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ALLOZYME VARIATION AMONG POPULATIONS OF THE GROUNDNUT SEED-BEETLE, *CARYEDON SERRATUS* (OL.) IN SENEGAL

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Abstract—Starch gel electrophoresis was used to compare eight loci in six enzymatic systems of 17 samples of the groundnut seed-beetle, *Caryedon serratus* (Ol.) (Coleoptera: Bruchidae) bred on five different host-plant species: *Arachis hypogaea, Bauhinia rufescens, Cassia sieberiana, Piliostigma reticulatum* and *Tamarindus indica*. The rate of polymorphism was 44.8%. The average genetic diversity (Hw) was 0.184. Allozyme variability analysis indicated that seed-beetles associated with *P. reticulatum* and groundnut, *Arachis hypogaea* were genetically similar, whereas other samples clustered according to their host plant species. Geographical distances less than 400 km were not decisive for the genetic structuring of samples associated with a given host plant.

Key Words: *Caryedon serratus*, seed-beetle, groundnut, population, genetics, electrophoresis, allozyme variation

Résumé—L'électrophorèse sur gel d'amidon a permis de comparer huit loci appartenant à six systèmes enzymatiques chez dix-sept échantillons de bruche de l'arachide provenant de cinq plantes hôtes différentes: *Arachis hypogaea, Bauhinia rufescens, Cassia sieberiana, Piliostigma reticulatum* et *Tamarindus indica*. Le taux de polymorphisme est de 44,8%. La diversité génétique moyenne (Hw) est de 0,184. L'analyse de la variabilité allozymique montre que les bruches inféodées à *P. reticulatum* et à l'arachide sont génétiquement très proches, alors que les autres échantillons se regroupent en fonction de leur espèce hôte. Les distances géographiques inférieures à 400 km ne sont pas déterminantes dans la structuration génétique des échantillons d'une plante hôte donnée.

Mots Clés: *Caryedon serratus* bruche, arachide, population, génétique, électrophorèse, allozyme

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) was introduced from South America to Africa towards the end of the sixteenth century. Its cultivation in West Africa remained low well into the early part of the nineteenth century. With the intensification of edible oil refining which occurred during the last third of the nineteenth century, groundnut farming experienced a dramatic increase, particularly in Senegal. The first infestations of stored groundnuts by the bruchid *Caryedon serratus* (Olivier) were reported in Senegal at the turn of the twentieth century (Davey, 1958; Delobel, 1995).

Caryedon serratus is widely distributed in Africa and southern Asia (Johnson, 1986). About 60 years after its first record as a pest of groundnut in West Africa (Roubaud, 1916), *C. serratus* has recently become a major primary groundnut pest in Central Africa (Matokot et al., 1987) and Asia (Dick, 1987). It is also recorded in Central and South America in the seeds of ornamental *Bauhinia*. Commonly

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known as the groundnut seed-beetle, *C. serratus* is nowadays responsible for heavy weight losses in stored groundnuts; in Senegal up to 83% losses after four months storage have been reported (Ndiaye, 1991).

Caryedon serratus larvae consume the seeds of wild Caesalpiniaceae belonging to a small number of species in four genera: *Bauhinia, Cassia, Piliostigma* and *Tamarindus* (Borowiec, 1987). The larvae bore through groundnut hulls, which favours attack by secondary pests such as *Oryzaephilus mercator, Tribolium confusum, Ephestia cautella* or *Corcyra cephalonica* as well as the spread of *Aspergillus flavus*, a mould which produces a toxic substance, aflatoxin (Gillier and Bockelée-Morvan, 1979).

Groundnut infestation by the seed-beetle raises the question of the mechanisms by which *A. hypogaea*, a plant of the family Fabaceae, became part of the insect's range of hosts. It is interesting to note that *C. serratus* is not a pest of stored groundnuts in all groundnut-growing regions in Africa (Gagnepain et al., 1986; Delobel and Matokot, 1991). Food-plant selection and larval development studies (Robert, 1984; Ali-Diallo, 1991) as well as morphometry (Sembène and Delobel, 1996) provide support to the hypothesis that there exists some genetic isolation between *C. serratus* populations with different feeding habits, and in particular between groundnut-feeding and Caesalpiniaceae-feeding forms.

Sound control methods, based on principles other than destruction by costly and dangerous chemicals cannot be developed without an understanding of the population biology of the pest. For example, females laying eggs on pods shortly after harvest are known to be responsible for primary field infestation of groundnut (Matokot et al., 1987; Ndiaye and Jarry, 1990; personal observations). Prevention will differ according to whether these females originate from groundnut stores, from wild hosts or whether they emerge from quiescence or diapause at the end of the rainy season. Questions such as whether stored groundnuts constitute a reservoir for the reinfestation of wild hosts at certain periods of the year, and the range over which such a reinfestation is possible, need to be answered.

In order to determine the degree of isolation between 'wild' forms and those feeding on groundnuts, we analysed the genetic variability of Senegalese populations of *C. serratus* feeding on seeds of five host plants species: *Piliostigma reticulatum, Bauhinia rufescens, Tamarindus indica,*

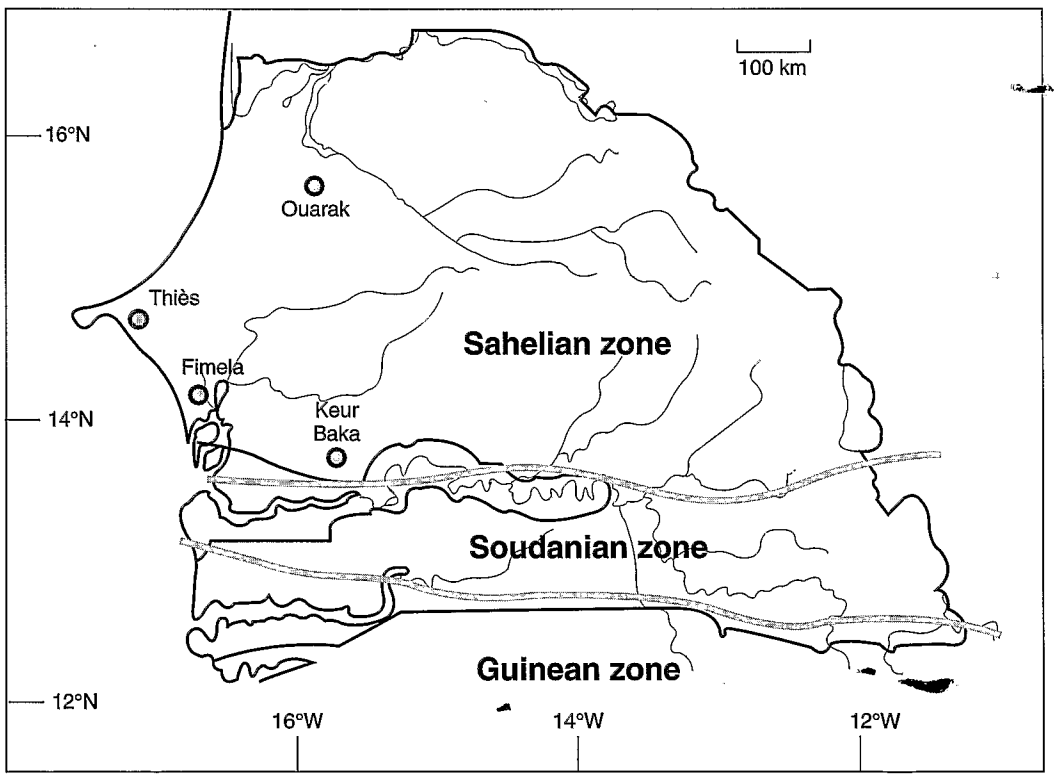

Fig. 1. Map of Senegal showing the geographical origin of *Caryedon serratus* samples

Cassia sieberiana and groundnut, *Arachis hypogaea*. *Bauhinia tomentosa*, an uncommon and introduced ornamental host, and *Piliostigma thonningii*, absent from the northern part of Senegal, were excluded from this study.

Materials and Methods

Study site

In Senegal, phytogeographic regions are basically determined by rainfall. Parallel isohyetes define, from north to south, the sahelian (less than 800 mm rainfall), soudanian (800 to 1000 mm rainfall) and guinean (more than 1000 mm rainfall) regions (Fig. 1). Samples originated from Ouarak (16°04'W, 15°33'N), Thiès (16°56'W, 14°48'N), Fimela (16°41'W, 14°08'N), and Keur Baka (15°57'W, 13°56'N) all within the sahelian zone.

C. serratus samples

Beetles used in this study were bred as eggs, larvae or pupae from pods collected on different hosts species. Samples were named after their host plant and geographic origin: in Fimela, sample Afi was obtained from *Arachis hypogaea*, Bfi from *Bauhinia rufescens*, Cfi from *Cassia sieberiana*, Pfi from *Piliostigma reticulatum* and Tfi from *Tamarindus 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*. Cfi and Bou were reared in the laboratory for one generation on their usual host.

Pods were collected as soon as they reached maturity, when new infestations started, except for '*C. sieberiana*' samples, which were collected 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 except Afi, which was taken from a farmer's store. For each host-plant species, enough pods were collected to obtain at least 40 *C. serratus* adults. Insects were used as soon as they emerged. Seventeen samples (total: 680 individuals of both sexes) were analysed. In case of doubt, genitalia were examined in order to avoid any confusion with *Caryedon crampeli* (Pic), a species which also feeds on *B. rufescens*, *C. sieberiana* and *P. reticulatum*. Voucher specimens, including genital parts, are kept in the I.F.A.N. (Institut Fondamental d'Afrique Noire Cheikh Anta Diop, Dakar) collections.

Starch gel electrophoresis

Sample preparation

Live individual seed-beetles were crushed in an ice bath in 100 µl of buffer (pH 7.4) made of 0.1M Tris, 0.04M L-cystein, and 10% Triton X100. Homogenates were collected in Eppendorf tubes and centrifuged at 26,000 rpm for 20 min. The extract was collected between the resulting supernatant (lipid) and the deposit (solid) with a syringe. It was then transferred to filter paper wicks (15 x 6 mm) which were immediately loaded into gels.

Running conditions and staining

Electrophoresis was performed in a 17 x 17 x 1 cm 12% potato starch gel. Gel preparation and migration techniques were those described by Moretti et al. (1957) and Pasteur et al. (1987). Gel buffer was made of Tris (0.02M) and maleic acid (0.01M). Gel slabs were run for 15 h in a 0.02M Tris and 0.01M maleic acid buffer of pH 7.3, under a constant 120 V potential. Specific staining solutions were prepared according to Lebrun and Chevalier (1990). To stop enzymatic activity, the staining solution was replaced by a 7% acetic acid solution for 1 hour. Gels were then kept for 12 h in the refrigerator in a 15% glycerol solution, then dried at 60°C for 4 h. Twelve enzymatic systems were tested: alcohol dehydrogenase (ADH), diaphorase (DIA), endopeptidase (ENDO), esterases (EST), glutamate oxalo-acetate transaminase (GOT), glucose-phosphate isomerase (GPI), hexokinase (HK), isocitrate dehydrogenase (ICD), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), and phosphoglucomutase (PGM).

Statistical analyses

Data were analysed using Genepop V1.2 (Raymond and Rousset, 1995) and Biosys-1 V1.7 (Swofford and Selander, 1981). The following parameters were estimated:

(1) genetic variability: allele frequencies, mean number of alleles per locus, and rates of polymorphism and heterozygosity. A locus

was considered as polymorphic if the frequency of its most common allele was less than 95%.

(2) genetic equilibrium: deviation from Hardy-Weinberg equilibrium, absence of linkage disequilibrium.

Genotype frequencies were tested against Hardy-Weinberg expectations with panmixia as the null hypothesis. The value of the consanguinity coefficient, *Fis*, represents the heterozygote deficiency of each population at each locus. 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 was used.

Correspondence factor analysis (CFA) was performed using STAT-ITCF V5 (Anonymous, 1991)

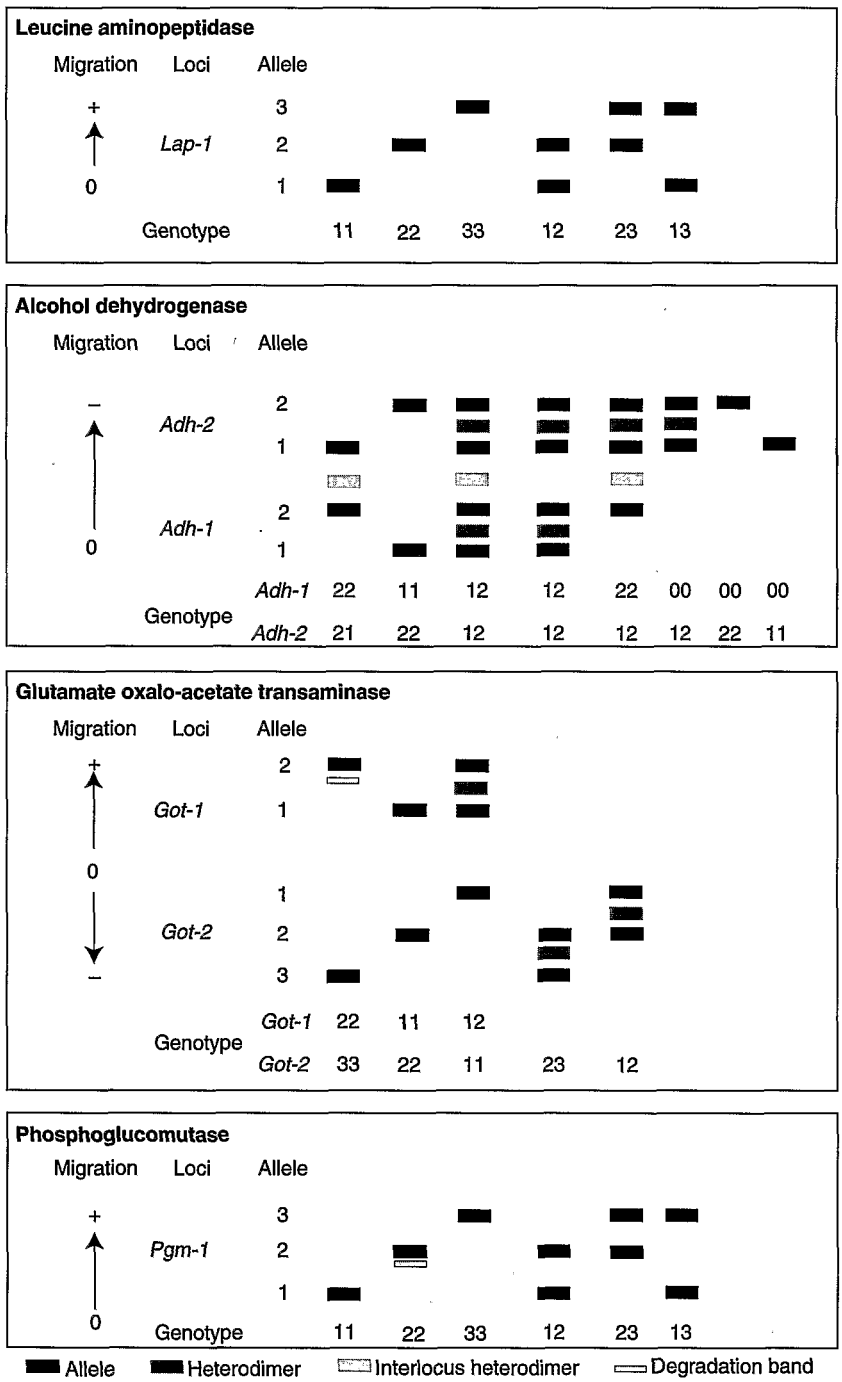

Fig. 2. Genetic interpretation of polymorphic zymograms of *Caryedon serratus* enzyme systems

to ascertain seed-beetle groupings and their relationships with allele frequencies at the various loci.

Results

Enzyme systems

Six enzyme systems (ENDO, PGD, GPI, DIA, HK and EST) failed to stain reliably or did not show clear bandings. Two of them (ICD and MDH) proved to be monomorphic. Only LAP, ADH, GOT and PGM showed scoreable polymorphic loci.

Polymorphic loci:

LAP, E.C. 3.4.1.1.: the profiles showed numerous migration zones which could be interpreted as two loci of a monomeric enzyme: a slow locus (*Lap-1*) coding for an active enzyme with three electromorphs, and a fast-migrating, highly polymorphic locus, with numerous bands of low intensity. The latter was not used.

ADH, E.C. 1.1.1.1.: zymograms exhibited two cathodally migrating activity zones, each with two alleles. The activity of the slowest enzyme (ADH-1), coded by *Adh-1*, was low. At both loci, heterozygotes had three well defined bands. The presence of inter-locus heterodimers suggested the existence of a dimeric enzyme with a duplicated gene, and a post-translational and/or post-transcriptional modification. ADH-1 was not observed in samples from *C. sieberiana*.

GOT, E.C. 2.6.1.1.: anodally migrating GOT-1 had two alleles. The fast allele, which was the most frequent, showed two electromorphs, the slowest of which was a degradation band. Cathodally migrating and dimeric GOT-2 was coded by *Got-2*, with three alleles. Heterozygotes appeared as more-or-less oval bands.

PGM, E.C. 5.4.2.2.: two groups of well separated electromorphs were distinguished. The faster was faint and could not be regularly scored. It was not used in the analysis. The slower enzyme (PGM-1) was coded by a monomeric locus with three alleles. The genotype which was homozygote for the median allele showed two close electromorphs, the slower of which was faint. The existence of double electromorphs in phosphoglucomutase has also been reported by Ouazzani et al. (1993).

Figure 2 shows the electrophoretic profiles of the four enzymatic systems and their genetic interpretation.

Table 1. Allelic frequencies, mean number of alleles (A) and percentage of polymorphic loci (P%) for 17 samples of *Caryedon serratus*

		Samples																	
		Fimela					Keur Baka				Ouarak				Thiès				
Loci	Allele	Afi	Bfi	Cfi	Pfi	Tfi	Akb	Ckb	Pkb	Tkb	Aou	Bou	Pou	Tou	Ath	Bth	Pth	Tth
Got-1	1	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.038	0.000	0.050	0.000	0.050	0.000	0.063	0.000	0.075	0.000
	2	1.000	1.000	1.000	1.000	1.000	0.962	1.000	0.962	1.000	0.950	1.000	0.950	1.000	0.938	1.000	0.925	1.000
Got-2	1	0.000	0.112	0.075	0.000	0.000	0.100	0.000	0.100	0.000	0.100	0.125	0.138	0.000	0.100	0.138	0.075	0.000
	2	1.000	0.887	0.925	1.000	1.000	0.825	1.000	0.813	1.000	0.825	0.875	0.788	1.000	0.850	0.863	0.825	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.075	0.000	0.087	0.000	0.075	0.000	0.075	0.000	0.050	0.000	0.100	0.000
Lap-1	1	0.000	0.125	0.250	0.000	0.063	0.275	0.225	0.237	0.075	0.275	0.138	0.262	0.162	0.250	0.188	0.200	0.063
	2	0.800	0.825	0.750	0.800	0.863	0.712	0.775	0.750	0.850	0.700	0.788	0.700	0.625	0.725	0.750	0.788	0.863
	3	0.200	0.050	0.000	0.200	0.075	0.013	0.000	0.013	0.075	0.025	0.075	0.038	0.213	0.025	0.063	0.013	0.075
Adh-1	1	0.320	0.138	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138	0.000	0.013	0.000	0.225	0.000	0.000
	2	0.680	0.863	0.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	0.863	1.000	0.988	1.000	0.775	1.000	1.000
Adh-2	1	0.962	0.938	0.525	0.962	1.000	0.962	0.213	0.950	1.000	0.962	0.938	0.950	1.000	0.950	0.938	0.938	1.000
	2	0.038	0.063	0.475	0.038	0.000	0.038	0.788	0.050	0.000	0.038	0.063	0.050	0.000	0.050	0.063	0.063	0.000
Pgm-1	1	0.338	0.150	0.075	0.338	0.338	0.262	0.075	0.300	0.325	0.275	0.150	0.262	0.363	0.287	0.112	0.425	0.338
	2	0.563	0.813	0.925	0.563	0.663	0.738	0.925	0.700	0.675	0.725	0.813	0.738	0.637	0.712	0.813	0.575	0.663
	3	0.100	0.038	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.075	0.000	0.000
A		1.7	2.2	1.7	1.7	1.5	2.2	1.5	2.2	1.5	2.2	2.2	2.2	1.7	2.2	2.2	2.2	1.5
P% (.95 criterion)		37.5	62.5	50.0	25.0	25.0	37.5	37.5	50.0	25.0	50.0	62.5	62.5	25.0	62.5	62.5	62.5	25.0

Table 2. Observed mean heterozygosity (Ho), expected mean heterozygosity (Hw), heterozygosity deficit (*Fis*) and deviation tests from Hardy-Weinberg equilibrium for each locus of seed-beetle samples from the different localities in Senegal. The deviations compared with expected values are determined by the χ^2 test. ** $P < 0.01$; * $P < 0.05$

		Samples																
		Fimela					Keur Baka				Ouarak				Thiès			
Loci		Afi	Bfi	Cfi	Pfi	Tfi	Akb	Ckb	Pkb	Tkb	Aou	Bou	Pou	Tou	Ath	Bth	Pth	Tth
Got-1	Ho	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.027	0.000	0.050	0.000	0.060	0.000	0.025	0.000	0.050	0.000
	Hw	0.000	0.000	0.000	0.000	0.000	0.072	0.000	0.073	0.000	0.095	0.000	0.095	0.000	0.117	0.000	0.139	0.000
	Fis	–	–	–	–	–	+0.661	–	+0.649	–	+0.483	–	+0.475	–	+0.791	–	+0.647	–
	p	–	–	–	–	–	0.38	–	0.38	–	0.75	–	0.75	–	0.002	–	0.007	–
Got-2	Ho	0.000	0.025	0.000	0.000	0.000	0.050	0.000	0.075	0.000	0.100	0.050	0.125	0.000	0.100	0.025	0.150	0.000
	Hw	0.000	0.200	0.139	0.000	0.000	0.304	0.000	0.322	0.000	0.304	0.219	0.355	0.000	0.265	0.237	0.304	0.000
	Fis	–	+0.878	+1.000	–	–	+0.839	–	+0.772	–	+0.678	+0.777	+0.655	–	+0.630	+0.897	+0.516	–
	p	–	0.000	0.000	–	–	0.000	–	0.000	–	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–
Lap-1	Ho	0.000	0.200	0.250	0.000	0.275	0.275	0.150	0.200	0.300	0.300	0.325	0.350	0.175	0.300	0.325	0.225	0.275
	Hw	0.320	0.301	0.375	0.320	0.247	0.417	0.349	0.381	0.266	0.434	0.355	0.440	0.538	0.411	0.398	0.340	0.247
	Fis	+1.00	+0.347	+0.345	+1.00	-0.103	+0.351	+0.578	+0.485	-0.114	+0.313	+0.098	+0.216	+0.681	+0.282	+0.197	+0.349	-0.103
	p	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Adh-1	Ho	0.000	0.275	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.275	0.000	0.025	0.000	0.300	0.000	0.000
	Hw	0.000	0.237	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.237	0.000	0.025	0.117	0.349	0.000	0.000
	Fis	–	-0.147	–	–	–	–	–	–	–	–	-0.147	–	0.000	–	+0.152	–	–
	p	–	1.00	–	–	–	–	–	–	–	–	1.00	–	1.00	–	0.37	–	–
Adh-2	Ho	0.075	0.125	0.700	0.075	0.000	0.025	0.425	0.050	0.000	0.025	0.125	0.050	0.000	0.000	0.075	0.075	0.000
	Hw	0.072	0.117	0.499	0.072	0.000	0.072	0.335	0.095	0.000	0.072	0.117	0.095	0.000	0.095	0.117	0.117	0.000
	Fis	-0.02	-0.054	-0.393	-0.026	–	+0.661	-0.258	+0.483	–	+0.661	-0.054	+0.483	–	+1.00	+0.371	+0.371	–
	p	1.00	1.00	0.02	1.00	–	0.038	0.16	0.007	–	0.038	1.00	0.007	–	0.000	0.12	0.12	–
Pgm-1	Ho	0.560	0.325	0.150	0.475	0.275	0.275	0.100	0.250	0.550	0.300	0.325	0.225	0.225	0.325	0.325	0.300	0.275
	Hw	0.475	0.316	0.139	0.560	0.447	0.387	0.139	0.420	0.439	0.399	0.316	0.387	0.462	0.410	0.322	0.489	0.447
	Fis	+0.164	-0.016	-0.068	+0.164	+0.396	+0.301	+0.291	+0.415	-0.242	+0.259	-0.016	+0.429	+0.522	+0.219	+0.002	+0.397	+0.396
	p	0.019	1.00	1.00	0.019	0.029	0.09	0.18	0.01	0.16	0.12	1.00	0.01	0.01	0.24	0.59	0.02	0.03
Overall	Ho	0.092	0.158	0.183	0.092	0.092	0.108	0.113	0.100	0.142	0.129	0.183	0.133	0.071	0.125	0.175	0.133	0.092
	Hw	0.159**	0.195	0.192*	0.161	0.116	0.209**	0.137*	0.215**	0.117	0.217**	0.207	0.229**	0.171*	0.216**	0.237	0.231**	0.116
	Fis	+0.379	+0.201	+0.221	+0.374	+0.146	+0.562	+0.203	+0.560	-0.178	+0.478	+0.131	+0.453	+0.601	+0.584	+0.323	+0.456	+0.146

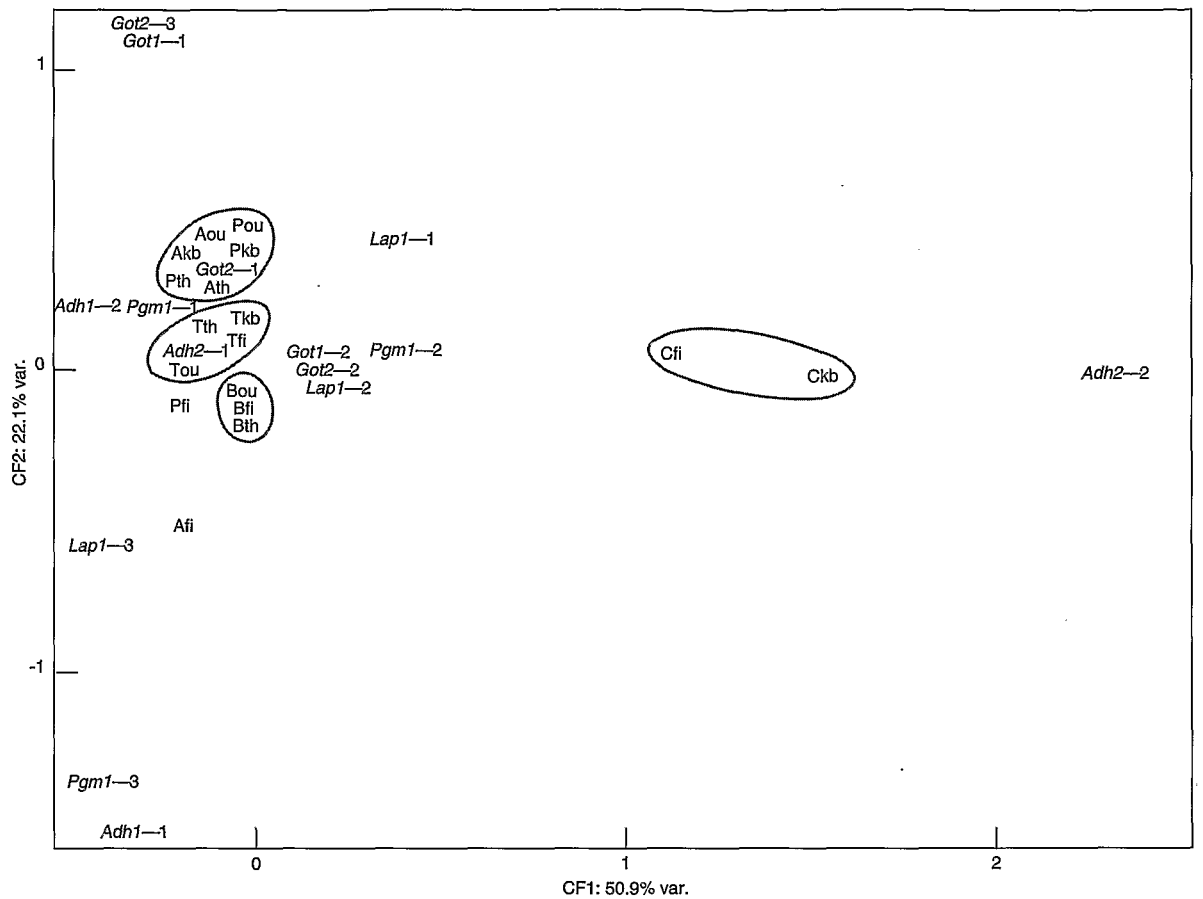

Fig. 3. CFA scattergram of seed-beetle samples from the different localities in Senegal. See Materials and Methods for naming codes. Alleles are numbered according to their migration speed; for example *Adh1*-1 is the slowest allele of *Adh-1*

Genetic analysis

Parameters of genetic variability of the 17 samples are given in Table 1. The number of alleles varied from 1.5 to 2.2 (mean: 1.8). 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, except for '*P. reticulatum*' and 'groundnut' samples. The proportion of polymorphic loci varied between 25.0 and 62.5%.

Observed (Ho) and expected (Hw) heterozygosities, together with deviations from Hardy-Weinberg expectations are shown in Table 2 for each locus and each sample. Over all loci, highly significant ($P < 0.001$) deviations from Hardy-Weinberg expectations were observed in all 'groundnut' and '*P. reticulatum*' samples, while '*C. sieberiana*' samples were in slight disequilibrium ($P < 0.05$). No significant deviation was found in samples from *B. rufescens* and *T. indica*. A slight excess of heterozygotes was however detected in Tkb (*Fis* multilocus = -0.242). Observed deviations were caused by a strong heterozygote deficiency (0.13 < *Fis* multilocus < 0.60). Over all loci, deviations from Hardy-Weinberg expectations occurred in populations from the same locality ($P < 0.01$). Finally, over all loci and samples, probability of deviation from Hardy-Weinberg expectations was highly significant ($\chi^2 = 622.5$, df = 32). No linkage disequilibrium was found among any pair of allozymes for any sample ($P > 0.05$).

Correspondence factorial analysis separated samples according to their host plants (Fig. 3). The most discriminating loci were *Adh-1* and -2 and *Got-1* and -2. The slow allele of *Adh-1* (*Adh1*-1) and the fastest allele of *Pgm-1* (*Pgm1*-3) were responsible for the clustering of '*B. rufescens*' samples, *Adh2*-2 for the clustering of '*C. sieberiana*' samples, *Adh2*-1 for the clustering of '*T. indica*'

samples, *Got1*-1 and *Got2*-3 for the grouping of '*P. reticulatum*' and 'groundnut' samples. Pfi and Afi did not cluster with the other '*P. reticulatum*' and 'groundnut' samples.

Discussion and Conclusion

With 1.8 alleles per locus, *C. serratus* populations show a rather low enzyme polymorphism. The percentage of polymorphic loci (44.8%) is similar to percentages commonly reported for other groups of insects: 56% in *Phlebotomus papatasi* (Kassem et al., 1993); 53.6% in *Hypera postica* (Hsiao and Stutz, 1985); 37.7% in *Dacus cucurbitae* (Yong, 1992), and 35% in *Yponomeuta* spp. (Menken, 1982). The comparison of polymorphism rates between different insect species is however, not straightforward as it depends not only on the criterion (95 or 99%) used to determine polymorphism, but also on the enzyme systems used. Polymorphism is maintained in laboratory samples of *C. serratus*. Samples Bou and Cfi, which were reared for one generation from a small number of females have rates of polymorphism equal to or even higher (50.0% and 62.5%, respectively) than most of the other samples. In these samples, one might have expected low genetic variability, resulting from a founding effect (Hartl, 1994; Kassem et al., 1993). The persistence of high variability suggests a low degree of consanguinity in field-collected individuals, at least in these two samples.

The overall average expected heterozygosity (0.184) of *C. serratus* in Senegal is somewhat lower than reported in most other Coleoptera: 0.160 in *Coccinella 7-punctata* (Krafsur et al., 1992), 0.206 in *Leptinotarsa decemlineata* (Jacobson and Hsiao, 1983), 0.231 in *Hypera postica* (Hsiao and Stutz, 1985), 0.236 in *Anthonomus grandis* (Terranova, 1981), all of which belong to the same superfamily (Phytophagoidea) as *C. serratus*. It is however higher than in most insects (mostly non-Phytophagoidea): 0.074 in 23 insects species (Nevo, 1978); 0.083 in *Yponomeuta* spp. (Menken, 1982); 0.116 in *Phlebotomus papatasi*, 0.137 in 170 insect species (Ward et al., 1992). Higher genetic variability is expected in species exploiting variable environments than those restricted to more stable environments. In the case of the Senegalese population of C. *serratus*, with five hosts having distinct phenologies, one may expect a rather high variability. It should also be mentioned that scoreable and informative enzyme systems vary from one insect species to the other.

Sample Tkb exhibits a homozygote deficiency which suggests an open genetic system in which females mate preferentially with heterozygous males. In all other samples, mean heterozygosity is lower than expected under Hardy-Weinberg predictions, which indicates a preferentially assortative reproductive behaviour. In spite of this high heterozygote deficiency, only '*A. hypogaea*' and '*P. reticulatum*' samples exhibited a significant deviation from Hardy-Weinberg expectations.

On *Piliostigma reticulatum*, the population dynamics of *C. serratus* seems to depend on the succession of a shorter period (November to February) when ripe pods are abundant in the field, and a longer period (March to October) when they become progressively less available, then absent. When the first pods reach maturity after the rainy season (in November), *C. serratus* population levels are usually very low. Infestation rates on *P. reticulatum* at that time are not higher than 2 to 4 eggs per 1000 pods, which suggests that '*P. reticulatum*' samples consist in a mixture of the F1 of a limited number of founding females. On newly harvested groundnut, infestation rates are similarly low, in the order of 1 egg per 10,000 seeds (Matokot et al., 1987 in Congo; personal observations in Senegal). This partly explains why '*A. hypogaea*' samples are not panmictic.

On the contrary, *B. rufescens* trees bear pods all year round. Infestation rates do not exhibit the wide fluctuations observed on *P. reticulatum*, a situation which is more favourable to panmixia. *Tamarindus indica* fruition reaches a peak in March–April. Inter-tree variability is high, so that a few ripe tamarind pods may be found in a given area at any time of the year (Ndiaye, 1991). Moreover, tamarind seeds are a common by-product of several local meals in Senegal. They are potential reservoirs for *C. serratus* before new pods mature. As indicated earlier, '*C. sieberiana*' samples were collected late in the season, after the *C. serratus* population had undergone several generations in the field. This precludes the development of an artificial founding effect and certainly explains the absence of deviation from Hardy-Weinberg expectations.

The relative genetic isolation between these populations is best explained by the fact that they feed on different host plants: samples from *B. rufescens*, which are all very similar, are characterised by allele frequencies which are different from other samples. The same is true for samples from *T. indica* and *C. sieberiana*. These

convergent results seem to indicate that host plants play a major part in the genetic structuring of the *C. serratus* population in Senegal. To the contrary, '*A. hypogaea*' samples cannot be differentiated from '*P. reticulatum*' samples by their allele frequencies. These samples show morphological similarities as indicated by morphometric analysis (Sembène and Delobel, 1996).

Geographic distances in the order of 200 to 400 km do not seem to play a decisive role in this structuration: except for groundnut and *P. reticulatum*-associated forms, samples from the same locality show a low degree of relatedness (Fig. 3). In this respect, samples collected in Fimela (Pfi and Afi) clearly stand out: Afi, with an unusually high frequency of *Adh1*-1 and *Lap1*-3, segregates far from other samples. This could be explained by the fact that Afi was collected in a farmer's store. This storage population may have originated from a very small initial infestation, with a high consanguinity level as a result. Fimela data also suggest that infestation of *P. reticulatum* may at times originate from *T. indica* and/or *B. rufescens*.

The generally strong association of *C. serratus* genotypes with particular hosts plants would indicate the existence of relatively isolated populations or biotypes. Seed-beetles associated with *P. reticulatum* exhibit strong genetic similarity with those associated with groundnut, indicating a close relationship between these two groups of insects. The peculiarity of *C. sieberiana*-associated forms questions the accuracy of the present classification of this group of insects. Hybridisation experiments are underway to elucidate its taxonomic status.

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