong genetic variations within populations of *B. atrolineatus*. The idea that commercialization may lead to homogenization in insect populations has been advanced in *Callosobruchus maculatus* (Kebe, 2013), a sympatric species of *B. atrolineatus* in storages with homogenous populations in Sahelian countries. In addition, the ability of dispersion of *B. atrolineatus* due mainly to its ability to fly seems to be a significant factor that can promote a high dispersion within populations and thus the homogeneity of populations. Our results showed that there is no significant genetic variation between agroclimatic zones. Comparable results were obtained by Diome and *al.* (2014) with *Tribolium castaneum*, a polyphagous insect pest whose genetic variation is found to be insignificant in all Senegal's agro-ecological zones. The structure of the haplotype network appears complex with many infrequent and little divergent haplotypes. This is a non-perfectly structured haplotype network with many unique and slightly divergent haplotypes, consistent with haplotypic diversity for quite a large sample set (0.931 ± 0.02). The main consequence of such a model, which is extremely rich in unique haplotypes, is the reduction of the statistical power of differentiation tests (Hauser *et al.,* 2001). The star shape indicates that there is a strong demographic expansion whose main consequence is the homogenization of *B. atrolineatus* populations. This structure of the obtained haplotype network seems to be consistent with negative and significant values of Tajima D (-2.475, P<0.01) and Fu FS (-51.996 P<0.0001), reflecting the appearance of a beneficial mutant in the population and is a sign of a population in demographic expansion. In fact, a negative Tajima D value corresponds to an excessive number of alleles (low frequency polymorphism), which would indicate a recent population expansion (Excoffier *et al.,* 2005) or a gene undergoing a genetic drive effect (selective sweep) and / or purifying selection (Holsinger, 2010). The hypothesis of a low-frequency polymorphism with the rapid appearance and spread of a mutant in the population seems likely and consistent with the "Mismatch distribution" patterns that favor an expanding population with unimodal distributions. Low frequency polymorphism might be assumed to be responsible for the large number of low divergent and low frequent haplotypes observed in this study.

6 APPRECIATION

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