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Comparative study of the genetic diversity of the cytochrome b gene of *Sitophilus zeamais* in 2 agro-climatic zones of West and Central Africa Sitophilus

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Abstract

This article aims to determine the genetic diversity of *Sitophilus zeamais*, a potential pest of maize stocks, in two different agro-climatic zones of West and Central Africa. The knowledge of the degree of genetic heterogeneity of each of these zones made it possible to apprehend the adaptive potential of the insect since the strong genetic diversity tends to increase the adaptation of a population while the genetic homogeneity presents effects inverse. To achieve this objective, some insects were sampled specifically from the humid and arid agro-climatic zone. The exploitation of 112 sequences of the cytochrome b gene of insects from these areas has made it possible to conclude that the genetic diversity is high in arid agro-climatic zones while it is low in humid agro-climatic zones. Thus the insects of the semi-arid zone would have more adaptive potentials than those of the humid zone.

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Introduction

For almost 2 decades, the reduction of poverty in the world, particularly in Africa, has been consecrated and relayed by international institutions. If this perspective is imperative for the avoidance of conflicts, immigration and development, it must be admitted that the decline of the poor requires a multisectoral approach. Since the tertiary sector, a real engine of viable growth is economically weak, the most successive governments in Africa have set their sights on the primary sector, where agriculture employs two-thirds of the working population (FAO, 2012). Unfortunately, African agricultural production is the victim of many constraints, including the deterioration of crops by insects. A variety of scientific studies have been commissioned and carried out to eliminate crop pests. We can cite the use of plants hostile to the blooming of insects: use of insecticide of *Azadirachta indica* on *Glossina fuscipes* (Makoundou *et al*., 1995), the use of pesticides. But the ineffectiveness of some and the negative impact of others on the environment (Hayo *et al*., 1996) should inspire some others studies, healthier and more effective. Our study falls within this perspective. Indeed, it aims to highlight the possibility of natural resistance of an agro-climatic zone on an insect through its effect on its genetic diversity. The principle underlying our thinking is that genetic diversity negatively influences the adaptive potential of a population (Frankham *et al*., 2002). For this, the maize, a cereal infested by *Sitophilus zeamais*, was chosen because of its socio-economic role in Africa. In each of the agro-climatic zones in question (humid and arid), some insects were collected and then stored. The sequences of the Cytochrome B gene corresponding to these insects were exploited by study software in population genetics (Bioedit, DNAsp, Mega, etc.), in relation to parameters of genetic variability (h, N, K, Pi, Hd, dn, ds, S, V).

Materials and methods

Sampling

Sampling localities: The individuals of *Sitophilus zeamais* were collected in nine (9) countries in the semi-arid and humid zones (White, 1983). These countries are Senegal, Mali, Burkina Faso, Guinea Conakry and Niger, belonging to the arid zone.

The other countries in the wetland where the harvest was made are Ghana, the Ivory Coast, Central Africa and Cameroon. Figs 1 and 2 summarize the sampling.

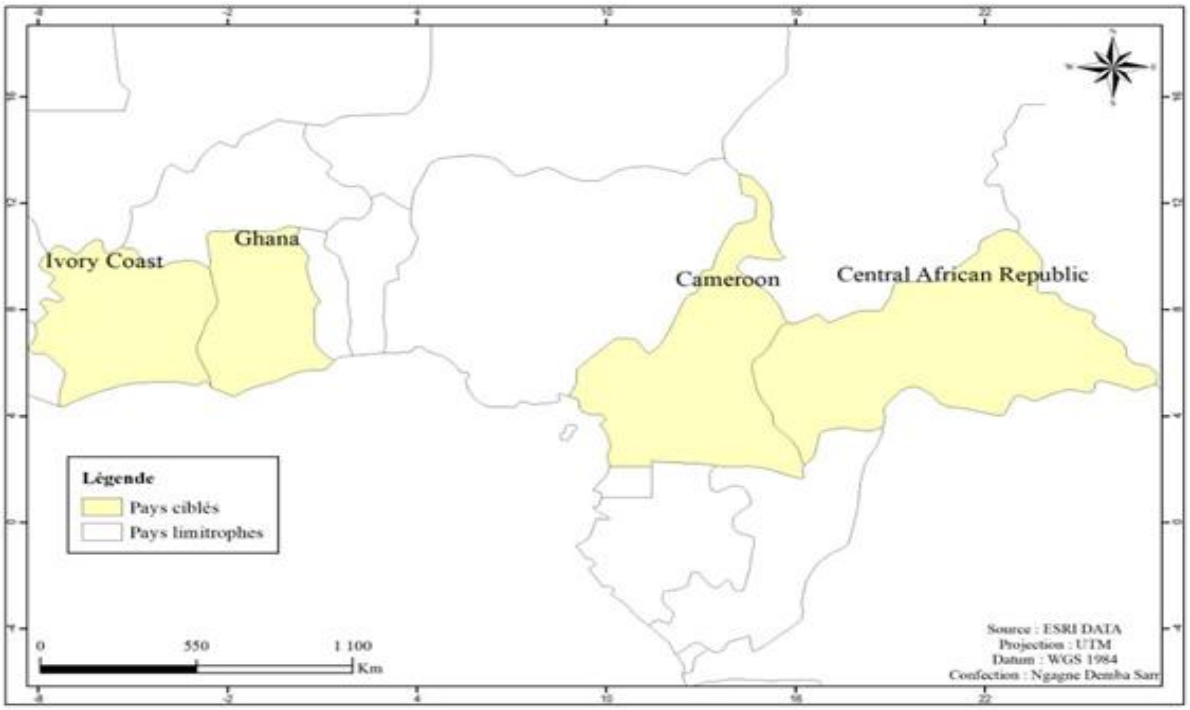

Fig. 1. Sampling country in the humid agroclimatic zone.

Harvest of individuals: In each of the above countries of the agroclimatic zones, some infested maize was collected from storage locations, through some partners of the project. The samples were taken to the laboratory where they are kept in jars with wire mesh covers for mass rearing. The insects collected from this breeding were stored in alcohol at 95 ° C, then transported to the laboratory for a genetic study. Each sample is identified by a code: the first 2 letters designate the binomial name of the species (S for *Sitophilus* and z for *zeamais*), the 2 letters which follow indicate the country of origin (example: SzSn, with S = *Sitophilus*, z = *zeamais*, Sn = Senegal, SzBf, with S = *Sitophilus*, z = *zeamais*, Bf = Burkina Faso.

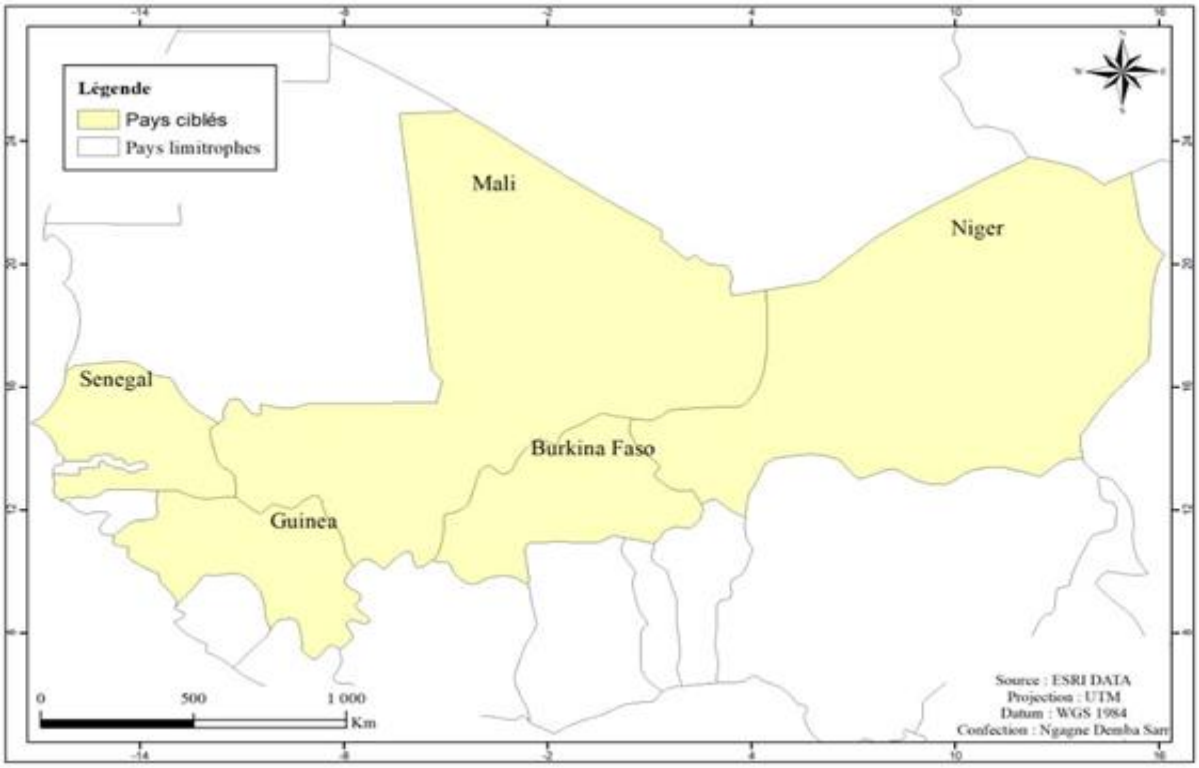

Fig. 2. Sampling country in the Arid Agroclimatic Zone.

Molecular method of analysis

The cytochrome B gene was chosen to be amplified. The choice is explained by its peculiarity to keep for a very long time without wear and it is used regularly in studies of insects. (Hillis *et al.*, 1996). DNA extraction: The extraction is the technique of releasing DNA from the cell. It includes the individualization of cells (digestion) and the destruction of their plasma and nuclear membranes (lysis). The digestion of the cells consisted of placing their legs and prothorax in tubes containing ATL buffer and proteinases K. After incubation, the tubes were centrifuged to separate the supernatant from the cell debris. To destroy cell membranes first cell lysis buffer (LA) was added, then ethanol (96%) after incubation, in the tubes. Then the tubes are crossed in columns with a silica membrane. Finally, the centrifugation of the tubes made it possible to retain the DNA on the siliceous membranes of the columns because it was negatively charged.

DNA purification: The DNA from the tubes was purified by adding 2 buffers AW1 and AW2 to each column. After centrifuging the tubes and precipitating the DNA from the bottom, the buffers and contaminants are discarded. The columns are then placed in other tubes in which the AE buffer has been added to detach the DNA. The DNA is thus removed and stored at -20 ° C.

PCR of the mitochondrial Cytochrome B gene: The PCR of the mitochondrial gene Cyt.B was carried out by 2 primers defined by Simon *et al.* (1996). For each sample (tube), amplification was made from a total

volume of 25µl, including a mixed volume of 23µl and a volume of 2µl of DNA extract. The mixed volume was constituted by 18.3 µl of milli water, 2.5 µl of 10X buffer, 1 µl of additional Mgcl2, 0.5 µl of Dntp, 0.25 µl of each primer and 0.2 µl of Taq polymerase.

Bioinformatics analyzes: The sequences were corrected and aligned by the Clustal software installed in the Bioedit version 7.2.5 programs (Hall, 1999). The evaluation of sequence diversity was made on the basis of certain parameters of genetic variability. These are, on the one hand, the standard indices which are among others the variable sites in parsimony and singleton, the number of haplotypes (h), the average number of nucleotide difference (k), the transition percentage (S) and transversion (V), non-synonymous (dn) and synonymous (ds) substitutions, the mutation rate (R) and on the other hand the Haplotypic (Hd) and nucleotide (Pi) diversity. These two indices have the particularity of highlighting the diversity and divergence of haplotypes. The parameters h, k, Hd, Pi were calculated by the DNAsp ver software. 5.10.01 (Librado and Rozas, 2009). While those such as dn, ds, S, V and r have been estimated by MEGA7 software ver. 7.0.18 (Kumar *et al.*, 2016).

Results

The table and the figure summarize some parameters of genetic variability of *Sitophilus zeamais* populations in 2 agroclimatic zones of West Africa, namely the semi-arid zone and the humid zone.

Table 1. Identification of the primers used and programming of the PCR.

Gene	Primer Names	Primer Sequences	PCR Program
Cyt.B	CB-J-10933(F)	5-TATGTACTACCATGAGGACAAATATC-3	1. Initial denaturation : 94°C, 3 min ; 35 denaturation cycles : 94°C, min
	CB-N-11367(R)	5-ATTACACCTCCTAATTTATTAGGAAT-3	2. Hybridization: 47°C, 1 min
			3.Elongation : 72°C, 2 min ; elongation finale : 72°C, 8 min

The haplotypic diversity of the semi-arid zone (0.820 ± 0.045) is higher than that of the humid zone (0.011 ± 0.002). The strong haplotypic diversity of the semi-arid zone is mainly due to the Niger and Burkina Faso populations. Their respective values are 21% and 20% of the total percentage. The low haplotypic diversity of the wetland is mainly due to the insects of the Ivory Coast (6%). The nucleotide diversity presents the same evolution. It is higher in the Sahelian zone (0.342 ± 0.082) than in the humid zone (0.005 ± 0.001). The high nucleotide diversity of the semi-arid zone is explained by the strong genetic diversity of the populations of this zone except in Guinea. The other genetic diversity parameters confirmed the greater genetic diversity of the arid agro-climatic zone compared to the humid agro-climatic zone.

Table 2. Other parameters of genetic diversity.

	Genetic Diversity Parameters										
	n	h	N	K	Dn/ds	S	V	R	Monomorphics sites	Variables sites	
Countries of Humid Climatic Zone										Singletons	Parcimony
Ivory Coast	15	2	442	0,248	3,60	99,68	0,34	322,4	441	0	1
Ghana	10	5	442	5,131	3,61	33,34	66,68	1,152	431	1	10
Centrafrique	20	1	442	0,000	3,60	57,3	42,7	0,451	442	0	0
Cameroon	7	1	442	0,000	3,60	33,34	66,68	0,451	442	0	0
HCZ	52	6	442	2,372	3,62	88,96	11,04	7,594	430	1	11
Countries of Arid Climatic Zone											
Senegal	20	8	442	4,932	3,63	66,54	33,46	1,83	420	12	10
Mali	10	1	442	0,000	3,60	33,33	66,66	0,451	442	0	0
Niger	10	6	442	7,089	3,62	31,88	68,12	0,299	420	9	13
Burkina Faso	10	7	442	5,911	3,60	58,71	41,3	0,977	427	5	10
Guinea	10	4	442	1,689	3,61	76,97	23,02	3,262	436	4	2
ACZ	60	22	442	4,99	3,62	61,31	38,68	1,353	391	23	28

n=number of individuals, h= numbrer of haplotypes, N= number of sites, K= average number of nucleotide differences, dn= non-synonymous type substitution, ds= synonymous type substitution, S= transition percentage, V= transversion percentage, R= mutation rate.

Even if the size of the 2 populations is not significantly different quantitatively, with 60 individuals in the arid zone and 52 in the humid zone, the number of haplotypes (h) is much higher in the Sahelian zone (n = 22 against 6 in a wet area). The average number of nucleotide differences (K), the number of variable sites, the percentage of transversion (V) are higher in the Sahelian zone than in the Guinean zone. On the other hand, the mutation rate (R), the transition percentage (S) are more important in humid zones.

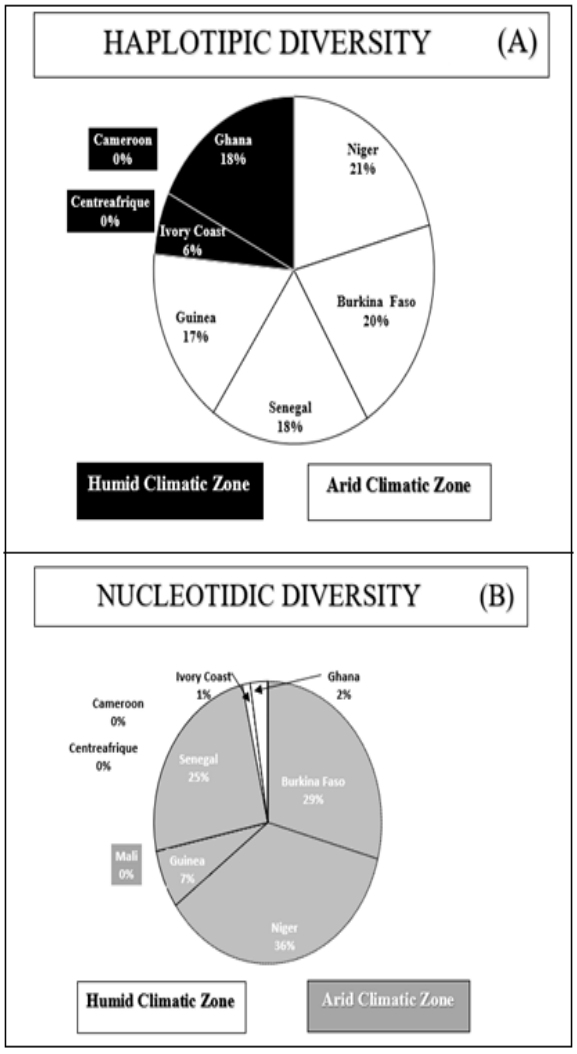

Fig. 3. Haplotypic (A) and nucleotide (B) diversity of *S. zeamais* in humid and Sahelian zones.

The haplotype distribution map indicates that the 2 agro-climatic zones share haplotype 10 in common, which is, therefore, the majority and regional haplotype. The other haplotypes are deprived of either agroclimatic zone. So there is little exchange of individuals between them.

Discussion

The knowledge of the degree of genetic heterogeneity of a biogeographic ensemble like these is very important insofar as it provides some information on the adaptive potential of living beings in the face of natural extinction factors. Indeed, according to Soulé (1987) and Frankham (1990), the level of genetic diversity of a population is a key factor for the long-term survival of the species and that its alteration can jeopardize its adaptability. Thus, the genetic diversity parameters of the two populations of agro-climatic zones were apprehended.

The values found are in line with those of Assane Ndong (2015) of the same insect, in Senegal and Guinea, but also with those of other insects living in climatic conditions similar to one or other of the agro-climatic zones in question. These are insects like *Busseola fusca* (Sézonlin *et al.*, 2006), Callosobruchus *maculatus* (Kébé, 2016). For the two populations of approximately equal size in the two zones, almost all of the genetic variability parameters highlighted a greater genetic diversity in the arid agroclimatic zone than in the humid agroclimatic zone. Knowing that these biogeographic sets are substantially different from ecological factors, we can conclude that precipitation, temperature, humidity ... would be at the origin of this difference in genetic diversity of the corn weevil in Central and West Africa. The high genetic diversity of the arid zone is mainly due to the significant genetic differentiation of insects from Burkina Faso and Niger. Indeed, these 2 countries compared to the others are characterized by a lower rainfall and a more arid climate. There are a cause and effect relationship between the level of genetic diversity of a population and its capacity for resilience. Thus the strong genetic variability of *Sitophilus zeamais* in an agro-climatic zone could increase its adaptive potential, as was the case with populations of *Zostera marina* which, possessing a greater genetic diversity compared to other populations, also have greater resistance high to disturbances (Hughes *et al.*, 2004; Reusch *et al.*, 2005). This is also the case with the study by Crutsinger *et al.* (2006) who showed a positive

relationship between the number of genotypes present in a population of goldenrods (*Solidago altissima*) and the primary production of the system. In the arid zone (Senegal), which is precisely the subject of this study, Simard *et al* demonstrated that the population of *Anopheles Arabiensis* from Barkedji, which is characterized by high genetic diversity (high rate of heterozygosity and allelic richness) is not subject to a bottleneck. But it is important to note that despite the positive correlation that exists between the genetic diversity and the adaptive potential of individuals, a correlation confirmed by several studies including those cited above, there are exceptions. Thus, McKay *et al.* (2001) for example showed in a study of Brassicaceae *Arabis fecunda*, that there was no link between the effective size and the adaptive potential. On the other hand, for the same principle, the low genetic diversity of the humid agro-climatic zone could be detrimental to the adaptive capacities of the insect.

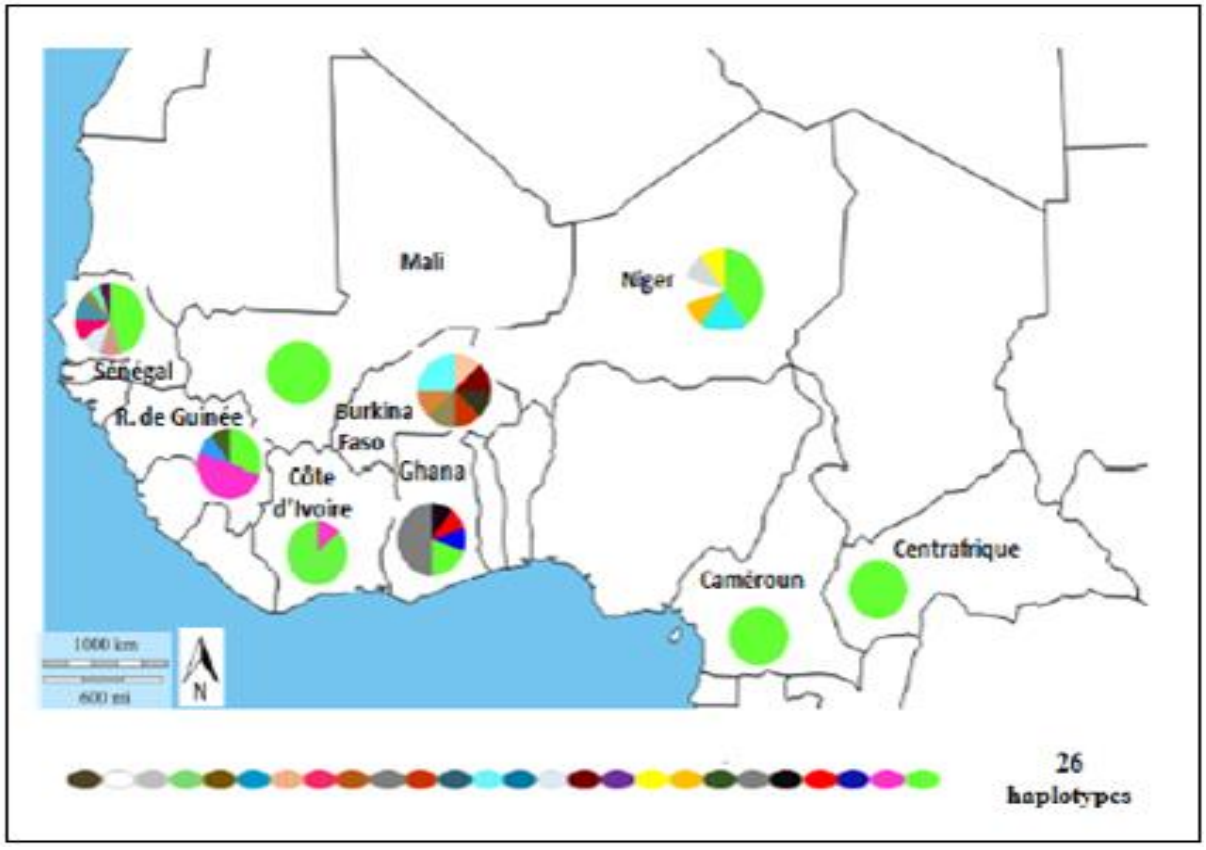

Fig. 4. Distribution of haplotypes in humid and arid zones.

A lack of heterozygotes has been shown to negatively influence the survival of living things.

According to Reed *et al.* (2003), a population limited in genetic diversity is less able to respond to changes in its environment and is more likely to become extinct. *Acinonyx jubatus* (Cheetah) is compromised due to its low genetic diversity (O'brien *et al.,* 1985). But the genetic homogeneity is not always the work of ecological factors. Indeed, the bottlenecks that are demographic collapses drastically reduce genetic variability (Mayr, 1963).

Conclusion

The study highlighted a high genetic diversity of *Sitophilus zeamais* in arid agro-climatic zones and low in humid agro-climatic zones. Knowing that the degree of homogeneity can influence the adaptability of a population, we can conclude that the insects of the arid zone would have more adaptive potentials than those of the wet zone.

References

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC.

1987. Intraspecific phylogeography: the mitochondrial DNA Bridge between population genetics and systematics.

Avise JC. 1994. Molecular Markers, Natural History, and Evolution. Chapman and Hall, (New York)., p 511.

Bossart JL, Prowell DP. 1998. Genetic estimates of population structure and gene flow: limitation, lessons, and new directions. Trends Ecology Evolution **13,** 202e206.

Delobel B, Grenier AM. 1993. Effect of non-cereal food on cereal weevils and tamarind pod weevil (Coleoptera: Curculionidae). Journal of Stored Products Reseach **(29),** 7–14.

Delobel A, Tran M. 1993. Les coléoptères des denrées alimentaires entreposées dans les régions chaudes, Paris, éditions ORSTOM. p 425.

Delobel A. 1995. The shift of *Caryedon serratus* Ol. From wild Caesalpiniaceae to groundnuts took place in West Africa (Coleoptera : Bruchidae). Journal of Stored Products Research **31(1),** 101-102.

Moyal P. 1994. Maize Cob-borer abundance and influence on yield in côte d'ivoire. International Journal of Tropical Insect Science **15(4-5),** 469-478. http://dx.doi.org/10.1017/S1742758400015836.

Moyal P. 1993. Maize crop Intensification and borer attacks in the Ivory Coast. Yields. Soil Boita, Nutvient cycling, and Farming Systems, CRC Press, Boca Raton, FL, chap. **22,** p 254.

Ndong A. 2015. Caractérisation génétique de *Sitophilus spp*., ravageur des stocks de maïs et essais d'éradication du virus de la mosaïque du manioc Manihot esculenta par la culture de tissus in vitro. Thèse de Doctorat. Université Cheikh Anta Diop de Dakar (Sénégal), p 314.

Reed DH, Frankham R. 2003. Correlation between Fitness and Genetic Diversity. Conservation Biology **17,** 230-237. http://dx.doi.org/10.1046/j.15231739.2003.01236.X.

Sarr AGRJ, Dia CAKM, Ndiaye MR, Sembène M. 2016. Genetic Structure of Two Sitophilus (Coleoptera, Curculionidae) Species According to Storage Infrastructures and Agro-ecological Areas. University Cheikh Anta DIOP of Dakar. International Journal of Science and Advanced Technology **6(4),** 1-11.
(ISSN 2221-8386).

Sembène M. 2000. Variabilité de l'Espaceur Interne Transcrit (ITS) de l'ADN ribosomique et polymorphisme des locus microsatellites chez le bruche *Caryedon serratus* (Olivier, 1790): différenciation en races hôtes et infestation de l'arachide au Sénégal. Thèse de Doctorat d'Etat es Sciences Université Cheikh Anta Diop de Dakar (Sénégal), p 180.

Sembène M, Kébé K, Delobel A. 2012. Effet structurant de la plante hôte chez le bruche de l'arachide, *Caryedon serratus* (Olivier, 1790) (Coleoptera : Bruchidae). Biotechnologie Agronomie Societe Environment **16(1),** 3-11.

Sembène M. 2012b. De la naissance des concepts à la génétique évolutive des populations. Cours de master II de spécialité « Génétique des populations ». Université Cheikh Anta DIOP de Dakar.

Sezonlin M, Dupas S, Moyal P, Calatayud PA, Giffard I, Faure N, Silvain JF. 2006. Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa. Molecular Ecology **15,** 407e420.

Tamgno BR, Ngamo T. 2018. Potentialisation de l'efficacité insecticide des. poudres de feuilles ou amandes de neemier *Azadirachta indica* A. juss par formulation avec la cendre de tiges de mil contre *Sitophilus zeamais* Motsch. et *Sitophilus oryzae* L(coleoptera : curculionidae). African Journal of Food Agriculture Nutrition and Development **18(1),** 13254-13270.
http://dx.doi.org/10.18697/ajfand.81.17095.