INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 6, p. 82-91, 2021

RESEARCH PAPER OPEN ACCESS

Characterization of populations *Bruchidius atrolineatus* (Coleoptera-Bruchinae) grown from varieties of cowpea (*Vigna unguiculata* Walp) through phylogenetic analysis

D.A. Moumouni[1*], A. Doumma[2], M. Sembene[3]

[1]*University of Tahoua, Faculty of Agricultural Sciences (FSA), B.P / 255 Tahoua, , Niger*

[2]*Abdou Moumouni University of Niamey, Faculty of Science and Technology*

[3]*Cheik Anta Diop University of Dakar (UCAD), Faculty of Science and Technology, Senegal*

Key words: Phylogenetic analysis, *Bruchidius atrolineatus*, Cowpea varieties, Niger.

http://dx.doi.org/10.12692/ijb/18.6.82-91 Article published on June 29, 2021

Abstract

Callobruchus maculatus and *Bruchidius atrolineatus* Peak are the two main pests of seeds of cowpea in the Sahel. The attacks start in the field and continue during storage where the damage can be considerable. During this study, several aspects concerning the phylogeny of *Bruchidius atrolineatus* were examined. Sampling was done in Niger and genetic analysis at IRD bel air Dakar. The mitochondrial marker (cytochrome B) was used. The results obtained showed that, by comparing the genetic differentiation pairs (Fst per pair), all populations are close to each other. Nearly 90% of the observed genetic variation is due to variation in the group (Variety) that was formed. So, there is no significant difference between individuals from the varieties tested. The consequence is that the population of *B. atrolineatus* appears to form a homogeneous genetic unit. These data constitute important biological information for researchers, agricultural technical services and other structures for the implementation of method strategies for the control of this pest.

* **Corresponding Author:** D.A. Moumouni ✉ admoumouni@yahoo.fr

Introduction

Describing the genetic variability within natural populations and understanding its evolution over time is essential for understanding the functioning of populations of species of economic interest. The ultimate goal of the analysis of genetic diversity is: i) to allow the development of strategies for conservation and pest control of cultivated plants. These pests are among others, Bruchinea beetles, pests of food legumes (Sembene and Delobel, 1998), Lepidoptera pests of crops (Timm *et al.,* 2005), Orthoptera pests of crops (Yang *et al.,* 2005), ii) to develop a conservation policy for the different races of an insect species of agronomic interest (Ladybirds predatory of Aphids (Chen *et al.,* 2000), Hymenoptera parasitoids (Baker *et al*., 2003), iii) to consider means of protecting endangered or threatened species. By analysis of DNA samples from individuals from different regions of the studied system, population genetics enables or not defining genetically distinct populations. An investigation on the genetic diversity of populations *Callosobruchus chinensis* (L.) from natural sites of pre and post-harvest showed that those from the post-harvest hosts and preserved mass were probably the refuge of a common haplotype while the populations of the host plants of natural habitats far from agricultural areas were characterized by local haplotypes (Tuda *et al*., 2004).

This study looked at *Bruchidius atrolineatus*, a major insect pest of cowpea in West Africa. Population genetics of this species is still little studied in Africa despite its economic importance. However, understanding the genetic aspects of the geographic variation and population structure of this species can provide important biological information for the deployment of control strategies against this pest. In *B. atrolineatus*, the literature provides little information on the populations that infest cowpea pods and seeds in Niger. Are these populations genetically different and/or are they homogeneous? We only know that Niger has four agro-ecological zones that may affect the population dynamics of insects. Added to this is also the diversity of ecotypes and varieties of cowpea, the main food substrate. This diversity is either seed exchanges either by trade or internally between Niger and neighboring countries. It is therefore important to know if the genetic structure of populations of *B. atrolineatus* grown from varieties of cowpea is homogeneous or different.

Methodology

Choice of individuals for each locality

For each locality, 10 individuals from two varieties of cowpea, KVX30-309-6G and TN5-78 were used with the exception of Bermo where only individuals from TN5 / 78 are used. For each population, the individuals were numbered from 1 to 10 (see Table 1 below).

Choice of codes for each individual

A code corresponding to each variety of the cowpea samples and the resulting beetle species was chosen. This coding has been recorded in Table 1

DNA extraction

B. atrolineatus DNA extractions were performed using the Qiagen DNeasy Kit and Puregene Protocol protocols. With the Kit Qiagen protocol, the DNA is pure and can be stored longer, but the kit is expensive. Whereas with the Puregene protocol the DNA is not pure but the kit is cheaper.

Choice of marker and primers used

With a view to providing answers to the various questions listed during the presentation of the problem of this study, a mitochondrial marker (cytochrome b) was selected. Cyt b is between 14747 and 15667th bp and exhibits properties similar to those of COI. It is shorter and can be amplified more easily even on degraded material (Borsa, 2009). Cytochrome b is a first-choice mitochondrial marker for the phylogenic analysis of a pest insect (Borsa, 2009; Diome, 2014).

The primers CB1 (5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3 ') and CB2 (5'-ATT ACA CCT CCT AAT TTA TTA GGA AT-3') were used for amplification of the cytochrome gene b.

Amplification of DNA by PCR (Polymerase Chain Reaction)

It is thanks to an Eppendorf type thermocycler that 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) extension of the complementary DNA strand at 72 ° C for 1 minute and a final extension at 72 ° C for 10 minutes ends the PCR.

Electrophoretic migration

Electrophoresis consists of separating DNA fragments according to their size by migration in a solid matrix called an agarose gel subjected to an electric field. The DNA molecule with a negative charge will migrate under the effect of the electrostatic field towards the anode. The distance traveled, measured from the deposition wells, will depend on the size of each fragment. Therefore, the larger the size of the fragment, the lower the distance traveled and vice versa. The samples, consisting of 7 µl of DNA extracts + 3 µl of bromophenol blue, were deposited on a 1.5% agarose gel and subjected to an electrostatic field at a voltage of 100 volts for 40 minutes.

The migrated DNA was revealed in a darkroom under UV after passing through an Ethidium Bromide (BET) bath. DNA size is estimated approximately using a Smart Ladder 200 bp size marker. The gel was prepared with 1.5 grams of agarose which is added to 100 ml of 0.5X TAE solution.

In this study, electrophoresis was performed before and after PCR to assess the quality of the DNA extracted before sequencing.

Sequence processing and analysis

Sequence alignment: DNA sequences were edited and aligned with BioEdit v. Software. 7.0.5.3 (Hall, 1999) using the Clustal W version 1.4 algorithm (Thompson *et al.*, 1994). The nucleotide sequences of the sequences were checked and carefully corrected with reference to the electropherogram.

Indices of genetic variability: The standard indices of genetic variations (number of polymorphic sites, number of informative sites, number of total haplotypes and by individual origins, genetic distances within/between haplotypes) are explained with the MEGA 5 software (Tamura *et al.*, 2011). In order to estimate the genetic diversity in each population, the haplotypic diversity (Hd) and the nucleotide diversity (π) (Nei and Tajima, 1981; Nei, 1987) were calculated under DNAsp version 5.10.01 (Librado and Rozas, 2009) and the software Arlequin Version 3.1 (Excoffier *et al.*, 2005).

Genetic distances and genetic structure: Intra / inter-population (Intra / inter-varietal) genetic distances were calculated using MEGA version 5.0 software (Tamura *et al.*, 2011).

The genetic structure of populations was understood by a hierarchical analysis, molecular analysis of variance (AMOVA), using the software Arlequin Version 3.1 (Excoffier *et al.*, 2005). This procedure estimates the proportion of total genetic variation attributable to different hierarchical levels based on a priori groupings and the degree of genetic divergence between the groupings. Thus the varietal effects of the individuals resulting therefrom on the genetic structure and diversity of populations of *B. atrolineatus* have been studied.

Finally, the relative degree of genetic differentiation was tested using the Arlequin software, the relative degree of genetic differentiation in order to describe the divergence between the different Nigerien populations of the species.

Demographic evolution: Neutrality tests, namely the D statistics of Tajima (Tajima, 1989) and Fs of Fu (Fu, 1997), were calculated in order to estimate population growth and test for divergence from equilibrium, with DNAsp and the Arlequin software. The D statistic is based on a comparison of two estimators of the measure of expected polymorphism (θ) in a sequence. When the genetic polymorphism is explained by a balance between mutation and drift, then the two

parameters are equal and Tajima's D is zero. If not, then the significant positivity of the D value is a reflection of a low level of low and high-frequency haplotypic polymorphism (= excess of intermediate frequency variants) indicating balanced selection (maintenance advantageous ancestral alleles) and/or a demographic decrease (bottleneck). On the other hand, if the value of D is significantly negative, we are in the presence of an excessive number of variants (haplotypes), infrequent, not very divergent (one or two bases of divergence). This may be due to selective scanning (emergence and rapid spread of a beneficial mutant in the population) or indicates recent population expansion (eg after a bottleneck) and/or purifying selection.

Fu's Fs Index (1997) uses information from the distribution of haplotypes to test population growth. A negative Fs value is evidence of an excessive number of haplotypes, due to recent population expansion or genetic hitchhiking; a positive Fs value indicates insufficiency of haplotypes, following a recent bottleneck in the population. Fu's simulations suggest that Fs is a more sensitive indicator of population growth and genetic hitchhiking than Tajima's D. These simulations also suggest that the classic *p*-value of 0.05 corresponds to a value from the coalescence simulation of 0.02. In other words, the value of Fs should be considered significant if *p* <0.02. The demographic expansion hypothesis and the plotting of the corresponding "Mismatch Distributions" graphs were analyzed using the model integrated into Dnasp (population expansion model).

Mismatch distributions correspond to the distribution of the number of differences between pairs of haplotypes. Unimodal distributions are interpreted as the signature of sudden population expansion, while multimodal distributions indicate demographic stability (Slatkin and Hudson, 1991).

Mantel test

The Mantel test was carried out under the 2015 version of XLSTAT software (Addinsoft, 2015). This test is used to calculate the linear correlation between two proximity matrices (dissimilarity or similarity).

This test is designed to test the correlation between genetic differentiation (Fst) and the geographical distance between sites sampled.

Results

Global genetic diversity

After the processing phase (cleaning and alignment) of the sequences, 77 sequences were retained from the 89 samples sequenced. The rest of the sequences were eliminated either because they were short or because they were incomplete or irregular.

Table 1. Origin of *B. atrolineatus* strains and codes used.

Codes used	
Individuals from TN5 / 78	Individuals from KVX
SMBaT1, SMBaT2, SMBaT3, SMBaT4, SMBaT5, SMBaT6, SMBaT7, SMBaT8, SMBaT9 et SMBaT10	SMBaK1 SMBaK1, SMBaK2, SMBaK3, SMBaK4, SMBaK5, SMBaK6, SMBaK7, SMBaK8, SMBaK9 et SMBaK10
SNBaT1, SNBaT2, SNBaT3, SNBaT4, SNBaT5, SNBaT6, SNBaT7, SNBaT8, SNBaT9 et SNBaT10	SNBaK1, SNBaK2, SNBaK3, SNBaK4, SNBaK5, SNBaK6, SNBaK7, SNBaK8, SNBaK9 et SNBaK10
SDBaT1, SDBaT2, SDBaT3, SDBaT4, SDBaT5, SDBaT6, SDBaT7, SDBaT8, SDBaT9 et SDBaT10	SDBaK1, SDBaK2, SDBaK3, SDBaK4, SDBaK5, SDBaK6, SDBaK7, SDBaK8, SDBaK9 et SDBaK10
SSGBaT1, SSGBaT2, SSGBaT3, SSGBaT4, SSGBaT5, SSGBaT6, SSGBaT7, SSGBaT8, SSGBaT9 et SSGBaT10	SSGBaK1, SSGBaK2, SSGBaK3, SSGBaK4, SSGBaK5, SSGBaK6, SSGBaK7, SSGBaK8, SSGBaK9 et SSGBaK10
SshBBaT1, SshBBaT2, SshBBaT3, SshBBaT4, SshBBaT5, SshBBaT6, SshBBaT7, SshBBaT8, SshBBaT9, SshBBaT10	

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

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 sparingly informative (Table 2).

Table 2. Parameters of the overall genetic diversity of populations of *B. atrolineatus*.

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

Demographic expansion of the overall population

Neutrality tests carried out on the overall population show significantly negative values of Tajima's D (-2.475, P<0.01) and Fu's FS (-51.996 P<0.0001) (Table 3), thus suggesting an expanding demographic of the population. The distribution disparity curves (Fig. 1) show a unimodal distribution of the number of differences observed between haplotypes taken in pairs. This unimodality is confirmed by the Rg and R2 irregularity indices of Ramos-Ouzin and Rozas which are significantly positive indicating that the population is in the full demographic expansion (Table 3).

Table 3. Neutrality indices (D of Tajima, Fs of Fu) and Irregularity indices (Rg, R2), of the Total population.

D Tajima	Fs Fu	Raggedness (r)	R2
-2,475	-51,996	0,0047	0,0275
P<0,01	P<0,0001	P=0,019	P<0,0001

Genetic diversity of two populations (populations from KVX and TN5 / 78)

The analysis of genetic diversity in populations of *B. atrolineatus* from two different varieties (KVX30-309-6G and TN5 / 78) shows that the haplotype diversity varies very slightly from 0.921 ± 0.040 to 0.931 ± 0.026. The greatest haplotypic diversity was obtained in the population of *B. atrolineatus* derived from variety TN5 / 78. Nucleotide diversity also varies very slightly depending on the variety. The greatest nucleotide diversity is obtained in the population derived from TN5 / 78 (0.0070 ± 0.0042) (Table 4).

Table 4. Genetic diversity of the different populations of *B. atrolineatus*.

Varieties	Sample size	Number of alleles	haplotype diversity	Nucleotide diversity
Varietie KVX	35	24	0.921±0.040	0.0069±0.0042
Varietie TN5/78	42	26	0.931±0.026	0.0070±0.0042

Genetic structure of B. atrolineatus populations

The analyzes of molecular variance (AMOVA) show a non-significant genetic variation between populations according to the varieties (Table 5). Indeed, 99.17% of the observed genetic variation is explained by the individual variations within populations and therefore less than 1% (0.83%) of the variation is related to the differences between populations.

Table 5. Results of the analysis of molecular variance (AMOVA) for populational analysis *Bruchidius atrolineatus*

Sources of variation	Sum of squares	Percentage of variation (%)	Fst (*P*-value)
Inter population	0.611	0.83	F_{st}=0.00829 (P=0.14)
Intra population	34.752	99.17	

The overall value of Fst is 0.0298 (P = 0.062). The value of Fst (0.008) obtained although not significant (*P*-value = 0.14) (Table 6) between the two populations shows that they are genetically very close. The genetic distance between the two populations is 0.007. On the other hand, when examining the intra-population genetic distance, the highest was observed in the population from the variety KVX30-309-6G and the smallest in the population from TN5 / 78 (Fig. 2).

Table 6. The values of Fst (bottom half-matrix) and genetic distance D (top half-matrix) between the two populations of *Bruchidius atrolineatus*.

Variety	Variety KVX30-309-6G	Variety TN5-78
Variety KVX30-309-6G	-	0,007
Variety TN5-78	0.008	-

Demographic expansion of these two populations

The neutrality tests show that the D values of Tajima and Fs of Fu are negative and significant for the two populations (Table 7). This results in the appearance of a beneficial mutant in the population and suggests a growing population. The disparity curves for the two populations (Fig. 3) show a unimodal appearance, a sign of an expanding population. The values of the sum of squared deviation (SSD) and the Radggness irregularity index (Rag) are positively insignificant for all populations. This results in a non-significant difference between the observed and expected values and confirms the unimodality of the "Mismatch distribution" curves and therefore the demographic expansion of the population.

Discussion

Various approaches have been used for the study of populations of *B. atrolineatus* (Huignard, 1985; Alzouma, 1987; Glitho, 1990; Doumma, 1998), all of these approaches relating to the biology and/or ecology of *B. atrolineatus*. This work is the first to deal with a genetic characterization of populations of *B. atrolineatus* in the Sahelian zone.

Table 7. Neutrality indices (D of Tajima, Fs of Fu), sum of the squares of deviation (SSD) and the Radggness irregularity index (Rag) for each population of *Bruchidius atrolineatus*.

Varietie	Varietie KVX30-309-6G	Varietie TN5-78
D de Tajima	-2.17 (P=0.002)	-1.95 (P= 0.008)
FS de Fu	-24.52 (P= 0.0000)	-25.31 (P=0.0000)
SSD	0.0034 (P= 0.470)	0.0005 (P= 0.940)
Raggedness (Rag)	0.038 (P= 0.61)	0.035 (P= 0.720)

The genetic characterization of *B. atrolineatus* populations based on the use of the cytochrome b gene shows a large genetic variation with 41 haplotypes out of the 77 specimens, including 31 individual haplotypes. This is not surprising since cytochrome b is a rapidly evolving, highly mutational mitochondrial gene in the assessment of the 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 function of its protein product (Esposti *et al.*, 1993). Comparable results

were obtained by Kébé (2013) who observed 44 haplotypes out of the 98 sequences in *Callosobruchus maculatus,* by Ndiaye (2014) who found 37 haplotypes for 46 sequences in *Caryedon serratus* and by 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 was obtained in other insect pests by Diome (2014) in *Tribolium castaneum*; Foster *et al.* (1997) in *Tribolium castaneum*; Kebe (2013) in *C. maculatus*). The number of haplotypes found in this study varied among varieties.

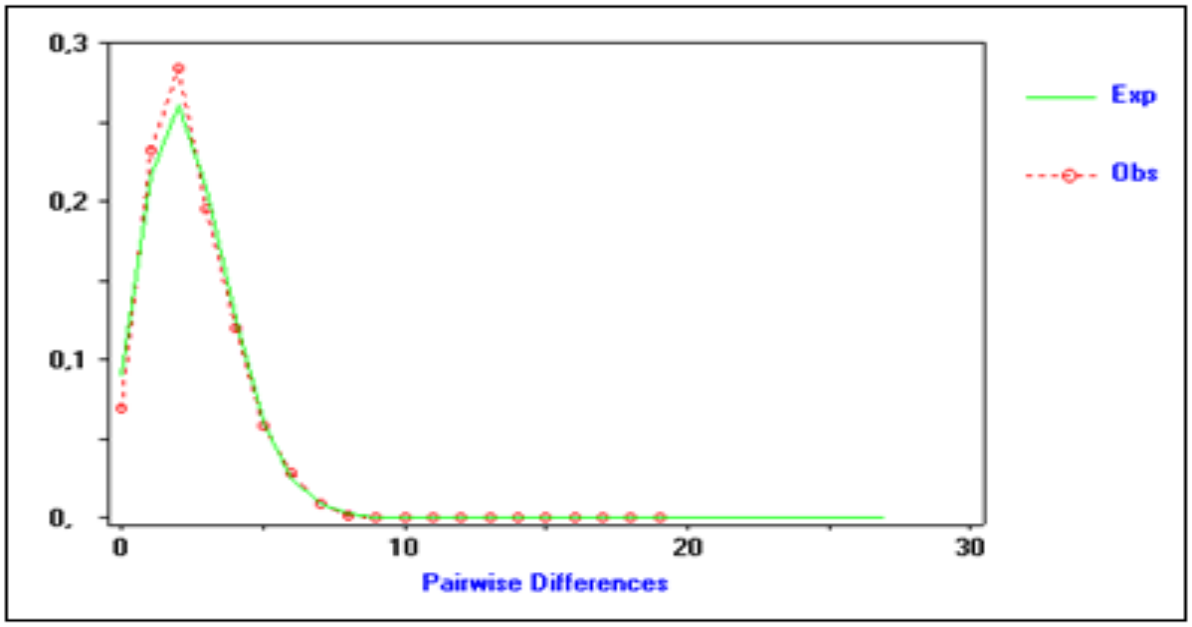

Fig. 1. Diagram of the population distribution disparity.

It should be noted that the imbalance of the sampling in favor of the Sahelian areas may have played a non-negligible role in the estimation of both haplotypic and nucleotide diversity.

The level of genetic diversity observed under 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 results of the analysis of molecular variance according to cowpea variety show that more than 90% of the observed genetic variation is explained by variation within populations. These results are confirmed by the values of the genetic differentiation (Fst) per pair and the genetic distances which are insignificant as a function of climatic zones, varieties, or localities. This could indicate that gene flow is occurring between different populations of *B. atrolineatus*. Similar results have been obtained on other insect pests such as the aphid *Sitobion avenae* F. in China (Xu *et al.*, 2011), Coleoptera *Phyllodecta vulgatissima* L. and *P. vitellinae* L. in the United Kingdom (Batley *et al.* ., 2004) or the lepidopteran *Plutela xylostella* (L.) (Endersby *et al.*, 2006).

The high value of the percentage of variance within the population is thought to be due to the fact that cytochrome b is a marker that evolves very quickly.

The marketing of cowpea seeds between different localities, exchanges of cowpea seeds, storage conditions, could be at the origin of the strong genetic variations within populations of *B. atrolineatus*.The idea that commercialization can lead to homogenization within populations of an insect has been put forward in *C. maculatus* (Kébé, 2013), a sympatric species of *B. atrolineatus* in stocks which has homogeneous populations in Sahelian countries.

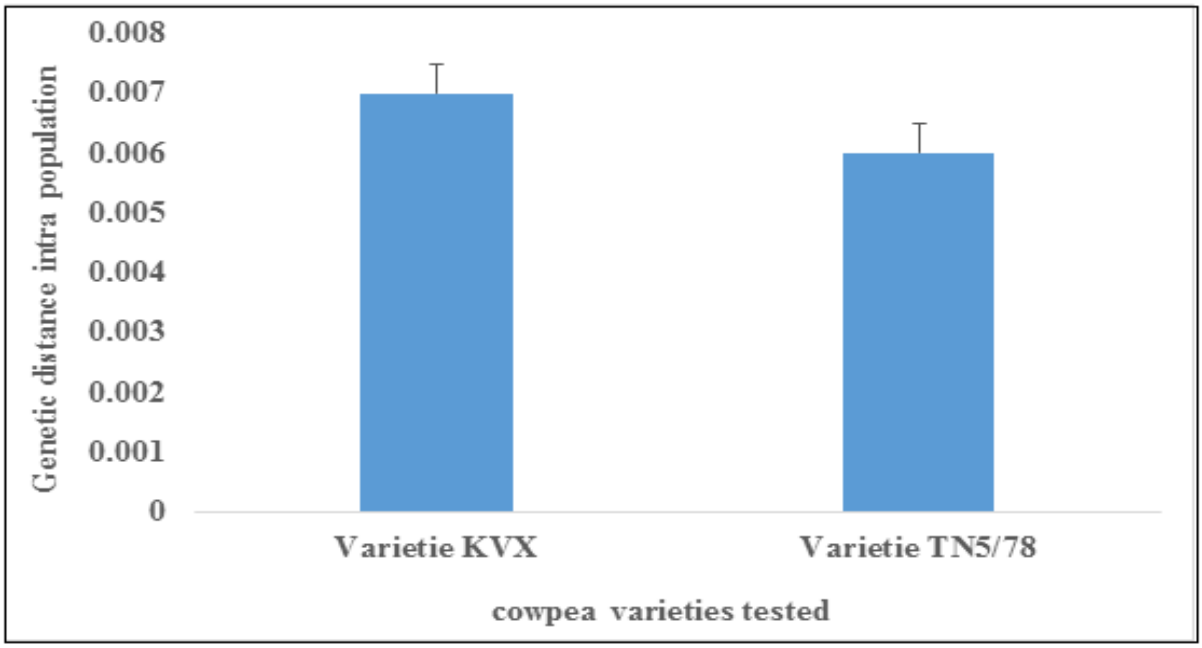

Fig. 2. Variation of the genetic distance of two populations of *B. atrolineatus*.

In addition, the dispersal capacity of *B. atrolineatus*, explained above all by its ability to fly, seems to be a significant factor that can promote strong dispersal within populations and therefore population homogeneity. Our results have shown that there is no significant genetic variation between varieties. Comparable results were obtained by Diome *et al.* (2014) with *Tribolium castaneum*, an insect that is nonetheless polyphagous but whose genetic variation found is not significant in all agro-ecological zones of Senegal.

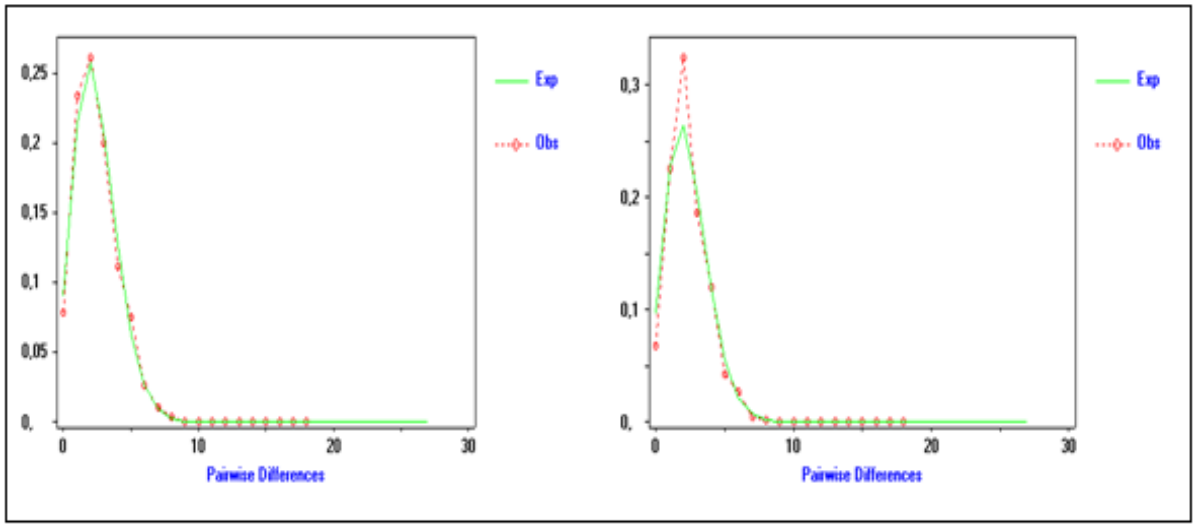

Fig. 3. Distribution of the number of differences between haplotypes taken in pairs (Mismatch distribution) for each population. (a) Population derived from variety KVX; (b) population derived from the variety TN5 / 78.

The values of D of Tajima (-2.475, P<0.01) and FS of Fu (-51.996 P<0.0001) negative and significant, reflecting the appearance of an advantageous mutant in the population and a sign of a growing population. Indeed, 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 knock-on effect (bottleneck or selective sweep) and/or purifying selection

(Holsinger, 2010). The hypothesis of a low-frequency polymorphism with rapid appearance and spread of a beneficial mutant in the population seems probable and agrees with the "Mismatch distribution" plots which favor an expanding population with unimodal distributions. One might speculate that low-frequency polymorphism is responsible for a large number of low-divergence and infrequent haplotypes observed in this study. All these results and hypotheses seem to be consistent when compared with the values of haplotypic and nucleotide diversity.

The relationship between haplotypic diversity and nucleotide diversity provides information on the demographic history of a population (Ndiaye, 2014). In the case of this study, the haplotypic diversities are high for all the samples while the nucleotide diversities are relatively low.

According to Grant and Bowen (1998) who consider the levels of diversity detected through these two indices under conditions of rapid expansion, haplotypic diversity grows faster than nucleotypic diversity, which leads to obtaining a large number of unique haplotypes. This current observation may reflect a low-frequency polymorphism, followed by moderate population expansion and accumulation of mutations.

References

Alzouma I. 1987. Reproduction and development of *Bruchidius atrolineatus* Pic. (Coleoptera: Bruchidae) at the expense of *Vigna unguiculata* L. Walp (Leguminosae: Papilionaceae) cultures in a Sahelian agrosystem in Niger. State Doctorate Thesis, Univ. François-Rabelais, Tours France, 162p.

Delobel A, Tran M. 1993. Coleoptera of food stored in hot regions. ORSTOM / CTA. Paris, p 312-316.

Doumma A. 1998. Contribution to the search for control methods against *Bruchidius atrolineatus* Pic. And *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae), cowpea pest (*Vigna unguiculata* (L.) Walp) in the Sahelian zone. Doctoral thesis. Univ. Abdou Moumouni from Niamey, p 136.

Doumma A. 2012. Influence of two control alternatives on the reproductive capacity of *Callosobruchus maculatus* Fab. (Coleoptera-Bruchidae), pest of cowpea (*Vigna unguiculata* Walp.): State Doctorate Thesis. United. Abdou Moumouni from Niamey, p 171.

Doumma A, Liman AI, Toudou A, Alzouma I. 2006. Behavior of 20 local varieties of cowpea (*V. unguiculata* (L.) Walp) vis-à-vis *B. atrolineatus* P. and *C. maculatus* F., cowpea pests in the Sahelian zone: Cahiers d'Agriculture, No. 15; Flight. 2.

Excoffier L, Laval G, Schneider S. 2005. Arlequin 3.01.An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online **1,** 47-50.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics **147,** 915-925.

Glitho IA. 1990. Les Bruchidae ravageurs de *Vigna unguiculata* Walp. en zone guinéenne. Analyse de la diapause reproductrice chez les mâles de *Bruchidus atrolineatus* Pic. Thèse de Doctorat, Université F. Rabelais, Tours, 100 p.

Hauser L, Turan C, Carvalho GR. 2001. Haplotype frequency distribution and discriminatory power of two mtDNA fragments in a marine pelagic teleost (Atlantic herring, *Clupea harengus)*. Heredity, **87,** 621-630.

Kébé K. 2013. Genetic diversity, evolutionary history and bioecology of *Callosobruchus maculatus* F. (Coleoptera, Bruchinea) pest of cowpea seeds (*Vigna unguiculata* Walp). PhD thesis at the Sciences Cheikh Anta Diop University of Dakar (Senegal), p 186.

Kergoat GJ, Silvain JF. 2004. Is the genus *Bruchidius* (Coleoptera: Bruchidae) monophyletic? Contributions of parsimony methods, maximum

likelihood and Bayesian inference. Biosystema 22: 113-125.

Kergoat GJ, Delobel A, Silvain JF. 2004. Phylogeny and host-specificity of European seed beetles (Coleoptera, Bruchidae), new insights from molecular and ecological data. Molecular Phylogeneticsand Evolution **32,** 855-865.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25(11),** 1451-1452.

Ndiaye A. 2013. Genetic characterization, evaluation of gene flow for different ecotypes of *Caryedon serratus*, peanut pest. PhD thesis at the Sciences Cheikh Anta Diop University of Dakar (Senegal), p 151.

Nei M. 1987. Molecular evolutionary genetics, Columbia University Press, New York. NY, USA.

Nei M, Tajima F. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics, 97: 145-163.

Sembène M, Kébé K, Delobel A, Rasplus JY. 2010. Phylogenetic information reveals the peculiarity of *Caryedon serratus* (Coleoptera, Chrysomelidae, Bruchinae) feeding on *Cassia sieberiana* DC (Caesalpinioideae). African Journal of Biotechnology **9,** 1470-1480.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123,** 585-595.

Tamura K, Peterson D, Peterson N, Stecher G, Nei MKS. 2011. "MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods". Molecular Biology and Evolution **28(10),** 2731-2739.

Thompson JD, Higgins DG, Gibson TJ. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research **22,** 4673-4680.

Tuda M, Wasano N, Kondo N, Horng SB, Chou LY, Tateishi Y. 2004. Habitat related mtDNA polymorphism in the stored-bean pest *Callosobruchus chinensis* (Coleoptera: Bruchidae). Bulletin of Entomological Research **94,** 75-80.

Xu ZH, Chen JL, Cheng DF, Liu Y, Francis F. 2011. Genetic variation among the geographic population of the grain aphid, *Sitobion avanae* (Hemiptera: Aphidae) in China inferred from mitochondrial COI gene sequence. Agricultural Science in China **10,** 1041-1048.