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Diversity and Genetic Differentiation of Senegalese Populations of *Caryedon serratus* (Olivier, 1790) Between 1998 and 2022

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Abstract

Groundnuts are an important food resource in Senegal, but post-harvest losses of this product are considerable and represent a major economic constraint for our country. Most often, these losses are due to beetles (pests) and among these, the one that causes the most damage to the stocks belongs to the Chrysomelidae family : *Caryedon serratus* (Olivier, 1790), commonly known as groundnut seed-beetle. Faced with these losses, it is becoming urgent to look for new methods of control. The objective of this research is therefore to study the diversity. The objective of this research is therefore to study the diversity and genetic differentiation of Senegalese populations of *C. serratus* associated with groundnuts (*Arachis hypogaea*) and wild host plants (*Piliostigma reticulatum*, *Tamarindus indica*, *Bauhinia rufescens* et *Cassia sieberiana*) between 1998 and 2022. For this, the Cytochrome B (Cyt-B) gene of different strains of *C. serratus* was sequenced. The results obtained reflect a high level of genetic diversity of *C. serratus* populations. A statistically significant genetic differentiation is also noted, thus a clear difference in genetic structuring at the intra- and inter-population levels. The AMOVA inferences show that most of the genetic variation observed on Cyt-B sequences is significantly explained by intra-population genetic differentiation.

Keywords: *Caryedon serratus*, groundnut, wild host plants, diversity, genetic differentiation, Senegal groundnut basin, Cytochrome B.

1. INTRODUCTION

Groundnuts are an important food resource in Senegal, but the post-harvest losses of this product are considerable and represent a major economic constraint for our country. Indeed, the contamination of groundnuts by insects greatly depreciates the nutrient content of this staple food. Among these insects, the one that causes the most damage to the stocks is a beetle belonging to the Chrysomelidae family : *Caryedon serratus* (Olivier, 1790), commonly known as groundnut seed-beetle. In Senegal, the damage caused by this

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seed-beetle to groundnuts can be up to 83% of quantitative loss over a storage period of 4 months (Ndiaye, 1991). Faced with these losses, it is becoming urgent to look for new methods of control.

In this perspective, different approaches have been used to study *Caryedon serratus* populations : morphometric studies, allozyme studies (Sembène & Delobel, 1996 ; 1998 ; Sembène *et al.*, 1998), and molecular : microsatellites (Sembène, 2000), Cytochrome B (Delobel *et al.*, 2003) and the Transcribed Internal Spacer, ITS1 (Sembène, 2004 ; Sembène *et al.*, 2010). In addition, it has been reported in numerous studies that *C. serratus* is an insect with a high level of diversity and genetic differentiation between its populations and biotypes (Delobel *et al.*, 2003 ; Sembène *et al.*, 2006 ; Diome *et al.*, 2011 ; Ndong *et al.*, 2011 ; Ndiaye, 2014). Thus, this work finds its originality in the fact that it is the first study to deal with the temporal aspect of the genetics of Senegalese populations of *C. serratus* (here, between 1998 and 2022, i.e. a duration of 24 years) through the Cyt-B gene.

The objective of this research is therefore to study the diversity and genetic differentiation of different populations of *C. serratus* associated with groundnuts (*Arachis hypogaea*) and wild host plants (*Piliostigma reticulatum*, *Tamarindus indica*, *Bauhinia rufescens* et *Cassia sieberiana*) between the years 1998 and 2022. Specifically, it is a question of assessing the diversity and genetic differentiation of two (2) groups of *C. serratus* populations (groundnut populations and global populations) between the years 1998 and 2022. For this, the Cytochrome B (Cyt-B) gene of different strains of *C. serratus* was sequenced. The results obtained will undoubtedly contribute to the protection of groundnut seeds against *C. serratus* populations without the use of synthetic pesticides but rather by the definition of effective and non-polluting control strategies.

2. MATERIALS AND METHODS

2.1. Sampling

The samples studied come from different storage sites in the Senegalese groundnut basin (targeted agro-ecological zone) (Figure 1).



Indeed, they were taken from four (04) localities in three (03) regions of Senegal : Diourbel (in Diourbel and Touba), Fatick (in Passy) and Kaolack (in Keur Ayib). These regions can be divided into two sub-zones : the North Groundnut Basin (Diourbel) and the South Groundnut Basin (Fatick and Kaolack) (Table 1).

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Table 1. Agro-ecological zones and geographic coordinates of the four (4) locations sampled in the groundnut basin between 2020 and 2022

Regions	Localities	Storage sites	Agro-ecological zones	Geographic coordinates	
				Latitude	Longitude
Diourbel	Diourbel	Stockroom	North groundnut basin	<u>14° 39' 21.438''</u> <u>N</u>	<u>16° 13' 31.089''</u> W
	Touba	Stockroom	North groundnut basin	<u>14° 54' 23.850''</u> <u>N</u>	<u>15° 51' 55.806''</u> W
Fatick	Passy	Granary	South groundnut basin	<u>13° 58' 42.369''</u> <u>N</u>	<u>16° 15' 52.136''</u> W
Kaolack	Keur Ayib	Granary	South groundnut basin	<u>13° 35' 45.800''</u> <u>N</u>	<u>15° 36' 02.100''</u> W

In this study, the sampling method used is the same regardless of the storage site. Indeed, it consists of taking a random sample of four kilograms (04 Kg) of groundnuts (about 1000 pods) from each locality. The pods are then placed in jars with wire lids and placed in a ventilated room in the laboratory where the ambient temperature follows the fluctuations of the outside temperature. Samples are kept for a minimum of 75 days to control the level of *C. serratus* infestation. Daily monitoring of this mass culture made it possible to collect the adults of *C. serratus* that emerge from the samples. The individuals collected in this way are preserved in 95° alcohol for the realization of the molecular protocol.

The *Caryedon serratus* specimens that were the subject of this study come from pods sampled in 1998 and between 2020 and 2022 from different host plant species. Pods collected between 2020 and 2022 are coded as 2022 (Table 2).

Table 2. Summary of the different sampling parameters between 1998 and 2022

Host plants	Harvest years	Sample codes	Number of individuals sequenced
<i>Arachis hypogaea</i>	2022	CsA-2022	52
	1998	CsA-1998	34
<i>Piliostigma reticulatum</i>	2022	CsP-2022	13
	1998	CsP-1998	9
<i>Tamarindus indica</i>	2022	CsT-2022	9
	1998	CsT-1998	6
<i>Bauhinia rufescens</i>	2022	CsB-2022	11
	1998	CsB-1998	5
<i>Cassia sieberiana</i>	2022	CsC-2022	8
	1998	CsC-1998	6
Total			153

CsA = *Caryedon serratus* from *Arachis hypogaea* (groundnut) ;

CsP = *Caryedon serratus* from *Piliostigma reticulatum* ;

CsT = *Caryedon serratus* from *Tamarindus indica* ;

CsB = *Caryedon serratus* from *Bauhinia rufescens* ;

CsC = *Caryedon serratus* from *Cassia sieberiana*.

2.2. Genetic studies

2.2.1. Choice of Cytochrome B (Cyt-B)

The Cyt-B gene is considered to be one of the most widely used genes for determining phylogenetic relationships due to its low sequence variability and probably the best known mitochondrial gene in terms of its structure and the function of the protein produced (Esposti *et al.*, 1993). It is distinguished by a high intra-specific variability (Avise, 1994) especially in insects (Simmons and Weller, 2001). It is easier to align

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between species because it does not have gaps and easier to use to estimate the divergence of dates between taxa.

2.2.2. Extraction of genomic DNA from *C. serratus*

The extraction of DNA from *C. serratus* was done using the standard Qiagen method (Qiagen Dneasy Tissue kit). The insect is first dissected to carefully remove the abdomen, elytra and fins. The other parts of the body (the head, thorax and legs) are crushed and then placed in a 1.5 ml tube. These three parts are therefore the only ones to be used in the extraction protocol which took place in 4 steps : digestion, cell lysis, purification and elution.

2.2.3. Amplification by PCR (Polymerase Chain Reaction)

PCR is a specific in vitro of a target DNA sequence ; that is to say, a repetition of cycles that ensures a doubling (02) of the target DNA at each cycle. In reality, PCR is based on a repetition of three (03) phases : DNA denaturation, hybridization of primers on templates, and elongation of the 3' ends of the neosynthesized strands. The primers used were defined by Simon *et al.* (1994) (Table 3) and the conditions summarized in Table 4.

Table 3. Identification of the sequences of the different primers used in PCR

Gene	Primer names	Primer sequences
Cyt-B	CB-J-10933 (F)	5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3'
	CB-N-11367 (R)	5'-ATT ACA CCT CCT AAT TTA TTA GGA AT-3'

Table 4. Summary of PCR conditions

Gene	PCR conditions
Cyt-B	(1) Initial denaturation : 94°C, 3 min ; 35 denaturation cycles : 94°C, 1 min ;
	(2) Hybridization : 47°C, 1 min ;
	(3) Elongation : 72°C, 1 min ; final elongation : 72°C, 10 min.

2.2.4. Sequencing

The sequencing of the Cyt-B gene was performed by the South Korean sequencing company MacroGen. This technique consists of determining the nucleotide sequence of a DNA fragment. It therefore makes it possible, by comparing the sequences of the same gene in different individuals of the same species or of different species, to highlight point mutations.

2.3. Genetic analyses

2.3.1. Sequence cleaning and alignment

Sequence cleaning is the visual verification of the correct automatic interpretation of chromatograms. First, the dataset was blasted to the Genbank to check if our sequences actually match those of the *Caryedon serratus* species. Subsequently, the resulting raw sequences were manually corrected and aligned with the BioEdit ver software. 5.0.6 (Hall, 2001) which uses the Clustal-W algorithm (Thompson *et al.*, 1997).

2.3.2. Polymorphism and genetic diversity

2.3.2.1. Basic parameters of genetic variability

Different parameters were determined in order to infer the genetic diversity and structure of *C. serratus* populations. All of these parameters were determined using the DnaSP ver software. 5.10.01 (Librado and Rozas, 2009) except for the transition and transversion percentages (%Trs and %Trv). The latter were determined with the ARLEQUIN ver. 3.1 (Excoffier & Heckel, 2006).

2.3.2.2. Nucleotide and amino acid frequency

Comparison of the average frequency of nucleotides and amino acids between populations provides insight into the diversity of the latter. For each population, the nucleotide frequencies are determined with the ARLEQUIN ver program. 3.1 (Excoffier & Heckel, 2006) and those of amino acids using the MEGA7 ver. 7.0.14 (Tamura *et al.*, 2016). The latter also allowed us to obtain the average frequencies of nucleotides and amino acids.

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2.3.2.3. Indices of genetic diversity : P_i , H_d and their standard deviations

P_i and H_d were determined using the DnaSP ver software. 5.10.01 (Librado & Rozas, 2009). These two parameters were combined to provide information on the evolution and provenance history of the populations from which our samples were taken.

2.3.3. Genetic structure of populations

2.3.3.1. Genetic differentiation

To assess genetic differentiation, Nei's (1987) genetic distance D was calculated to the detriment of the F_{ST} (degree of genetic differentiation) because the latter is more appropriate to loci than to sequences. Its intra- and inter-population values, as a function of the years of harvest of the individuals from the groundnut and the overall population, were generated using the MEGA7 worm software. 7.0.14 (Tamura *et al.*, 2016) with the Kimura-2-P model to account for the non-equiprobability of mitochondrial DNA transitions and transversions.

2.3.3.2. Analysis of molecular variance

The genetic structuring of populations was also evaluated by a molecular analysis of variance (AMOVA : Analysis of MOlecular VAriance, Excoffier *et al.*, 1992) in order to detect the source of variance at the molecular level. This molecular variance between the different sources of variation was calculated using the ARLEQUIN ver program. 3.1 (Excoffier & Heckel, 2006). The F_{ST} fixation index, which expresses the decrease in heterozygosity related to the divergence between the subpopulation and the total population, was determined : $F_{ST} = (H_T - H_S) / H_T$. This index is therefore used as an index of genetic differentiation between subpopulations (Weir & Cockerham, 1984).

3. RESULTS

3.1. Polymorphism and genetic diversity

3.1.1. Genetic variability of *Cyt-B*

The sequenced individuals come from five (05) populations of *C. serratus*, therefore dependent on five (05) host plants, one (01) of which is cultivated (groundnut) and four (04) wild (*Piliostigma reticulatum*, *Tamarindus indica*, *Bauhinia rufescens* and *Cassia sieberiana*). One hundred and fifty-three (153) *Cyt-B* sequences make up the dataset to be analyzed. For each sequence, the total number of unambiguously aligned nucleotides is 412 bp (four hundred and twelve base pairs). In total, 30 (thirty) haplotypes were noted, of which 10 (ten) were individual, 264 (64.08%) were invariable sites and 148 (35.92%) were polymorphic sites, of which 145 (97.97%) were informational sites in parsimony and 3 (02.03%) were singleton sites. In addition, the total number of mutations observed is 179 (one hundred and seventy-nine) ; Overall, transition-type mutations (52.93%) are more numerous than those of transversion types (47.07%). Overall, the mean number of nucleotide differences is 38.086 (Table 5).

Table 5. Parameters of *Cyt-B* genetic variability as a function of harvest years of individuals from groundnut populations and overall (global) populations

Parameters of genetic variability	Groundnut populations		Global populations		Total
	CsA-2022	CsA-1998	Pop-2022	Pop-1998	
SQ	52	34	93	60	153
S	412	412	412	412	412
SI	305	352	265	344	264
SP	107	60	147	68	148
SIP	104	60	145	66	145
SS	3	0	2	2	3
H	14	3	24	16	30
HI	8	0	8	8	10
Eta	123	61	175	75	179
%Trs	53.66	50.82	52.57	54.67	52.93
%Trv	46.34	49.18	47.43	45.33	47.07
K	26.219	25.781	37.712	28.216	38.086

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CsA : *Caryedon serratus* from *Arachis hypogaea* (groundnut) ; Pop : global Population ; SQ : number of sequences ; S : number of sites ; SI : number of invariable sites ; SP : number of polymorphic sites ; SIP : number of information sites in parsimony ; SS : Number of singleton sites ; H : number of haplotypes ; HI : number of individual haplotypes ; Eta : number of mutations ; Trs : transition ; Trv : transversion ; K : mean number of nucleotide differences.

3.1.2. Nucleotide and amino acid frequency

Analysis of the nucleotide composition (percentage of adenine, thymine, guanine and cytosine) of the Cyt-B gene reveals that for all populations studied (groundnut populations and global populations), the frequency in A+T (68.60%) is higher than that in G+C (31.40%) (Table 6). In addition, analysis of the amino acid composition (average amino acid frequency) of the same gene shows that all 153 individuals of *C. serratus* have an amino acid number between 16 and 18 except for three (CsA49-2022, CsC2-1998 and CsC3-1998) which have 19 with the absence of only Tryptophan for CsA49-2022 and Glycine for CsC2-1998 and CsC3-1998.

Table 6. Nucleotide composition and frequency in A+T and G+C of the Cyt-B gene in *C. serratus* populations.

Populations studied		% Adenine	% Thymine	% Guanine	% Cytosine	% A+T	% G+C
Groundnut populations	CsA-2022	29.71	38.04	10.84	21.42	67.75	32.26
	CsA-1998	31.45	37.64	10.75	20.16	69.09	30.91
Global populations	Pop-2022	30.26	38.01	10.83	20.90	68.27	31.73
	Pop-1998	31.26	37.78	10.78	20.17	69.04	30.95
Total		30.70	37.90	10.80	20.60	68.60	31.40

CsA = *Caryedon serratus* from *Arachis hypogaea* (groundnut) ; Pop = global Population.

3.1.3. Genetic diversity indices : P_i (π), H_d and their standard deviations

The genetic diversity indices of Cyt-B were calculated according to the years of harvest of the individuals from the groundnut and the overall population (all host plants). Overall, high haplotypic diversity ($H_d > 50\%$) and nucleotide diversity ($P_i > 0.5\%$) were noted. Similarly, the Cyt-B gene reveals a high haplotypic diversity ($H_d > 50\%$) and a high nucleotide diversity ($P_i > 0.5\%$) both in individuals from groundnut populations (CsA-2022 and CsA-1998) and in individuals from global populations (Pop-2022 and Pop-1998). However, it should also be noted that within each of the two population groups (groundnut populations and global populations), the H_d and P_i indices are higher in individuals collected in 2022 (CsA-2022 and Pop-2022) than in individuals collected in 1998 (CsA-1998 and Pop-1998) (Table 7).

Table 7. Indices of Cyt-B genetic diversity according to harvest years of individuals from groundnut populations and overall populations

Populations studied		Number of individuals	Genetic diversity indices	
			H_d +/- SD	P_i +/- SD
Groundnut populations	CsA-2022	52	0.796 +/- 0.050	0.06364 +/- 0.01017
	CsA-1998	34	0.540 +/- 0.067	0.06257 +/- 0.00966
Global populations	Pop-2022	93	0.907 +/- 0.018	0.09153 +/- 0.00719
	Pop-1998	60	0.755 +/- 0.053	0.06848 +/- 0.00585
Total		153	0.876 +/- 0.018	0.09244 +/- 0.00414

CsA = *Caryedon serratus* from *Arachis hypogaea* (groundnut) ; Pop = global Population ; H_d : haplotypic diversity ; P_i : nucleotide diversity ; SD : Standard Deviation

3.2. Genetic structure of populations

3.2.1. Genetic distance D from Nei

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The results obtained with Cyt-B sequences reveal significant genetic distances within and between groundnut populations (0.06988 +/- 0.00869 in 1998 ; 0.07030 +/- 0.00681 in 2022 and 0.14465 +/- 0.01478 between 1998 and 2022). The same is true for genetic distances within and between global populations (0.07578 +/- 0.00966 in 1998 ; 0.10254 +/- 0.00936 in 2022 and 0.11342 +/- 0.01212 between 1998 and 2022). In addition, it should also be noted that in each of the two (02) population groups (groundnut populations and global populations), the genetic distances within the populations collected in 2022 (CsA-2022 and Pop-2022) are greater than those of the populations collected in 1998 (CsA-1998 and Pop-1998). In addition, the genetic distance between groundnut populations (CsA-2022 and CsA-1998) is greater than that between the overall populations (Pop-2022 and Pop-1998) (Table 8).

Table 8. Intra- and inter-population Nei D genetic distances calculated from Cyt-B gene sequences

Populations studied	Genetic distances within populations		Genetic distances between populations	
Groundnut populations	CsA-2022	0.07030 +/- 0.00681	CsA-1998	
	CsA-1998	0.06988 +/- 0.00869	CsA-2022	0.14465 +/- 0.01478
Global populations	Pop-2022	0.10254 +/- 0.00936	Pop-1998	
	Pop-1998	0.07578 +/- 0.00966	Pop-2022	0.11342 +/- 0.01212

CsA = *Caryedon serratus* from *Arachis hypogaea* (groundnut) ; Pop = global Population

3.2.2. Molecular analysis of variance (AMOVA)

Considering the year of harvest and the origin of the individuals, 2 population groups were defined : the groundnut population group (CsA-2022 and CsA-1998) and the global population group (Pop-2022 and Pop-1998). Thus, AMOVAs reveal that the source of the molecular variance is essentially due to genetic diversity between individuals in the same population representing 79.39% of the total variance ($F_{ST} = 0.20607$; $P = 0$). In addition, 37.92% of the observed molecular variance is significantly explained by genetic variation between populations ($F_{SC} = 0.32326$; $P = 0$). Finally, there is no significant genetic differentiation between the two population groups ($F_{CT} = -0.17316$; $P = 0.67937$ +/- 0.01182) (Table 9).

Table 9. Analysis of Molecular Variance for Cyt-B. Significant P indices and values ($P < 0.05$) are marked with the asterisk symbol (*).

Source of variance	Variance components	Percentage (%) of variance	Fixation indices	P-value
Between population groups	$V_a = -3.39793$	- 17.32	$F_{CT} = -0.17316$	0.67937 +/- 0.01182
Between populations within groups	$V_b = 7.44154$	37.92	$F_{SC} = 0.32326$ ***	0.00000 +/- 0.00000 ***
Within populations	$V_c = 15.57905$	79.39	$F_{ST} = 0.20607$ ***	0.00000 +/- 0.00000 ***

4. DISCUSSION

4.1. Polymorphism and genetic diversity

The analysis of the 153 sequences of the Cyt-B gene reflects a high level of genetic diversity of *C. serratus* populations. Indeed, the nucleotide replacements observed reveal that at least 30 haplotypes of *C. serratus* circulate in the groundnut basin of Senegal. This result is similar to those of Ndiaye (2014) with 37 haplotypes of *C. serratus* found on 46 Cyt-B sequences and Ndiaye (2018) with 26 haplotypes of *Sitophilus zeamais* obtained on 112 sequences of the same gene. Compared to the above-mentioned results of Ndiaye (2014) who sampled in four (4) countries, the difference observed in the number of haplotypes would be related to the size of the samples (153 against 46) and/or the number of countries sampled (4 against 1) ; compared to Ndiaye (2018) who sampled in nine (9) countries, this difference would be due to the nature of

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the specimen sequenced in his studies (*Sitophilus zeamais*). It is also important to remember that Cyt-B is a highly mutational mitochondrial gene with rapid evolution commonly used in the evaluation of genetic diversity in beetles (Alvarez *et al.*, 2003 ; Sembène *et al.*, 2010 ; Kébé, 2013 ; Ndiaye, 2014).

In addition, there is a high genetic diversity in all four (4) subgroups of populations except with CsA-1998 which has only three (3) haplotypes. Within each of the two (2) population groups (groundnut populations and global populations), genetic variation is higher in 2022 (with 24 and 14 haplotypes respectively on the 93 and 52 sequences of Pop-2022 and CsA-2022) than in 1998 (with 16 and 3 haplotypes respectively on the 60 and 34 sequences of Pop-1998 and CsA-1998). Here, the level of genetic variation obtained could be explained by genetic cycling in already infested granaries and storage stores (Sembène, 2000 ; Diome, 2010) but also by the time that elapsed between 1998 and 2022 (i.e. a temporal genetic variability lasting 24 years). These results are corroborated by the 19 haplotypes of Sembène (2004) found in the different biotypes of *C. serratus* encountered in Senegal, the 15 Senegalese haplotypes of Ndiaye (2014) for 18 Cyt-B sequences, the 16 and 18 haplotypes of Ndong (2015) respectively for 24 and 49 Cyt-B sequences and the 20 haplotypes of Sarr (2019) for 102 sequences of the same gene. In addition, in each of the four (4) subgroups of populations, eight (8) individual haplotypes were counted except for CsA-1998 which has none. This constant number of individual haplotypes could be linked to the proximity of the sampled localities but also to the scarcity or even the total absence of groundnut imports within the groundnut basin, which is a production area ; This scarcity or absence of imports would not only prevent the introduction of new haplotypes but would also promote the conservation of old ones.

Analysis of the nucleotide composition (mean nucleotide frequency) of Cyt-B showed that for all populations (groundnut populations, global populations and total population), the mean frequency in A+T (68.60%) is higher than that in G+C (31.40%), which is the rule in insects. Indeed, there is a high level of A+T saturation in the mitochondrial DNA of insects (Perna and Kocher, 1995 ; Pedersen, 2002). In addition, in the total population as well as in the two (2) population groups (groundnut populations and global populations), the Cyt-B gene reveals that transition-type mutations are more numerous than those of transversion-type mutations. These results are well in line with those obtained in other work on phytophagous insects such as *C. serratus* (Ndiaye, 2014), *S. oryzae* (Ndong, 2015) and *S. zeamais* (Ndong, 2015 and Sarr, 2019). According to Gibson and Muse (2002), transitions are less likely to change the nature of amino acids than transversions ; According to these same authors, transitions are more likely to be conserved at the coding region level than transversions.

The results obtained with the calculation of the mean numbers of nucleotide (K) differences show that within each of the two (2) groups, the 2022 populations are more heterogeneous (with K=37.712 and K=26.219 respectively for Pop-2022 and CsA-2022) than those of 1998 (with K=28.216 and K=25.781 respectively for Pop-1998 and CsA-1998). However, these K values are much higher than those obtained by Ndiaye (2018) between Sahelo-Sudanian and Guinean-Congolese populations of *Sitophilus zeamais* and by Lo (2021) between *Corcyra cephalonica* populations from the central groundnut basin and the Senegal River delta. The genetic differences observed (especially between global populations) could be justified by a possible shift in the food spectrum of groundnut seed-beetle between 1998 and 2022. In addition, many authors argue that there is at least as much genetic variability related to host choice in sympatric populations as there is between clearly isolated allopatric populations (Sembène, 2000).

The levels of haplotypic and nucleotide diversity highlighted in this study fall well within the standard ranges of genetic diversity of insect pests (Kébé, 2013 ; Ndiaye, 2014 ; Ndiaye, 2018 ; Lô, 2021). Indeed, in the whole as in each of the two (2) population groups (groundnut populations and global populations), there is a high haplotypic ($H_d > 0.5$) and nucleotide ($P_i > 0.005$) diversity with the Cyt-B gene. However, within each of these two (2) groups, the H_d and P_i indices are higher in individuals collected in 2022 (CsA-2022 and Pop-2022) than in individuals collected in 1998 (CsA-1998 and Pop-1998). This difference is simply explained by a higher level of genetic diversity in individuals in 2022 than in individuals in 1998.

4.2. Genetic structure of populations

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Any method aimed at eliminating or mitigating the impact of a harmful species on others necessarily requires a good knowledge of its structure (Kébé, 2013). Today, there are still many points to be elucidated about the genetic structuring of *C. serratus* populations. In this study, the large genetic distances noted within and between groundnut populations (CsA-2022 and CsA-1998) and global (Pop-2022 and Pop-1998) indicate that there is a statistically significant genetic differentiation, i.e. a clear difference in genetic structuring at the intra and inter-population levels (i.e. between the years 1998 and 2022). This difference would probably be linked to a genetic evolution of populations (between 1998 and 2022) in an unstable environment. Indeed, the sustainability of a population depends on its ability to adapt to a changing environment (Montaigne, 2011 ; Greenbaum *et al.*, 2014). In addition, in a changing environment, the ecosystem requires a group of individuals with varying genetic makeup (Usher, 2007).

In addition, in each of these two (2) population groups, the differentiation observed is not only greater in 2022 than in 1998 but also more pronounced between groundnut populations than between global populations. These results are significantly supported by the degree of genetic differentiation in the analysis of molecular variance ($F_{ST} = 0.20607$; $P = 0$). Indeed, according to Wright (1978), a F_{ST} between 0.15 and 0.25 reflects significant genetic differentiation. However, these results are not in line with that of Sarr *et al.* (2016) according to which there is no genetic differentiation (according to agro-ecological zones) between *Sitophilus zeamais* populations for Cyt-B. In the same vein, using microsatellite sequences, Sembène (2000) showed that intra-host genetic differentiation is not significant in *C. serratus*, which means that individuals dependent on the same host plant are genetically very close to the same host plant.

The AMOVA inferences suggest that 79.39% of the genetic variation observed on Cyt-B sequences is significantly explained by intra-population genetic differentiation ; it can be deduced that there is therefore a high molecular variance within the different populations of *C. serratus* (CsA-2022 ; CSA-1998 ; Pop-2022 and Pop-1998). This variance may result from the fact that Cyt-B is a rapidly evolving gene (Diome, 2014). On the other hand, 37.92% of genetic diversity is attributed to inter-population variation, which indicates a significant difference in genetic structuring between populations within the two (2) groups (groundnut populations and global populations), i.e. between the years 1998 and 2022. Finally, -17.32% variance is recorded between these two groups mentioned above, which means that there is no significant genetic differentiation between the two population groups. In addition, Kébé (2013) and Ndiaye (2014) showed that more than 73% and 80% of the molecular variance observed in *Callosobruchus maculatus* and *C. serratus* was explained by variation within populations for Cyt-B sequences, respectively. In addition, using microsatellite sequences of 16 populations of *C. serratus*, Sembène (2000) found that 32.5% of the total variability was due to genetic differentiation between samples.

5. CONCLUSION

Thanks to the Cyt-B gene, this part of the research is oriented towards the study of the diversity and genetic differentiation of different populations of *C. serratus* associated with groundnut and wild host plants between the years 1998 and 2022. The results of this study reveal a high level of genetic diversity with more mutations of the transition type than those of the transversionary type and a frequency in A+T higher than that in G+C ; Genetic variation is higher in 2022 than in 1998. There is also a high haplotypic and nucleotide diversity in *C. serratus* populations ; the Hd and Pi indices are higher in individuals collected in 2022 than in individuals collected in 1998. Thus, we can deduce that the mitochondrial Cyt-B gene is a good intraspecific marker. In addition, the large genetic distances found (within and between populations) indicate a statistically significant genetic differentiation ; i.e. a clear difference in genetic structuring at the intra- and inter-population levels, i.e. between the years 1998 and 2022. The AMOVA inferences suggest that most of the genetic variation observed on Cyt-B sequences is significantly explained by intra-population genetic differentiation.

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