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Genetic differentiation of groundnut seed-beetle populations in Senegal

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Abstract

Caryedon serratus, the groundnut seed-beetle, is a major pest of groundnut (*Arachis hypogaea*), an introduced legume in the subfamily Papilionoideae. 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.

The biology and natural history of *C. serratus* suggest that it is a candidate for population differentiation via host-race formation. Evidence for host-tree associated differentiation in *C. serratus* would be important for the design of rational pest management practices.

To test this possibility, we analyzed the genetic structure of 20 adult collections of *C. serratus* from six sites in Western Senegal, on its five hosts. Results show a strong differentiation of insects from different host trees, with specimens from *C. sieberiana* possibly representing a sibling species and insects from *B. rufescens* a distinct host-race.

Introduction

There is growing evidence that host-race differentiation within populations, or genetic differentiation in host use, is a widespread phenomenon among herbivorous insects. Although the possibility of its resulting in speciation is still debated, it may well be one of the major mechanisms which led to present-day diversity in herbivorous insects.

In a number of cases, host-range expansion is known to have followed the introduction of a new host plant, and in a few well-documented cases, this has been shown to lead to the differentiation of distinct host races: in *Leptinotarsa decemlineata*, host-adapted differentiation occurred in North America following the introduction of the cultivated *Solanum tuberosum* (Hsiao, 1978). Host race differentiation also occurred in the American hawthorn maggot fly *Rhagoletis pomonella* (Feder & Bush, 1989) and other *Rhagoletis* species (for review, see Diehl & Bush, 1984) when populations became adapted to European fruit trees. A certain degree of differentiation has also been found in

the Pierid butterfly *Colias philodice* after its shift from ancestral legume hosts to alfalfa (Tabashnik, 1983).

Groundnut (*Arachis hypogaea* L., a plant in the legume subfamily Papilionoideae) was introduced from South America to Africa towards the end of the XVIth century. The first infestations of groundnut by the Old World seed-beetle *Caryedon serratus* Olivier (Coleoptera: Bruchidae) were reported at the turn of the XXth century (Delobel & Matokot, 1991). Commonly known as the groundnut seed-beetle, *C. serratus* is responsible for important weight losses in stored groundnuts, reaching up to 83% in four months storage (Ndiaye, 1991).

The ancestral hosts of *C. serratus* are all members of the Caesalpinioideae (a legume subfamily closely related to Papilionoideae) and constitute a small number of species in four genera: *Bauhinia*, *Cassia*, *Piliostigma* and *Tamarindus* (Delobel et al., 1995). Although information on its spread in West and Central Africa is scanty, it appears that some areas in the central and eastern parts of Sahel have been reached only recently by the pest. In Congo, in-

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festation of groundnut by the beetle occurred around 1975 as a result of the introduction of infested seeds from Senegal. This is documented by historical record (Delobel & Matokot, 1991) and by the fact that several groundnut growing regions in Congo were still free from the pest in 1986, even though native populations of *C. serratus* could be found in these areas on the local host *Piliostigma thonningii* (Matokot et al., 1987; Delobel & Matokot, 1991). Since then, isolated populations of *C. serratus* have been found to infest groundnut in Ivory Coast (Pollet, 1984), in Central African Republic (Koyabay, 1988) and in India (Dick, 1987). Studies on food-plant selection and larval development (Robert, 1984; Ali-Diallo, 1991) as well as morphometry (Sembène, & Delobel, 1996), also suggest that genetic isolation exists between populations feeding on different host trees, and in particular between groundnut-feeding and Caesalpinoideae-feeding forms.

A novel control method, based on principles other than insect destruction by costly and dangerous chemicals cannot be developed without a detailed knowledge of the population biology of the pest. For example, primary infestation of groundnut is known to result from females laying eggs on pods shortly after harvest (Matokot et al., 1987). Prevention will differ according to whether ovipositing females emerge from groundnut stores or from wild hosts.

To determine the extent of gene flow and isolation between native forms and forms feeding on groundnut, we analyzed allozyme variation within and among Senegalese populations of *C. serratus* feeding on its five major host plants. Comparisons involved both allopatric (but feeding on the same host) and sympatric (but feeding on different hosts) seed-beetle populations. Our primary question was whether groundnut-infesting populations of *C. serratus* represent a newly formed host race of the species. However, we were also interested in determining whether populations of the beetle feeding on other native hosts might constitute cryptic sibling species (or host races) and whether *C. serratus* shows geographic substructuring across its range.

Material and methods

Study site. In Senegal, phytogeographic regions are basically determined by rainfall. Parallel isohyets define from north to south the sahelian, soudanian and guinean regions (Figure 1). Samples were collected



Figure 1. Map of Senegal showing the sites of *C. serratus* collections.

in Ouarak ($16^{\circ}04' W$, $15^{\circ}33' N$), Thiès ($16^{\circ}56' W$, $14^{\circ}48' N$), Fimela ($16^{\circ}41' W$, $14^{\circ}08' N$), Keur Baka ($15^{\circ}57' W$, $13^{\circ}56' N$), Sokone ($16^{\circ}22' W$, $13^{\circ}52' N$) and Bignona ($16^{\circ}13' W$, $12^{\circ}49' N$). The first four sites are within the sahelian zone of Senegal, where the rainy season lasts from July to September (500 to 700 mm rainfall). Sokone is in the soudanian zone, with a slightly longer rainy season (700 to 900 mm rainfall). Bignona, in the guinean region, receives around 1000 mm rainfall during a 4 to 5 months rainy season.

Biology of *C. serratus* on its five host plants. The pods of the five hosts of *C. serratus* are non-dehiscent. Females lay their eggs on the surface of ripe pods, and newly hatched larvae bore through the husk and into the seed, where larval development takes place. Five to seven weeks after oviposition, mature larvae exit the pods and fall to the ground where they pupate after spinning a whitish silk cocoon. Emergence of the adults takes place about two weeks after pupation. The period during which ripe pods of *P. reticulatum* and *C. sieberiana* remain on the tree does not exceed, in a given area, 5 or 6 months, usually between November and May. After pods have fallen to the ground, they are often eaten by cattle or burnt, and only a few seeds remain accessible to *C. serratus* females (Ndiaye, 1991). Towards the end of the dry season and during the rainy season, seeds become progressively less available. When the first new *P. reticulatum* or *C. sieberiana* pods reach maturity after the rainy season, in November, *C. serratus* levels are at their lowest. Infestation rates observed on *P. reticulatum* at that time are not higher than 2 to 4 eggs per 1000

Pods (Sembène, 1997). On hosts which bear pods all year round (*B. rufescens* and, to a lesser extent, *T. indica*), generations follow one another in the pods attached to the tree, and infestation rates do not exhibit the wide fluctuations observed on *Piliostigma*. Populations, however, experience a definite decline during the rainy season, probably due to increased adult mortality (Pierre & Huignard, 1990; Ndiaye, 1991). Pods of the derived host groundnut are removed from the soil shortly after the end of the rainy season, in October. Oviposition takes place on drying pods within a week after harvest. Infestation rates at that time are very low, in the order of 1 egg per 10 000 pods (Matokot et al., 1987 in Congo; personal observations in Senegal). After the harvest is taken to the stores, infestation continues and population levels rapidly increase.

C. serratus samples. Beetles used in this study were reared from eggs, larvae or pupae on/inside pods collected on the different host trees between 1995 and 1997. The term 'population' refers hereafter to all *C. serratus* specimens examined, and the term 'subpopulation' to samples from the same geographic origin, whatever their host plant. Samples were named after their host plant species and geographic origin: 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 Thiès: Ath from *A. hypogaea*, Pth from *P. reticulatum* and Tth from *T. indica*. Two additional samples were collected: a '*C. sieberiana*' sample in Sokone, and a '*T. indica*' sample in Bignona. *C. sieberiana* was not sampled in Ouarak and Thiès.

Pods were taken from the trees as soon as they reached maturity, at the start of new infestations (between November and January) except for '*C. sieberiana*' samples, which were collected in March, at a period when seed-beetle populations reached their highest levels, after at least one generation in the pods. Groundnut samples were collected from the field during drying. Adult beetles were genetically analyzed almost immediately after they emerged from pods. First, however, we examined genital parts following Prevett (1965) in order to avoid confusion with *Caryedon crampeli* Pic (*C. cassiae* in Prevett), a species

which also feeds on *B. rufescens*, *C. sieberiana* and *P. reticulatum*. Voucher specimens are kept in the I.F.A.N. Cheikh Anta Diop (Dakar) collections. In two instances (Cfi and Bou), parasitism by the spider mite *Pyemotes tritici* led to high mortality, and seed-beetles had to be reared on their original host-plant for one generation. In all, a total of 20 different samples (800 individuals of both sexes) were genetically scored in the study.

Electrophoretic studies. Sample preparation: individual seed-beetles were crushed alive in 100 µl of grinding buffer (0.1 M Tris, 0.04 M L-cystein, and 10% Triton, pH 7.4). Homogenates were centrifuged, at 26 000 rpm for 20 min and the resulting supernatants were transferred to filter wicks which were immediately loaded into gels.

Running conditions and staining: electrophoresis was performed in 12% potato starch gel. Gel preparation and migration techniques were those described by Moretti et al. (1957) and Pasteur et al. (1987). Gel slabs were run for 15 h in a 0.02 M Tris and 0.01 M maleic acid buffer (pH 7.3), under constant 120 V. Staining solutions were prepared according to Lebrun & Chevalier (1990). To stop enzymatic activity, the staining solution was replaced by a 7% acetic acid solution for 1 h. Gels were then placed in a 15% glycerol solution at 5 °C for 12 h, and then dried at 60 °C for 4 h. We stained for a total of 12 enzyme systems: alcohol dehydrogenase (ADH), diaphorase (DIA), endopeptidase (ENDO), esterases (EST), glutamate oxalo-acetate transaminase (GOT), glucose-phosphate isomerase (GPI), isocitrate dehydrogenase (ICD), hexokinase (HK), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), and phosphoglucomutase (PGM).

Statistical analyses. Quantitative analysis of electrophoretic data was performed using Biosys-1 V1.7 (Swofford & Selander, 1981). Genotype frequencies were tested against Hardy-Weinberg genotypic expectations with panmixia as null hypothesis using Genepop V1.2 (Raymond & Rousset, 1995). In the test of linkage disequilibrium, the null hypothesis was independence between genotypes at different loci. 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 at

the six loci studied (Sokal & Rohlf, 1981). Fstat V1.2 (Goudet, 1995) was used to compute Weir & Cockerham's (1984) estimators f and θ of F -statistics (Wright, 1978) in order to evaluate substructuring among hosts and sites. Parameter f (consanguinity coefficient) corresponds to Wright's F_{IS} . Parameter θ (degree of genetic differentiation between populations) corresponds to Wright's F_{ST} . Two scales of population subdivision were used in the analysis: host-plant and geographic origin. The significance of the deviations of θ from zero was tested using the formula $\chi^2 = 2N\theta(k-1)$ for a total population of N individuals with k alleles and s samples, with $(k-1)(s-1)$ degrees of freedom. Gene flow between samples from groundnut and each of the other host plants was computed from $\theta = 1/(4N_m + 1)$, where N_m is the number of migrants per generation (Wright, 1931).

Relationships among samples based on gene frequencies were displayed using UPGMA and neighbor-joining (Saitou & Nei, 1987) methods of clustering available in procedure 'NEIGHBOR' of Felsenstein's (1995) PHYLIP V3.57c package. Node stability was evaluated using 100 bootstrapping replications, and majority-rule consensus trees were obtained using procedures 'SEQBOOT' and 'CONSENSE' of PHYLIP. Swofford & Berlocher (1987) frequency parsimony program (FREQPARS) was used to build tree(s) based on a modified Wagner algorithm (Farris, 1970) using modified Roger's (1972) distances and also to compare the different topologies obtained. Nei's (1978) genetic distances were computed for comparative purposes.

Results

Enzyme systems. Of the 12 enzymes we attempted to resolve, 6 systems (ENDO, PGD, GPI, DIA, HK and EST) failed to stain reliably. In addition, 2 enzymes (ICD and MDH) were monomorphic. LAP, ADH, GOT and PGM proved to be polymorphic. At LAP, two monomeric enzymes were resolved: a slow system (LAP-1) and a fast migrating, highly polymorphic isozyme with numerous bands of very low intensity. The latter system could not be scored reliably and so was not used in the study. ADH showed two cathodally migrating isozymes (ADH-1 and ADH-2), each with two alleles. ADH-1 was not observed in samples from *C. sieberiana* (see Sembène et al., 1998). Two GOT isozymes were resolved, GOT-1 anodally migrating and GOT-2 cathodally migrating. Two PGM

isozymes were distinguished, of which the slowest only (PGM-1) could be reliably scored.

Genetic analysis. A total of 6 different polymorphic allozyme loci (*Got*-1 and 2, *Lap*-1, *Adh*-1 and 2, and *Pgm*-1) were scored. Allele frequencies of the 20 samples are given in Table 1. Deviations from Hardy-Weinberg expectations for each allozyme and each sample are given elsewhere (Sembène et al., 1997). Significant deviations were observed in 19.21% of the 146 tests: for 'groundnut' and '*P. reticulatum*' at *Got*-2 and *Adh*-2, '*C. sieberiana*' at *Got*-2 and *Pgm*-1, and '*B. rufescens*' at *Adh*-2. Deviations were all due to a deficiency of heterozygotes ($0.13 < f < 0.60$). No significant deviation was found for '*T. indica*'. In fact, a slight excess of heterozygotes was detected in Tkb ($f = -0.242$). No linkage disequilibrium was found among any pair of allozyme loci for any sample ($P > 0.05$).

Each subpopulation showed a highly significant allele frequency heterogeneity among host plants at all allozymes except *Adh*-2 in Ouarak (Table 2). Allele frequencies in '*B. rufescens*', '*C. sieberiana*' and '*T. indica*' samples were on the whole homogeneous (except '*T. indica*' samples at *Lap* and '*C. sieberiana*' samples at *Got*-2 and *Adh*-2). 'Groundnut' and '*P. reticulatum*' samples showed significant or nearly significant heterogeneity at *Got*, *Lap*-1 and *Pgm*-1, and were globally heterogeneous. However, this was largely due to Fimela samples: when these were excluded from the original data set, overall G-values became non-significant (1.962 for 'groundnut' and 9.738 for '*P. reticulatum*').

Similar results were obtained from the analysis of θ values within and between host-plants and within and between localities (Table 3). Within-host genetic differentiation was low: 0.023 for 'groundnut', -0.010 for '*B. rufescens*', 0.025 for '*P. reticulatum*', 0.007 for '*T. indica*' and 0.014 for *C. sieberiana*. Genetic differentiation between hosts was highly significant (average $\theta = 0.283$) and due for a large part to '*C. sieberiana*', which differed from all other samples at *Adh* and *Pgm*-1. '*B. rufescens*' also differed from other samples at *Adh*-1 and *Pgm*-1. All loci except *Lap*-1 contributed to the differentiation between hosts (Table 3). Differentiation between subpopulations (sites) was significant.

When compared to similar studies in other Coleoptera, the average value of θ (0.258) for all samples is high. In *Tetraopes tetraophthalmus* (Nearctic populations), $F_{ST} = 0.154$ (McCauley & Eanes,

Table 1. Allele frequencies at six polymorphic loci from six locations of *C. serratus* (see text for abbreviations of samples)

Loci	Allele	Samples																			
		Fimela					Keur Baka					Ouarak				Thies				Bignona	Sokone
		Afi	Bfi	Cfi	Pfi	Tfi	Akb	Bkb	Ckb	Pkb	Tkb	Aou	Bou	Pou	Tou	Ath	Bth	Pth	Tth	Tbi	Cso
<i>Got-1</i>	1	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.038	0.000	0.050	0.000	0.050	0.000	0.063	0.000	0.075	0.000	0.000	0.025
	2	1.000	1.000	1.000	1.000	1.000	0.962	1.000	1.000	0.962	1.000	0.950	1.000	0.950	1.000	0.938	1.000	0.925	1.000	1.000	0.975
<i>Got-2</i>	1	0.000	0.112	0.075	0.000	0.000	0.100	0.138	0.000	0.100	0.000	0.100	0.125	0.138	0.000	0.100	0.138	0.075	0.000	0.000	0.125
	2	1.000	0.887	0.925	1.000	1.000	0.825	0.863	1.000	0.813	1.000	0.825	0.875	0.788	1.000	0.850	0.863	0.825	1.000	1.000	0.875
	3	0.000	0.000	0.000	0.000	0.000	0.075	0.000	0.000	0.087	0.000	0.075	0.000	0.075	0.000	0.050	0.000	0.100	0.000	0.000	0.000
<i>Lap-1</i>	1	0.000	0.125	0.250	0.000	0.063	0.275	0.125	0.225	0.237	0.075	0.275	0.138	0.262	0.162	0.250	0.188	0.200	0.063	0.112	0.250
	2	0.800	0.825	0.750	0.800	0.863	0.712	0.813	0.775	0.750	0.850	0.700	0.788	0.700	0.625	0.725	0.750	0.788	0.863	0.738	0.750
	3	0.200	0.050	0.000	0.200	0.075	0.013	0.063	0.000	0.013	0.075	0.025	0.075	0.038	0.213	0.025	0.063	0.013	0.075	0.150	0.000
<i>Adh-1</i>	1	0.000	0.138	-	0.000	0.000	0.000	0.150	-	0.000	0.000	0.000	0.138	0.000	0.013	0.000	0.225	0.000	0.000	0.000	-
	2	1.000	0.863	-	1.000	1.000	1.000	0.850	-	1.000	1.000	1.000	0.863	1.000	0.988	1.000	0.775	1.000	1.000	1.000	-
<i>Adh-2</i>	1	0.962	0.938	0.525	0.962	1.000	0.962	0.913	0.213	0.950	1.000	0.962	0.938	0.950	1.000	0.950	0.938	0.938	1.000	1.000	0.512
	2	0.038	0.063	0.475	0.038	0.000	0.038	0.087	0.788	0.050	0.000	0.038	0.063	0.050	0.000	0.050	0.063	0.063	0.000	0.000	0.488
<i>Pgm-1</i>	1	0.338	0.150	0.075	0.338	0.338	0.262	0.150	0.075	0.300	0.325	0.275	0.150	0.262	0.363	0.287	0.112	0.425	0.338	0.338	0.075
	2	0.563	0.813	0.925	0.563	0.663	0.738	0.800	0.925	0.700	0.675	0.725	0.813	0.738	0.637	0.712	0.813	0.575	0.663	0.663	0.925
	3	0.100	0.038	0.000	0.100	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000

1987). In *Oreina cacalliae*, a beetle with a very low dispersal ability, and which feeds on a plant with a patchy distribution, $F_{ST} = 0.234$ (Rowell-Rahier, 1992). $F_{ST} = 0.290$ in Eurasian colonies of *Coccinella 7-punctata* (Krafsur et al., 1992).

Considering that *C. serratus* samples raised from *C. sieberiana* might represent a separate species, we excluded them from the original data set and reanalyzed the data. G values fell but remained significant ($P < 0.001$) in both cases. Overall θ fell from 0.258 to 0.035 (Table 3). Genetic differentiation between localities (subpopulations) became non-significant ($\theta = 0.009$). Genetic differentiation between hosts decreased ($\theta = 0.032$) but remained significant.

Similar patterns of relationships were obtained from UPGMA and neighbor-joining analysis (Figure 2) and from Wagner parsimony analysis (Figure 3). Samples typically clustered according to host plant, except for groundnut and *P. reticulatum*, which clustered together. Afi and Pfi were, however, clearly separated from all other samples reared from groundnut and *P. reticulatum*. As expected from Tables 2 and 3, *C. sieberiana* samples were clearly separated from all other samples in the Wagner tree and showed high bootstrap values in the UPGMA and neighbour-joining analysis. '*B. rufescens*' samples clustered together, with a bootstrap value of 99. The '*T. indica*' cluster was supported by a lower bootstrap value, and was split in two by the parsimony analysis. All dendrograms had similar lengths. In order of increasing lengths, they were the Wagner tree ($l = 10.13$); the UPGMA tree ($l = 10.79$); the neighbor-joining tree ($l = 11.06$).

The average Nei's genetic distance between '*C. sieberiana*' and the other samples was 0.50. Gene flow value was highest ($N_m = 250$) between 'groundnut' and '*P. reticulatum*'. It was much lower between 'groundnut' and '*T. indica*' (11), and between 'groundnut' and '*B. rufescens*' (6). It was very low between 'groundnut' and '*C. sieberiana*' (0.2).

Discussion

Host associated differentiation in C. serratus. We have identified different levels of genetic differentiation between co-occurring *Bauhinia*, *Cassia*, *Piliostigma*, and *Tamarindus* wild populations, and groundnut populations of *C. serratus*. There is evidence of at least four distinct biotypes in Senegal, with restricted gene flow between each other.

Table 2. G-tests values for allele frequency homogeneity among subpopulations (sites) and host plant samples calculated on the basis of 6 loci. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Loci	Sites		Host plants				
	Fimela	Thiès	Groundnut	<i>B. rufescens</i>	<i>C. sieberiana</i>	<i>P. reticulatum</i>	<i>T. indica</i>
Got-1	-	14.662**	7.131	-	3.3318	8.241*	-
Got-2	28.919***	33.801***	24.348***	0.309	14.650**	14.950	-
Lap-1	75.503***	15.948*	60.425***	2.093	0.193	57.234***	19.719
Adh-1	-	51.431***	-	2.888	-	-	1.710
Adh-2	95.904***	7.995***	0.198	0.497	18.166***	0.499	-
Pgm-1	55.818***	33.928***	24.119***	1.989	-	26.970***	0.273
Overall	364.670***	157.438***	110.978***	7.018	32.186	103.209***	16.342

Table 3. Values of θ at various hierarchical levels of differentiation estimated between sites, between hosts and for all *C. serratus* samples. Numbers within parentheses indicate θ values after removal of *C. sieberiana* samples. Negative values at loci are an artefact of the method for estimating θ when the value of the latter is close to zero. Significance of deviation from zero of θ values was tested using $\chi^2 = 2N(\theta)(k - 1)$ for k alleles (see Table 1) and s populations with $(k - 1)(s - 1)$ df. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Loci	Between sites	Between hosts	All samples
<i>Got-1</i>	0.008 (0.006)	0.021* (0.023**)	0.020* (0.020*)
<i>Got-2</i>	0.010 (0.014)	0.039** (0.047***)	0.046*** (0.049**)
<i>Lap-1</i>	0.013 (0.021*)	0.010* (0.006)	0.023** (0.030**)
<i>Adh-1</i>	0.087** (-0.001)	0.851*** (0.159***)	0.827*** (0.131***)
<i>Adh-2</i>	0.053** (-0.001)	0.420*** (0.021*)	0.402*** (0.005)
<i>Pgm-1</i>	0.002 (0.001)	0.061*** (0.029**)	0.050*** (0.023**)
Overall	0.028* (0.009)	0.283*** (0.032**)	0.258*** (0.035**)

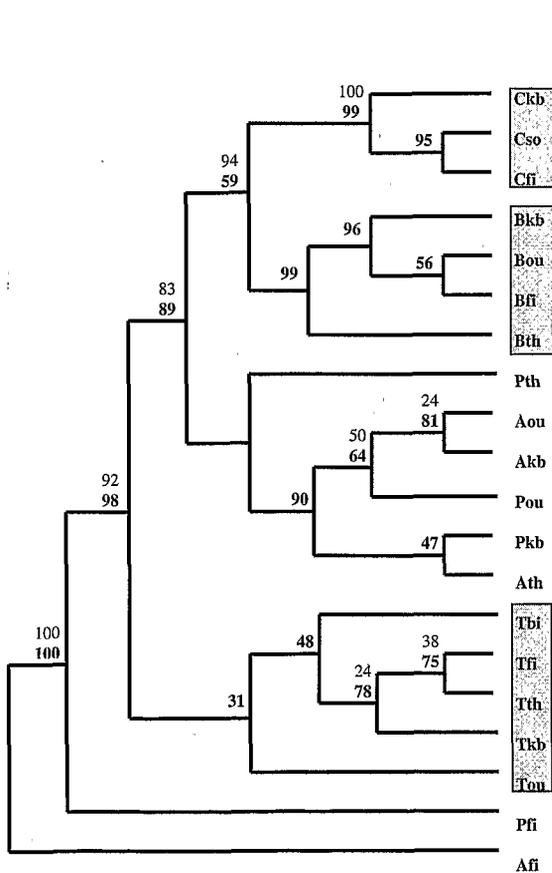


Figure 2. Relationships among *C. serratus* samples from different sites and host plants (see text). This tree was generated by UPGMA analysis of the six loci. It derives from a 50% majority-rule consensus of 100 bootstrap replicates. Bootstrap proportions correspond to (in bold) the UPGMA analysis and the neighbor-joining analysis (where the topologies of the two agree).

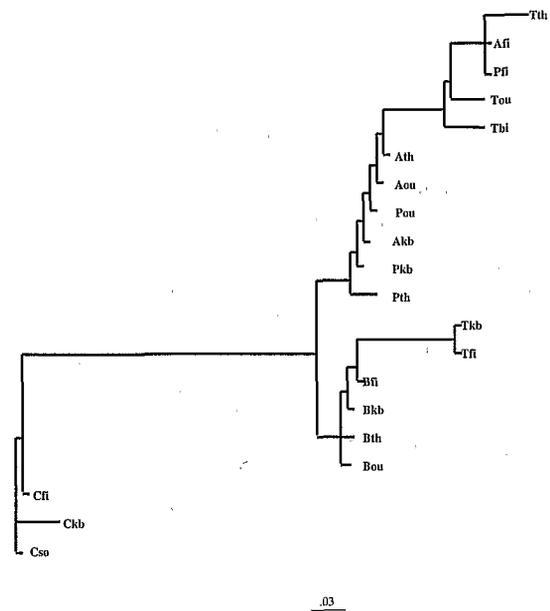


Figure 3. Distance Wagner tree, based on modified Roger's (1972) distance, of *C. serratus* samples.

A strong differentiation clearly exists between *C. serratus* feeding on *C. sieberiana* and on the four other host plants. The average value of Nei's genetic distance between the two groups (0.5) exceeds genetic distances typical of geographical populations or subspecies in Invertebrates (Klein & Seitz, 1994; Emelianov et al., 1995). The singularity of '*C. sieberiana*' samples, as seen from allele distribution patterns at *Adh* and *Pgm* (Table 1), may be explained by an incipient sympatric speciation or by the existence on *C. sieberiana* of a sibling species of *C. serratus* as a result of a previous differentiation of populations

in allopatry, with present-day overlap of *C. sieberiana* and other host plants. These hypotheses cannot be tested without further knowledge of biogeographic and temporal variations in host plant distribution.

The population on *Bauhinia* is strongly differentiated from *C. serratus* populations on other host plants, but the distribution of its allele frequencies is very homogeneous between localities. It possibly represents a distinct host race. The *Tamarindus* population is less clearly differentiated from populations on other host plants, and a slight geographical variation exists in the *Lap* allele frequencies. Groundnut and *P. reticulatum* populations are indistinguishable on the basis of allozymes. A high gene flow probably exists between the two populations, and it may be hypothesized that beetles feeding on *Piliostigma* were responsible for initial groundnut infestation at the turn of the XXth century. Contrary to Ndiaye's (1991) assumptions, *B. rufescens* and *T. indica* seem to play a secondary role in groundnut infestation in Senegal, with only a limited number of migrants per generation. Is the situation similar over the whole range of the groundnut seed-beetle? Experiments held in Niger (Ali-Diallo, 1991) indicate that local ovipositing groundnut-origin females have a significant preference for *T. indica* pods, which could be explained by the fact that in Niger the shift was from *T. indica* to groundnut, and not from *P. reticulatum* as was probably the case in Senegal. This would point to multiple shifts, and not to a single focus from which infestation subsequently spread to other parts of Africa. Ali Diallo's (1991) experimental conditions, however, were too different from natural conditions for definite conclusions to be drawn.

Maintenance of genetic heterogeneity among C. serratus populations. The main factors that could possibly maintain allele frequency differences among ancestral populations and between them and derived groundnut populations are the following: post-mating reproductive isolation between races, differential larval survivorship within the seed, differential host recognition by adult beetles, temporal, and spatial differences in adult emergence and host seed availability.

Preliminary 'hybridization' experiments between individuals from *C. sieberiana* and *P. reticulatum*, which are genetically the most distant, indicate that post-mating isolation is not probable, as laboratory F1 progeny is fertile. Robert et al. (1982) also obtained fertile F1 progeny from crosses between groundnut and *B. rufescens*-origin beetles. No data are currently available on the differential survivorship of larvae of

the different host populations, even though we have been able to rear individuals belonging to each of the different host plant populations on the seeds of all other hosts.

Host fidelity, the tendency of an herbivorous insect to reproduce on the same host species that it used in earlier stages is one of the major requirements for sympatric isolation, and mating on the original host is a key mechanism of host fidelity (Feder et al., 1994). The site of mating is unfortunately unknown in *C. serratus*. Experiments in Niger (Ali-Diallo, 1991), however, indicate that a certain level of adaptation to their different native host plants exists in different *C. serratus* strains: exposure to pods of the original host significantly increased fecundity in *C. sieberiana*, *B. rufescens*, *T. indica* and groundnut-origin females. Only *P. reticulatum*-origin females were more fecund when given groundnut pods. When given a choice, each strain laid more eggs on the pods of its native host, except, as mentioned earlier, that groundnut-origin females laid more eggs on *T. indica*. Similar results were obtained by Robert et al. (1982) with *B. rufescens* and groundnut-origin beetles.

Temporal and spatial differences in adult emergence and host availability certainly play a major part in maintaining genetic homogeneity within native host plant populations and between *P. reticulatum* and groundnut populations. On *Piliostigma*, very few insects probably survive during the period of extremely low host availability (from March to October), which they spend as adults on the soil, under fallen leaves, or in the cracks of host tree bark (Sembène, 1997). Heavy summer rainfalls probably further reduce beetle population levels. It may therefore be assumed that gene flow towards the only available host at that time, *B. rufescens*, is very low or non significant. In fact, no freshly laid eggs were found on this particular host during the rainy season, neither in Niger (Pierre & Huignard, 1990) nor in Senegal (personal observations). The low infestation rates observed on early maturing *P. reticulatum* pods suggest that '*P. reticulatum*' samples consist of a mixture of the F1 of a limited number of founding females. Similarly, low numbers of eggs found on groundnut immediately after harvest may explain the deviation of 'groundnut' samples from Hardy-Weinberg equilibrium at *Got-2* and *Adh-2*. The strong consanguinity of groundnut-infesting beetles probably also results from the succession of generations within farmers' stores, without genetic connections with native populations.

C. sieberiana trees bear pods during a short period of time, from December to February. *C. serratus* females keep laying eggs on the pods after abscission, but populations must then face interspecific competition with the congeneric *C. crampeli*. In mid-January 1997 we collected in Sokone a sample of *C. sieberiana* pods (on the tree) which yielded 100 seed-beetle adults, of which 87 were *C. serratus* and 13 were *C. crampeli*. A sample of fallen pods collected in March under the same tree yielded a total of 327 beetles, of which only 56 (17%) were *C. serratus*, and the rest (83%) was *C. crampeli*. It may be assumed that this drastic population reduction has a negative impact on the beetle's capability to utilize other host plant species.

A limited amount of research work has been so far devoted to *C. serratus*, and its biology is still poorly known. A better knowledge of its population dynamics on the different host plants, under a variety of climatic conditions, and of its dispersal capacities would undoubtedly improve our understanding of the groundnut beetle story. Admittedly our current data set is not complete and more extensive sampling is needed, especially across years, to confirm the existence of host races in *C. serratus*. But the allozymes are suggestive of host-associated differentiation in the beetle.

The ability of *C. serratus* to colonize groundnut must be considered in connection with recent advances in our knowledge of the phylogeny of the genus *Caryedon*. In effect, morphological and molecular data indicate that *C. serratus* belongs to a clade of Mimosoideae feeding species, and is therefore not directly related with other Caesalpinioideae feeders (J.F. Silvain & A. Delobel, unpubl.). It may then be hypothesized that *C. serratus* acquired at some time during evolution a higher secondary compound detoxification ability than its relatives in the Mimosoideae feeding clade, and that this ability in turn gave that ancestral species a high potential to expand its host spectrum beyond both Mimosoideae (ancestral host range) and Caesalpinioideae (extant host range).

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