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Full Length Research Paper

DNA successions and microsatellites loci recount field groundnut infestation via Caryedon serratus Ol.

***Idrissa E. Evra, Ousmane Maal, Safi B. Diome and Lamine I. Sylva**

Department of Cell Biology Faculty of Microbiology, Mansoura University, Mansoura, Egypt.

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The first infestations of stored groundnuts by the seed-beetle *Caryedon serratus* were reported in this country at the turn of the 20th century. This bruchid has a wide distribution in Africa, from Senegal to South Africa and in southern Asia. Native hosts of *C. serratus* in Senegal include *Bauhinia rufescens*, *Cassia sieberiana*, *Piliostigma reticulatum* and *Tamarindus indica*, all of which belong to the legume subfamily Caesalpinioideae. Molecular marker, DNA sequences and microsatellites loci polymorphism were used to investigate the mechanisms of first groundnut infestation by *C. serratus*. Sequence analysis of ribosomal DNA nuclear (ITS1) and mitochondrial coding DNA (Cytochrome b) reveal several biotypes in Senegal, with restricted past and/or present gene flow between each other. Samples typically clustered according to host plant, except for groundnut and *P. reticulatum*, which clustered together. Polymorphic microsatellites loci confirm the allelic proximity between *P. reticulatum* and groundnut *C. serratus*. These strains, genetically very close, begin however to diverge but the number of migrants between them keeps relatively important. Historical hypothesis of the first groundnut infestation in West Africa is also debated in this study.

Key words: groundnut, *Caryedon serratus*, *Piliostigma reticulatum*, groundnut, infestation, ITS, Cyt. B, DNA sequences, microsatellite loci.

INTRODUCTION

The disruption of biological communities creates several problems for people and can greatly affect productivity and profitability of agricultural systems. Also, conservation of food stored by the appearance of species or biotypes from other regions or agro-ecological zones creates several problems, but by increase populations of native species favored by global change. Nowadays, production of groundnut is provided to more than 85% of small farms in Africa and Asia. The conservation of crops ensures the availability of food resources which is a key factor in the food security of a country. Unfortunately, agricultural production is generally seasonal, while the consumer needs are extended throughout the year. Thus, the establishment of an adequate policy to keep the plant populations from the risks of food shortages during the off-season farming is the most important thing that developing countries should achieve. In this context, particular emphasis should be placed on controlling insect pests of crops in stocks because damage caused by insects can lead to financial loss, hunger and risks associated with intoxication consumption or damaged goods. As such, crops should be treated with pesticides

*Corresponding author. E-mail: evra.idrissa26 @ird.fr.

(Zuoxin et al., 2005). In countries like those in the Sahel, where the dry season lasts most of the year, storage of crops is a matter of survival.

Groundnut (*Arachis hypogaea*) is an herbaceous, annual legume, originating in Peru. Currently, it is cultivated in sub-tropical savanna regions in sub-saharan Africa, USA, Middle East and Asia. It was introduced into Africa from Brazil by the Portuguese at the end of the 15th century (Adrian and Jacquot 1968; Perhaut, 1976). The grain contains 38 - 50% oil and is also rich in protein. It is consumed by humans as grain, flour, paste and oil. Groundnut cake and foliage are used as animal fodder and the oil is also used in soap production.

In Africa, the first infestations of stored groundnuts by the bruchid *Caryedon serratus* (Olivier) were reported in this country at the turn of the 20th century (Davey, 1958; Delobel, 1995). *C. serratus* has a wide distribution in Africa, from Senegal to South Africa and in southern Asia (Johnson, 1986) . Its larvae feeds on the seeds of wild Caesalpiniaceae belonging to four genera: *Bauhinia, Cassia, Piliostigma* and *Tamarindus* (Borowiec, 1987). The groundnut seed-beetle is responsible for important weight losses in stored groundnuts, thereby reaching about 83% in four months storage in Senegal (Ndiaye, 1991). About 60 years after its first record as a pest of groundnut in West Africa (Roubaud, 1916), it has become a major primary groundnut pest in Central Africa (Matokot et al., 1987) and Asia (Dick, 1987). It was also recorded in Central and South America in the seeds of ornamental *Bauhinia.* Its larvae bore through groundnut hulls and favour attacks by secondary pests. In the same time, they favour the spread of *Aspergillus flavus*, a mould which produces a toxic substance, aflatoxin (Gillier and Bockelée-Morvan, 1979). Groundnut infestation by the seed-beetle puts the question of the mechanisms by which *A. hypogaea*, a plant of the family Fabaceae, became part of the insect's range of hosts.

Food-plant selection and larval development researches (Robert, 1985) as well as morphometric (Sembène and Delobel, 1996), allozyme variation (Sembène et al., 1998; Sembène and Delobel, 1998) and DNA sequences (Sembène et al., 2008 and 2010) provided support to the hypothesis that a certain amount of genetic isolation exists between populations with different feeding habits, and in particular, between groundnut- feeding and Caesalpiniaceae-feeding forms. In this study, we are particularly interested in the historic and present modalities of the infestation of the groundnut dried in fields. Our primary question was whether groundnut-infesting populations of *C. serratus* represent a newly formed host race of the species. In evolutionary biology, it is evident that the differentiation in host races is a phenomenon widely discussed to phytophagus insects. Indeed in a lot of case, it was observed after the introduction of a new plant. We are also interested in knowing the intensity of gene flow and migrants between the beetles on *Piliostigma reticulatum* and those on groundnut dried in the field.

MATERIALS AND METHODS

C. serratus sampling

Beetles were sampled from eggs, larvae or pupae on/inside pods collected from the different host trees between 2005 and 2010. Samples were collected in Bignona (16°13'W, 12°48'N), Fimela (16°41'W, 14°08'N), Keur Baka (15°57'W, 13°56'N), Linguere (15°25'W, 15°88'N), Ouarak (16°04'W, 15°33'N) and Thies (16°56'W, 14°48'N). Samples were named after their host plant species and geographic origin (Table I) . Pods were collected from the trees between January and May. Groundnut samples were collected from the field during drying. Adult beetles were genetically analysed almost immediately after they emerged from the pods. In all, a total of 1200 individuals of both sexes corresponding to 30 different populations with 40 individuals per population were genetically scored in the study.

DNA extraction and PCR

DNA was extracted, amplified and sequenced with standard protocols described elsewhere (Sembène et al., 2010). Each sequence was obtained from the DNA of a single seed- beetle. The abdomen, elytra and antennae were kept apart to avoid contamination by fungi and nematodes and to permit subsequent morphological observations.

A partial Cytochrome B (Cyt. B) end region was PCR-amplified using the primers CB1 (5' – TATGTACTACCATGAGGACAAATATC - 3') and CB2 (5'-ATTACACCTCCTAATTTATTAGGAAT - 3') . The ITS1 ribosomal DNA was targeted for PCR, amplified and sequencing with primer CIL (5' GCGTTCGAARTGCGATGATCAA 3') and CIU (5'GTAGGTGAACCTGCAGAAGG3').

For both markers, PCR amplification were performed in 25 µl reaction volume 2.5 µl enzyme buffer supplied by the manufacturer, 2.5 mM $MgCl_2$, 0.6 unit of Taq polymerase (Promega), 17.5 pM of each primer, 25 nM of each DNTP and 1µl of DNA extract. After an initial denaturation step at 92°C for 3 min, reaction were subjected to 35 cycles for 1 min at 92°C, 1 min 30 s at 48°C and 1 min at 72°C. PCR products were loaded on a 1.3% agar gel. The PCR band was cut and then purified with Quiaquick PCR gel purification kit (Qiagen) and directly sequenced on an Abi 373 automated sequencer using TaqFS and Dye-labeled terminators (Perkin-Elmer).

Radioactive polymerase chain reaction (PCR) amplifications of microsatellite loci were carried out on 10 µl as previously described in Estoup et al. (1995). Ten microsatellite loci were scored: M193, M2149, M66, M625, M836, M97, M984, M12o mega, M13113 and M1425. As such, primer sequences and annealing temperatures were used for each locus (Sembène et al., 2003).

Sequence alignment and phylogenetic analyses

Sequence alignment was performed using ClustalW (Thompson et al., 1994) as implemented in BioEdit. Alignment of coding sequences (Cyt. B) was unambiguous as no gap event was detected. For ITS1, we proceeded to several multiple alignments using ClustalW under different gap opening and gap extension coast. Aligned sequences were finally entered in McClade 3.06 (Maddison and Maddison, 1992) for subsequent treatments.

The resulting data set was used to calculate Kimura 2-parameter genetic distances between the haplotypes. G-tests (log-likelihood ratio test) were performed to test for genetic distance homogeneity among hosts at the same site and among sites for the same host(Sokal and Rohlf, 1981).

Phylogenetic relationships were reconstructed with PAUP $4^*{}_B8$ (Swofford, 2001) using the maximum parsimony method (MP). The MP analysis was carried out with the heuristic search option with 50

Table 1. Population sampling: origin, host plant, abbreviations and numbers of scored individuals (between parentheses).

Sites and coordinates	Sampling plants, abbreviations of populations and number of scored individuals									
	A. hypogaea		***B. rufescens***		***C. sieberiana***		***P. reticulatum***		***T. indica***	
Bignona (16°13'W, 12°48'N)	Abi	(40)	Bbi	(40)	Cbi	(40)	Pbi	(40)	Tbi	(40)
Fimela (16°41'W, 14°08'N)	Afi	(40)	Bfi	(40)	Cfi	(40)	Pfi	(40)	Tfi	(40)
Keur Baka (15°57'W, 13°56'N)	Akb	(40)	Bkb	(40)	Ckb	(40)	Pkb	(40)	Tkb	(40)
Linguere (15°25'W, 15°88'N)	Ali	(40)	Bli	(40)	Cli	(40)	Pli	(40)	Tli	(40)
Ourack (16°04'W, 15°33'N)	Aou	(40)	Bou	(40)	Cou	(40)	Pou	(40)	Tou	(40)
Thies (16°56'W, 14°48'N)	Ath	(40)	Bth	(40)	Cth	(40)	Pth	(40)	Tth	(40)

In Bignona, Abi was obtained from *A. hypogaea*, Cbi from *C. sieberiana*, Pbi from *P. reticulatum* and Tbi from *T. indica.* In Fimela, sample Afi was obtained from *A. hypogaea*, Bfi from *Bauhinia rufescens*, Cfi from *Cassia sieberiana*, Pfi from *P. reticulatum* and Tfi from *T. indica*. In Keur Baka, Akb was obtained from *A. hypogaea*, Ckb from *C. sieberiana*, Pkb from *P. reticulatum* and Tkb from *T. indica*. In Ouarak, Aou from *A. hypogaea*, Bou from *B. rufescens*, Pou from *P. reticulatum* and Tou from *T. indica*. In Linguere sample Ali was obtained from *A. hypogaea*, Bli from *Bauhinia rufescens*, Cli from *Cassia sieberiana*, Pli from *P. reticulatum* and Tli from *T. indica*. In Thies: Ath from *A. hypogaea*, Pth from *P. reticulatum* and Tth from *T. indica*.

random stepwise taxon addition replicates, using the branch swapping tree bisection-reconnection (TBR) option. A bootstrap procedure (1000 iterations with the same option of heuristic search) was used to establish the score of each node (Felsenstein, 1985) by retaining group compatible with the 50% majority rule consensus. A strict consensus tree was computed whenever multiple equal parsimonious trees were obtained.

The molecular clock hypothesis was tested following Possada and Crandall (2001) by comparing the log likehood scores of the bests trees obtained with molecular clock enforced and no enforced. The Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) using reel Bootstrap with 1000 replications was used to test for a significant difference between the score of the different trees obtained.

Analyses were also conducted using the distance-matrix method with the Neighbour-Joining (NJ) algorithm (Saitou and Nei, 1987) on Kimura 2- parameter distances with PAUP 4*$_B$8 (Swofford, 2001). The robustness of inferences was assessed through bootstrap resampling (1000 replicates). The consistency index CI and the retention index RI (Farris, 1994) were also calculated. The two analyses were made separately with Cyt.B and the ITS1. The partition homogeneity test (Farris, 1994), as implemented in PAUP was used to determine the appropriateness of combining both partial Cyt.B and ITS1 genes into a single analysis with the same options. The Wilcoxon's signed-rank test (Templeton, 1983) was applied to compare the statistical significance of the best tree produced by each tree reconstruction method to one another. Two out groups were used in our study: *Bruchidius atrolineatus* which belongs to the family Bruchidae and *Caryedon gonagra* obtained from *Bauhinia variegata* from Egypt (BvC). *C. gonagra* (F.) is a species which may be synonymous with *C. serratus* (Delobel et al., 2003).

Polymorphic microsatellite data

Quantitative analysis of microsatellite data, tests for Hardy-Weinberg equilibrium and genotypic linkage disequilibrium were computed using Genepop V1.2 (Raymond and Rousset, 1995). In both cases, Fisher's exact test available in Genepop V1.2 was used after pooling the data by sample for a given locus and by locus for a given sample. G-tests (log-likelihood ratio test) were performed to test allele frequency homogeneity among hosts at the same site and among sites for the same host (Sokal and Rohlf, 1981). Fstat V1.2 (Goudet, 1995) was used to compute Weir and Cockerham's (1984) estimators f and □ of F-statistics (Wright, 1931) in order to evaluate sub-structuration among hosts and sites. Parameter f (consanguinity coefficient) corresponds to Wright's F_{IS}. Parameter □ (degree of genetic differentiation between populations) corresponds to Wright's F_{ST}. Multiple test significance was assessed using Fisher exact test. The numbers of migrants (F1 and F2) between *C. serratus* feeding on the native hosts and the groundnut form is evaluated using Strcture.exe. Relationships among populations based on gene frequencies were displayed using the neighbour-joining algorithm (Saitou and Nei, 1987) and the mean chord distance (Cavalli-Sforza and Edwards, 1967). This distance is one of the most efficient for obtaining the correct tree topology under both the stepwise mutation model (SMM) and the infinite allele model (IAM) (Takezaki and Nei, 1996). Node stability was evaluated using 1000 bootstrap replicates (Hedges, 1992) re-sampling loci and/or individuals and majority-rule consensus trees were obtained using procedures "SEQBOOT" and "CONSENSE" in Felsenstein's (1993) PHYLIP V3.57c package. Swofford and Berlocher (1987) frequency parsimony program (FREQPARS) was used to build trees based on a modified Wagner algorithm (Farris, 1970) and also to compare the different topologies obtained. A strict consensus tree was computed after NJ and FREQPARS analysis. Tree of individuals (only with samples reared from *P. reticulatum* and groundnut) were constructed with Population.exe using the DAS (shared allele) distance (Chakraborty and Jin, 1993) using the distance-matrix method with the neighbour-joining algorithm. The robustness of inferences was assessed through bootstrap re-sampling (1000 replicates). For this tree, a classification index was calculated to establish the validity of the clusters of individuals on tree branches according to their membership to a known population. An individual is 'well classified' when it is attached to his group of origin (Estoup et al., 1994).

RESULTS

Alignment and genetic distance

We obtained 518 bp of the partial Cyt. B gene in 30 *C. serratus* populations. The alignment was straightforward and involved no insertions/deletions. The sequences could be unambiguously aligned and showed 22 different haplotypes due to 51 polymorphic sites. Of these sites, 94% were parsimony informative. The number of

nucleotide differences in pairwise comparisons of *C. serratus* populations ranged from 0 to 16.1% due to a large part of *C. serratus* sampled on *C. sieberiana* and the others. Within the same host species, the number ranged from 0 to 0. 02%.

Sequences obtained from ITS1 domain were 954 bp in *C. serratus* feeding on *C. sieberiana* and 879 bp feeding on the others including, in both cases 6 bp in 18 S and 49 bp in 5.8 S. Only 68.2% of the total could be aligned between both groups. Of these sites, 35.9% were variable and 89% of these were parsimony informative. The number of nucleotide differences in pairwise comparisons *C. serratus* populations ranged from 0 to 34, 6%. Within the same host species, the number ranged from 0 to 0. 03%.

Among the *C. serratus*, the genetic distances measured on the total alignment of Cyt.B + ITS1 (1418 bp) ranged between 0 and 0.204, but clearly fall in two classes: One group comprises haplotypes sampled in *A. hypogaea*, *B. rufescens*, *P. reticulatum* and *T. indica* from 0 to 0.032 and a second group gathers the haplotypes raised from *C. sieberiana* from 0.16 to 0.204; separating *C. serratus* into two major groups. The original data set was reanalysed without *C. serratus* sampled in *C. sieberiana*. Within host genetic distance decreased and was low: 0 - 0.004 for "groundnut", 0 - 0.002 for "Bauhinia", 0 – 0.002 for "Piliostigma*"* 0 – 0.002 for "Tamarindus". Genetic distance between hosts (0.036) decreased but remained significant (G-test $p < 0.01$). Genetic distance between localities was non-significant.

Phylogenetic relationships

The maximum parsimony (MP) analysis on Cyt.B nucleotide data yielded 32 equally parsimonious trees that were 274 steps long (CI = 0.882; RI = 0.785). The same methods on the ITS1 data set yielded 7 equally parsimonious trees, 263 steps long (CI = 0.967; RI = 0.992). Finally, analysis of the combined data set yielded 178 steps long (CI = 0.932; RI 0.988). Similar patterns of relationships were obtained with Neighbour-joining (NJ) analysis. Samples typically clustered according to host plant, except for groundnut and *P. reticulatum*, which clustered together. *C. sieberiana* samples were clearly separated from all other samples and showed high bootstrap values. Bootstrap values were all over 50%. Separation according to host plant is clear. For each data set, the topology obtained with MP and NJ methods were compared using the Shimodaira-Hasewaga test and the Wilcoxon's signed-rank test. These tests did not support the significant difference between the trees. The strict consensus tree of MP and NJ trees is presented in Figure 1 for the pooled data.

Polymorphic microsatellites

The microsatellite loci M12omega failed to sustain reliability. M66 and M13113 proved to be monomorphic. Only M625, M836, M97, M984 M193, M2149 and M1425 showed scoreable polymorphic loci.

The number of alleles varied from 4.8 to 6.9 (mean 5.85). The difference between allele frequencies at a given locus was lower between geographically distant samples from the same host plant than between sympatric samples from different host plants. All individual populations were in Hardy-Weinberg equilibrium at all loci with the exceptions of Ali and Afi. Deviation was due to a deficiency of heterozygotes ($0.23 < f < 0.67$). Over all loci, deviations from Hardy-Weinberg expectations occurred in populations from the same locality ($p < 0.01$).

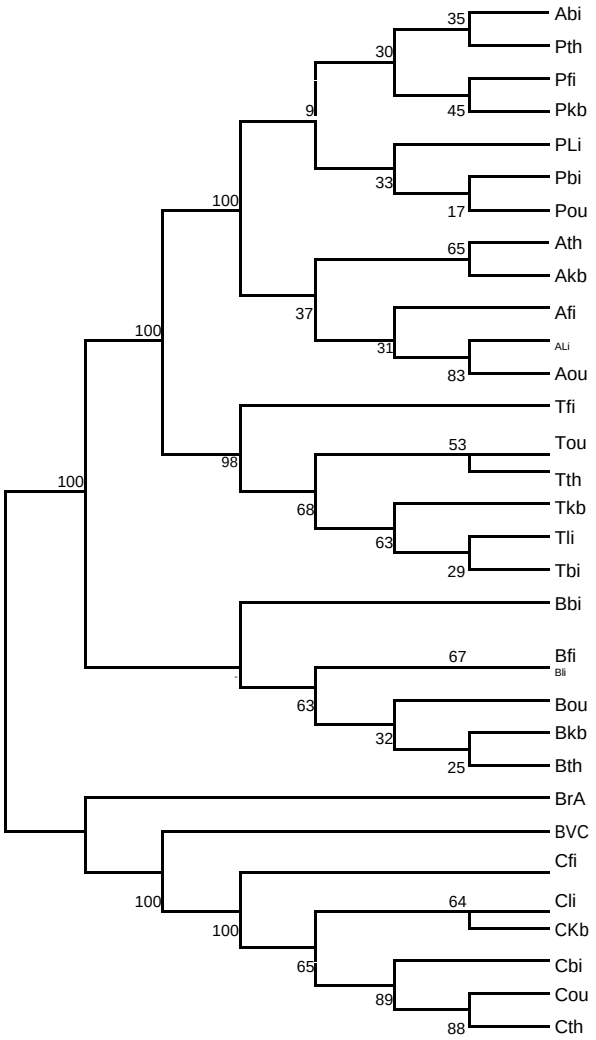

Figure 1. Phylogenetic relationships among nucleotide sequences of the pooled data (partial cytochrome b and ITS1 genes) of 30 specimens of *C. serratus* populations. This tree is the consensus between maximum parsimony (MP) consensus tree and neighbour- jonning (NJ) consensus tree. The numbers above/under branches are the means of MP and NJ % bootstrap values (1,000 replicates). *Bruchidius atrolineatus* (BrA) an *Caryedon gonagra* (BVC) are the out group.

Table 2. *C. serratus*, □ at various hierarchical levels of differentiation estimated for the host plants (a) the localities (b). Significance of deviation from zero of □ values was tested using $□^2 = 2N(□)(k-1)$ for k alleles (see Table 1) and s populations with (k-1)(s-1) df. * P < 0.001.

Locus	Bignona	Fimela	Localities					
			Keur Baka	Linguere	Ouarak	Thies	Between localities	Between samples
M1425	0.332*	0.308*	0.384*	0.403*	0.336*	0.364*	0.008	0.312*
M625	0.318*	0.316*	0.314*	0.381*	0.317*	0.312*	0.007	0.291*
M836	0.321*	0.321*	0.326*	0.310*	0.282*	0.303*	0.005	0.262*
M97	0.398*	0.432*	0.415*	0.475*	0.430*	0.418*	0.002	0.389*
M984	0.390*	0.419*	0.392*	0.452*	0.380*	0.382*	0.006	0.358*
M2149	0.312*	0.324*	0.342*	0.352*	0.299*	0.315*	0.006	0.349*
M193	0.242*	0.298	0.247*	0.288*	0.251*	0.249*	0.007	0.301*
Overall	0.355*	0.368*	0.362*	o.405*	0.344*	0.358*	0.006	0.321*

Locus	Host plants					
	A. hypogaea	*B. rufescens*	*P. reticulatum*	*T. indica*	Between host	Between samples
M1425	0.007	0.004	0.024	0.004	0.359*	0.312*
M625	0.017	0.016	0.003	0.009	0.336*	0.291*
M836	0.005	0.022	0.007	0.007	0.314*	0.262*
M97	0.009	0.007	0.059	0.007	0.447*	0.389*
M984	0.063	0.012	0.010	0.012	0.412*	0.358*
M2149	0.007	0.006	0.008	0.009	0.447*	0.349*
M193	0.006	0.004	0.006	0.002	0.412*	0.301*
Overall	0.011	0.006	0.009	0.006	0.368*	0.321*

Finally, over all loci and samples, the probability of deviations from Hardy-Weinberg expectations was highly significant ($□^2$ = 246.7; ddl = 154). No linkage disequilibrium was found among any pair of microsatellite loci for any sample (P > 0.05). The large positive f value indicated a lower number of heterozygous individuals relative to that expected when data was pooled for all populations.

In "*Bauhinia* " and "*Tamarindus*" samples, allele frequencies were on the whole homogeneous. "*Groundnut*" and "*Piliostigma*" samples showed signify-cant or nearly significant heterogeneity and were globally homogeneous. Comparable results were obtained from the analysis of □ values within and between host plants and within and between localities (Table 2). Genetic differentiation between hosts was highly significant (average □ = 0.368) and was due to a large part of "*Bauhinia*" samples, which differed from all other samples. All loci contributed to the genetic differentiation between hosts. Differentiation between sites was not significant.

Similar patterns of relationships between populations were obtained from neighbour-joining analysis and from Wagner parsimony analysis. The consensus tree generated by the two analyses is presented in Figure 2. Samples typically clustered according to host plant as results obtained and largely discussed by Sembène et al. (2008*)*. The individuals tree (Figure 3) show a slight divergence between *C. serratus* feeding on *P. reticulatum* and those feeding on groundnut.

Results obtained with the "Structure.exe" reveal 12 migrants of *P. reticulatum C. serratus* to groundnut in the first generation. At the same time, 3 migrants left the groundnut lying on the residual seeds of *P. reticulatum* in its nature, and 23 infested the recently harvested groundnut. In the second generation, all these values increase approximately with 15%.

DISCUSSION AND CONCLUSION

It is evidence that a strong genetic differentiation clearly exists between *C. serratus* feeding on different host plant. Phylogenetic hypotheses and the relative genetic isolation between these populations are best explained by the fact that they feed on different host plants. These host race differentiation and host race formation mechanisms are largely debated in Sembène et al. (2008 and 2010).

In this study, we were particularly interested in understanding the origin of dried groundnut infestation in the field. Our results show that Groundnut and *P. reticulatum C. serratus* populations are indistinguishable on the basis of sequence sets. These samples show however similarities and was genetically very close. High gene flow probably exists between the two populations, as period. The introduction of groundnut into the environ-

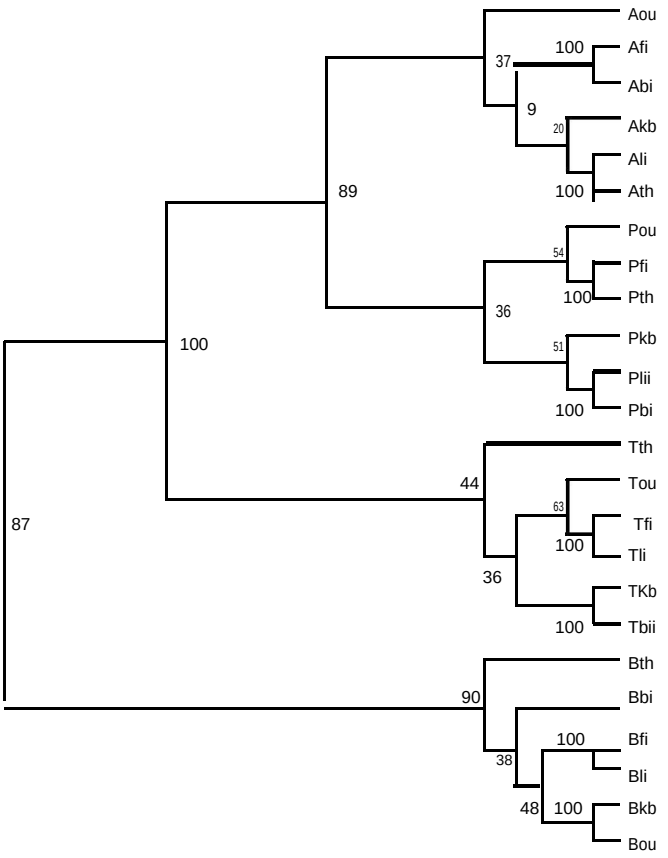

Figure 2. Relationships among *C. serratus* samples from different sites and host plants (see Table 1). *C. sieberiana* samples are excluded. This tree is a consensus between neighbour- joining consensus tree and FREQPARS consensus tree using 7 microsatellites loci. Node stability was evaluated using 1,000 bootstrapping replications re-sampling loci or populations. The numbers above branches are the neighbour-joining % bootstrap values (1,000 replicates).

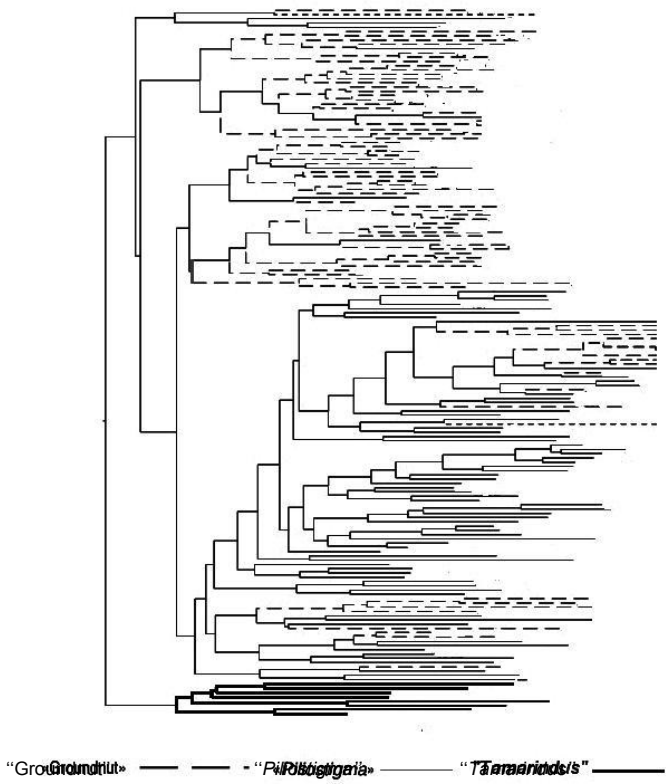

Figure 3. Relationships among "*Piliostigma reticulatum*" and "Groundnut" *C. serratus* individuals. This tree was constructed with the DAS (shared allele) distance using 7 microsatellite loci. It derives from 50% majority- rule consensus of 1,000 bootstrap replicates. "Tamarindus" individuals are the out group.

ment of wild plants supported the transfer of "Piliostigma" indicated by morphometric (Sembène and Delobel, 1996) and allozymic (Sembène and Delobel, 1998) analysis,and it may be hypothesized that beetles feeding on *P. reticulatum* were responsible for the initial infestation of groundnuts at the turn of the 20th century. Polymorphic microsatellites loci confirm and explain this hypothesis. Beetles feeding on *P. reticulatum* and those infesting the groundnut, although genetically very close begin to diverge. The number of individuals not being able to be brought back to the one or the other group is however 10 %. The rate of "well classified" is 90 % for each of its two stumps.

The depth of knots in the individuals tree between these two stumps and the big resemblance of the allele frequencies of the individuals belonging to these two origins show that both strain began to diverge only recently on the scale of time. This situation may be explained by the absence of wild pods during the rainy on groundnut and the "cycling" of the groundnut stock in the residual pods of stores. Currently, adults of *C. serratus* which came from stores and those which emerged from residual seeds of *P. reticulatum* at the end of the dry season, when there were still some pods on trees, or from the few pods of *P. reticulatum* in decom-posItion on the ground, were responsible for the re-infestation of new harvests. These beetles feed on pollen or nectar during the rainy season. In the presence of adequate food (pollen, water), beetles can survive 80 to 90 days in the laboratory (Delobel, 1989), and so in nature may also live 3 months or even longer. In periods of intense heat or strong rains (factors enhancing mortality of *C. serratus*) the beetles shelter, by negative phototropism, under the trees in litter. This finding points to the importance of residual populations in stores in the re-infestation of newly collected groundnuts.

Even though, we do not have references for estimate divergence dates on Cyt. B transversions, but molecular data make it possible today to propose how food preferences evolved in certain groups of insects and to identify the possible host plant ancestor of a studied group. In the case of groundnut infestation, we can

suggest that *C. serratus* feeding on *P. reticulatum* were the origin of groundnut infestation at the beginning of the century, by allotrophy in Senegal. This population, undoubtedly, extended to infest groundnut harvests in most of Western Africa. Historical data reveal that it is in Western Africa (Senegal) that the first groundnut attacks by *C. serratus* were reported (Roubaud, 1916). Until the beginning of the 1970's, when damage in Congo was first reported (Delobel, 1989), this was the only area of the world where *C. serratus* infested groundnut stocks. Today, *C. serratus* is a pest of stored groundnuts from Senegal to Chad and southwards to the Central African Republic and Congo (Matokot et al., 1987). It has become a pest of groundnuts in Asia (Dick, 1987) and has colonized parts of the New World tropics in the seeds of tamarind and ornamental *Bauhinia* species (Johnson, 1966; Nilsson and Johnson, 1992) . The present-day distribution of *C. serratus* as a pest of groundnuts may be explained by successive introductions of infested material, as was the likely cause in the Congo (Delobel and Matokot, 1991).

The groundnut crop was first stepped in Senegal without major difficulty, and was even free from plant health problems. In 1910, however, a sudden deterioration of commodities in the metropolis was reported. In 1912, Azemard, sub-inspector of agriculture Diambour (Senegal), draws the study's attention to the Senegalese head of agriculture report on the plant health of peanuts stocks and he echoed the concerns of traders with the subsequent receipt of stocks heavily damaged and diagnosed, following attacks by termites, millipedes of elaterides and secondary infestations by *Plodia interpunctella*, *Tribolium confusum* and *Oryzaephilus surinamensis*. To remedy the deterioration of stocks, Azemard advocates the elimination of grain "stuck or broken". Finally in 1913, Roubaud, a prominent expert on sleeping sickness, then head of laboratory at the Pasteur institute, was sent to Senegal, where he reported a significant amount of data. His list of insect pests in groundnut shell is much more comprehensive and more accurate than those previously published. It includes, in particular, groundnut bruchid (under the name *Pachymaerus acaciae* Gyll. However, this is the first time *Caryedon serratus* is associated with groundnut. The damage as indicated by Roubaud, may significantly prolong storage, but the author is very far from it according to the weevil importance he gives to termites, in that *C* . *serratus* grows slowly only in recent literature. In 1914, an increase in insect damage was attributed to *Ephestia cautella*. Eight years later, the weevil is still relatively uncommon in stocks, but passes unnoticed in stores mills of Bordeaux, which annually treat 80,000 tons of peanuts from Senegal (Feytaud, 1924). Yet more than 15 years after the first observations of echoes, it has become the main enemy of peanut stocks in Senegal in 1935 and Sagot Bouffil emphasize three crucial points concerning the mode of contamination in groundnut: (i) *C. serratus* from peanut thrives in tamarind seeds (ii) This host is infested wilderness in nature by *C*. *serratus* (iii) Infestation of peanut field occurs during drying in windrows. Guiraud, especially in a study on the economics of groundnut cultivation, cites a pest of stocks, and this is the weevil, which, he says, "is more and more damage. These are particularly serious when they are made in stocks *of* foresight, because the seeds attacked are quickly incapable of germination "(Guiraud, 1938).

The preceding history highlights the difficulty of interpreting the evidence left by the early century entomologists. Our only certainty lies in the fact that *C*. *serratus* had already attacked the Senegalese groundnut in 1913 at least in some stocks. If we can assume that low levels of infestation have escaped in 1912 and 1913 to a non-specialist like Azemard, it is surprising that in 1910 Perez could not detect *C*. *serratus* had he been present in the stocks of Metropolitan mills. Indeed, the time limits imposed on stocks before their arrival in Metropole probably reached several months or one year; such delays could only encourage the growth of the weevil and the appearance of witnesses and compelling the obvious presence of *C*. *serratus*: pupa cocoons, outlet of larvae and adults. And it should be noted that ten years later, Bordeaux still does not know the ground-nut bruchid, although its warehouses are continuously fed with peanuts from West Africa (Feytaud, 1924).

One can imagine two explanations for this apparent contradiction: The first is that there has been rejection by buyers of seed lots infested or sort particularly effective. But on one hand the chronic left no trace of such practices on the other hand, it is difficult to imagine that they could be effective as to obscure *C*. *serratus* metropolitan entomologists for so long. The second explanation is more plausible: It is likely that in 1913 the Senegalese groundnut infestation by the weevil was far from widespread and affected only a very limited number of production areas. That is why it escaped the successors of Roubaud. The geographically discontinuous nature of infection corresponds to a reality that had since been observed elsewhere, particularly in Congo.

It is difficult to specify the date of onset of weevil in Senegalese stocks because if we can date the onset of weevil in stocks for export around 1910, this does not mean that the passage populations of *C. serratus* on groundnut date precisely from this period. In fact, it perhaps became effective after multiple failed attempts that have occurred over several centuries. However, it is clear that the widespread infestation throughout West Africa took place in 15 or 20 years.

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