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Research Article

Biological and Molecular Evidence for the Existence of Bruchid Ecotypes in the Nigerian Cowpea Ecosystem

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Abstract

The oviposition, growth and development of six populations of *Callosobruchus maculatus* (IT, PH, UM, MD-1, MD-2, MD-3) from different localities in Nigeria were studied on a resistant (TVu 2027) and susceptible (Ife Brown) cowpea cultivar and four cultivars of African yam bean, Progenies obtained by cross-mating individuals from the different populations were also studied.. Oviposition was highest in the MD insect beetle population and lowest in the UM population. IT and PH populations had the highest adult emergence and shortest development times on the resistant control. Genomic differences among bruchid populations were confirmed by the Random Amplified Polymorphic DNA (RAPD) fingerprinting technique with PH population being the most distant. All progenies with IT as the female parent also had better emergence and shorter development time than their reciprocal crosses or those involving other populations, but this varied with the fitness of the male parent. The UM population averaged <10% adult emergence on TVu 2027 compared to 71.9 % on the susceptible control, but was better adapted to the AYB (66 % emergence) than were other populations. These results suggest possible existence of ecotypes, of this bruchid species in Nigeria with potentially important implications for the development and deployment of resistant cowpea varieties.

Keyword

Introduction

Grain legumes suffer drastic reduction in market value and nutritional properties because of damage in storage by bruchids of the genus *Callosobruchus* spp. [1-4]. This genus has a pantropical distribution and includes the most important post-harvest pest species of cowpea worldwide [5-6]. Most species are multivoltine making them very destructive in storage [7]. The pest species in the genus worldwide include *C. maculatus Fabricius*, *C. chinensis (Linnaeus), C. analis* (Fabricius), *C. rhodesianus* (Pic), *C. subinotatus* (Pic), and *C. phaseoli* (Gyll). These species have been reported to attack cowpea [6-8], causing up to 70 % damage to seeds of susceptible cultivars after six months of storage. Although several insecticides are used to protect stored cowpea, alternative control strategies such as the use of resistant cultivars (e.g., TVu nos. 2027, 11952 and 11953, IT86D-4980), solar heating, and the use of a wide range of liquid or powdered plant extracts and various plant oils and bagging systems greatly reduced the need for synthetic insecticide use in storage [9, 10, 3, 11]. Bruchid populations from different parts of the world have been shown to vary in their basic bionomics [12-14]. Various workers have also reported increased survival in different bruchid populations on the resistant cowpea, TVu 2027 [15, 1, and 16]. The bruchid populations in these studies were obtained either from different localities in the same country, or from different countries. These differences in feeding behaviour on the resistant cowpea are thought to be, at least in part, due to geographical isolation and possible adaptation to different ecological conditions [17, 12, and 18]. If this were the case, it mis possible that in a large country with wide-ranging ecological zones (Figure 1), distinct bionomic differences may be prevail among bruchid populations from widely disjointed regions. In addition, in bruchid populations attacking more than one host plant, adaptation to different defense compounds may be an important factor leading to evolution of differences between either sympatric or allopatric populations. Other workers have referred to such sub-populations as strains [12-19], biotypes [16] ecotypes (Shade et al., 1996; Tarver et al. 2006), or simply "cultures" [20-21].

Irrespective of the mechanism(s) that have given rise to new and distinctive pest populations, their existence poses a serious threat to agricultural production, and the success of newly developed resistant cultivars which may not have any pre-adaptive mechanisms to protect themselves. Cowpea resistance to *C, maculatus* has been sustained for several years, but it cannot be predicted how long it will continue to be effective because of its narrow genetic base [22]. New and more diversified resistance genes are needed to broaden the genetic base of this resistance. The search for new sources of resistance genes need not be limited to cowpea since, other plant genera can also be exploited. One of the potential sources for resistance to cowpea pests is the African yam bean, *Sphenostylis stenocarpa* Hochst A. Richard (Harms), an under-exploited African legume that appears to be resistant to a wide range of cowpea pests [23-24]. This legume grows in the humid forest and transition zones of West and Central Africa, and the savanna of east and southern Africa [25-26]. *C. maculatus* survives on some varieties of *S. stenocarpa* better than on others. This behaviour is common from an ecological standpoint but the interaction between C. maculatus and the African yam bean was striking. We became interested in the interaction because beetle populations from different parts of Nigeria varied drastically in their ability to survive on *S. stenocarpa*. This led us to investigate the extent to which this phenomenon also occurs with the same beetle species on cowpea in Nigeria, and how it affected the search for pest resistant cowpea. There is some published evidence of intra-specific variation within *C. maculatus* [12, 13, 27, 18, 28,29]. These and other studies used various techniques. In this study, we investigated the possible existence of intra-specific variation among *C. maculatus* from different parts of Nigeria on cowpea
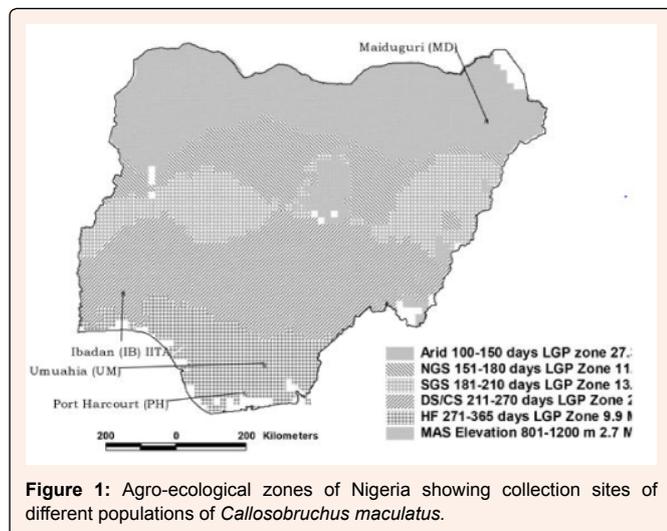
Figure 1: Agro-ecological zones of Nigeria showing collection sites of different populations of *Callosobruchus maculatus*.

and African yam bean hosts using biological and molecular approaches to detect small differences between populations [30-34].

Materials and Methods

Insects

C. maculatus adults and eggs laid on cowpea or African yam bean were collected from four localities in Nigeria, namely Port Harcourt [PH], Umuahia [UM], Maiduguri-1 to 3 [MD-1, MD-2, MD-3], and Ibadan (IITA-laboratory population [IT]) (Figure 1). There were three different beetle collections in the Maiduguri area at different times of the year, from different cowpea cultivars (known only as "local cowpea") in different village markets. The Maiduguri collections were treated as different beetle populations because of the proximity to the Cameroon cowpea market.. All the populations were subsequently maintained on the cowpea variety Ife Brown, following procedures described by Singh & Jackai in the Storage Entomology Laboratory of the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria. Ambient rearing temperature was 27 ±2 ºC and relative humidity ranged from 70-80 %. Insects from the different localities are hereafter referred to as 'populations'.

Beetle development on cowpea and African yam bean

Twenty seeds each of the resistant cowpea cultivar, TVu 2027, the susceptible local cowpea cultivar (Ife Brown), and five local cultivars of African yam bean (um1, um2, um3, um4, and um5) were purchased from a local market at Umuahia (Figure 1). These were each placed in a plastic box measuring 6.5 x 6.5 x 2.5 cm. There were thus five replications of each of the seven cultivars. The seeds were fumigated with aluminium phosphide pellets for 24h to eliminate any concealed infestation and then left in an airy, insect-free environment for 48h to dispel any residues of the fumigant. The seeds in each box were subsequently exposed for 24h to one pair of newly emerged adults from the stock culture of each beetle population and ovi posited eggs were left to incubate for seven days after which they were counted. From 20 days after ovi position, the boxes with seeds were examined daily for adult emergence until 55 days after ovi position, or until the second generation, progeny started ovi positing on the susceptible cultivar.

Demographic parameters of beetle progeny on TVu 2027 and Ife Brown

Reciprocal crosses of the six beetle populations were made using insects from the laboratory culture of each population. The F1 adults were then kept on Ife Brown for one generation before being used in this experiment. Newly emerged adults were exposed to seeds for 24h as above but using only the cultivars TVu 2027 and Ife Brown. Indices taken in the two experiments above were number of eggs laid per container, development time from oviposition to adult and percent adult emergence. An index of suitability of a substrate for insect development, Growth Index (GI), was calculated as log % AE / TDT [35]. Where AE is the percent adult emergence and TDT is the development time. This was used to compare the F1 and reciprocal crosses

Molecular differentiation of *C. maculatus* populations using RAPD markers

DNA was isolated using a method adapted from [32]. The head capsule and prothorax of one beetle from each population were removed and placed individually in

an Eppendorf tube and homogenized using a conical glass pestle. One ml homogenate consisted of 80 μl 0.008 M NaCl, 0.16 M sucrose, 0.06 M EDTA, 0.5 % SDS, 0.1M Tris-HCl buffer, pH 8.6. The homogenate was incubated at 65ºC for 30 minutes and then 40 μl of potassium acetate (8M) was added to the final solution. This was then incubated for 30 minutes at 4ºC. Samples were centrifuged at 12000-x g for 10 minutes at room temperature in an Eppendorf centrifuge. The supernatant was removed into a fresh tube, 100-μl 95 % ethanol added and the solution was again centrifuged at 12000 xg for 10 minutes. The supernatant was then discarded and the pellet washed with 70 % ethanol, dried and re-suspended in de-ionized water and stored at -70 ºC until used.

PCR and agarose gel electrophoresis

Oligonucleotide primers (10 mers each) tested in this study were purchased from Operon Technologies (Alamenda, Ca, USA). RAPD-PCR analysis was done using the method described by [36]. Two concentrations of each template DNA (24 μg and 96cμg per reaction) were used to test reproducibility and eliminate sporadic amplification products from the analysis. Amplifications were performed in a 25 μl reaction mixture consisting of 10 μg of diluted genomic DNA (5 μl), 2.5 μl of 10 X reaction buffer (Promega),100 μM each of dATP, dCTP, dGTP, and dTTP, 0.2 mM MgCl2, 0.5% Tween-20 and 2 units of Taq polymerase (Promega). A single primer was used in each reaction. The reaction mixture was overlaid with a drop of mineral oil to prevent evaporation. Amplification was performed in a Perkin Elmer Thermocycler, Model 9600. The cycling program: (1) one cycle at 94 ºC for 1 minute, followed by 40 cycles at 94ºC for 1 minute and 72 ºC for 1 minute; (2) one cycle at 72 ºC for 5 minutes. Amplification products were maintained at 4 ºC until electrophoresed. The reaction products were resolved by electrophoresis in a 1.4 % agarose gel using 1 x TAE buffer at 150 constant V for 2.5 hours. A 1kb ladder (Life Technologies, Gaithersburg, MD, USA) was included as a size marker. Gels were visualized by staining with Ethidium Bromide solution (0.5 μg/ml) and banding patterns were photographed using a UV light and a red filter. RAPD markers that were consistently reproduced in at least two PCR reactions were taken into account for further analysis. Fragment sizes of RAPD bands were estimated from the gel photograph by comparison with the 1 kb ladder marker. From the whole set of reproducible RAPD bands generated in the eight populations (two more than the number used in the biological tests) by the different primers, only bright bands were considered for scoring as either present (1) or absent (0). A data matrix with primers in columns and ecotypes was assembled from these scores. All DNA fragments were scored and used to estimate the Genetic Similarity Coefficients (GS) according to Jaccard (1908). This does not consider the joint absence of a marker as an indication of similarity (Sneath & Sokal, 1973). GSxy = a / (a + b + c) where; GSxy represents genetic similarity between two individuals x and y; a indicates the number of polymorphic bands present in both individuals; b is the bands present in x but absent in y; and c is the number of bands present in y but absent in x. Further analysis was carried out with Unweighted Pair Group Method of Arithmetic Average (UPGMA) algorithm and a similarity dendrogram was generated from this. This was designed to group the populations into clusters based on genetic similarity.

Statistical Analyses

All data from experiments 1 and 2 were analysed using General Linear Model ANOVA, and where significant results were obtained, multiple comparisons were performed using Fisher's Protected LSD. Percentage data were arcsine transformed before analysis [37]. Comparisons of parental and progeny data were made using Chi-square analysis. Zero values were not included in any of the analyses. Primed PCR fragments were analysed using GENSTAT 5 Release 3.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, and Hertfordshire, UK).

Results

Oviposition

Overall, without regard to the host plant (cultivar), the UM beetle population was least fecund while the ovi position levels among the other populations were generally similar. Of the other populations, MDs were the most fecund across cultivars. The MD-2 and 3 had the highest ovi position on the two cowpea cultivars. There was however, no clear-cut preference for any of the cultivars (Table 1), within the different host plants.

Larval development

Based on the development data from *S. stenocarpa*, we distinguished two population groups (Table 2). The first group (beetles from PH, IT, MD-1 and MD-3) completed larval development in over 30 days on the *S. stenocarpa* cultivars from Umuahia. Larvae in the other group, UM and MD-2 populations, developed faster (less than 28 days) on these same host plant genotypes. Two other groups were distinguished based on the developmental time on the resistant cowpea, TVu 2027. The first was the IT population which developed in a relatively short time (27.9 days) compared to the PH population

Table 1: Mean number of eggs laid by *Callosobruchus maculatus* collected from Different Localities in Nigeria+ on Seeds of *S. stenocarpa* and *Vigna unguiculata.* (n = 25-50 adults).

Cultivar	*Callosobruchus Maculatus* Population					
	UM	PH	IT	MD-1	MD-2	MD-3
S. Stenocarpa um1						
	27.3aA	56.0aC	63.7bC	62.3bBC	52.7aBC	40.3aAB
um2	37.7abA	56.0aA	51.3abA	58.0abA	52.3aA	56.3bcA
um3	44.3abAB	45.0aAB	34.3aA	63.3bC	57.7aBC	46.3abAB
um4	52.0bA	48.3aA	39.7aA	62.0bA	57.0aA	48.0abA
um5	30.7aA	62.3aBC	38.7aAB	63.0bC	65.0aC	55.0abcBC
V. Unguiculata						
TVu 2027 (rc)	29.0aA	49.0aBC	53.3abC	35.7aAB	60.7aC	58.7bcC
Ife Brown (sc)	40.3abA	46.0aAB	50.0abABC 48.3abAC		58.3aBC	64.3cC
Mean (across all cultivars)	37.3A	51.8BC	47.3B	56.1C	57.7C	52.7BC

rc = resistant control; sc = susceptible control; +see map of Nigeria: UM = Umuahia; PH = Port Harcourt; IB = IITA Ibadan; MD1,2,3 = Maiduguri. Values followed by the same lowercase (columns) or uppercase (rows) letters are not significantly different at P ≤ 0.05 (Fisher's Protected

Table 2: Mean development time from egg to adult *Callosobruchus maculatus* from different localities in Nigeria+ kept on seeds of *S. stenocarpa* and *Vigna unguiculata.* (n = 40).

Cultivar	*Callosobruchus Maculatus* Population					
	UM	PH IT		MD-1	MD-2	MD-3
S. Stenocarpa um1						
	26.9abcA	34.0abcB	34.5cB	26.8aA	25.0aA	36.1cB
um2	26.9abcA	29.5abAB	34.9cB	34.5abB	25.3abA	32.7bcB
um3	27.7bcB	31.0abcC	34.2cD	44.0cE	25.7abA	31.6bC
um4	26.5abA	26.2aA	34.5cC	40.0bcD	25.0aA	30.7bB
um5	27.8bcAB	36.5bcCD	34.0cC	43.0cD	25.5abA	33.2bcBC
V. Unguiculata						
TVu 2027 (rc)	31.0cAB	37.6cB	27.9bA	*	30.0cAB	31.6bAB
Ife Brown (sc)	23.4aB	26.1aC	22.7aA	26.9aD	26.7bD	26.6aCD
Mean (across all cultivars)	27.0A	31.4B	32.0B	34.1B	26.2A	31.8B

*no survivors; rc = resistant control; sc = susceptible control; +see map of Nigeria: UM = Umuahia; PH = Port Harcourt; IT = IITA Ibadan; MD1, 2, 3 = Maiduguri. Values followed by the same lowercase (columns) or uppercase (rows) letters are not significantly different at P ≤ 0.05 (Fisher's Protected LSD).

which took (37.6 days) (F = 7.999, d.f. = 5.99, p = 0.0001). All other populations had larval developmental times between 27.9-37.6 days but similar to the PH population (Table 2).

Adult emergence

The development time of UM & MD-2 population was relatively short on *S. stenocarpa* (Table 2). However, daily emergence data revealed that only a few MD-2 individuals actually contributed to the population (Figure 2). In addition, survivorship to the adult stage on *S. stenocarpa* was high only in the UM population (Table 3). The daily emergence patterns of this population were also not similar on all the cultivars (Figure 2). All other beetle populations had a distinct emergence peak on Ife Brown within the first week. Most of the eggs that were laid on the seeds hatched and larvae penetrated the seeds. Therefore, suitability of the seeds for larval development (within-seed mortality of [29-35], rather than larval penetration into the seed played an important role. All beetle populations performed poorly on TVu 2027 compared to Ife Brown (Table 3), even though the IT and PH beetles were relatively more successful than others in developing on this resistant cultivar. Furthermore, although all MD populations appeared to be susceptible (F = 13.307, d.f. = 4, 6, p = 0.0038) to TVu 2027, MD-1 was the most sensitive and produced no adults (Table 3).
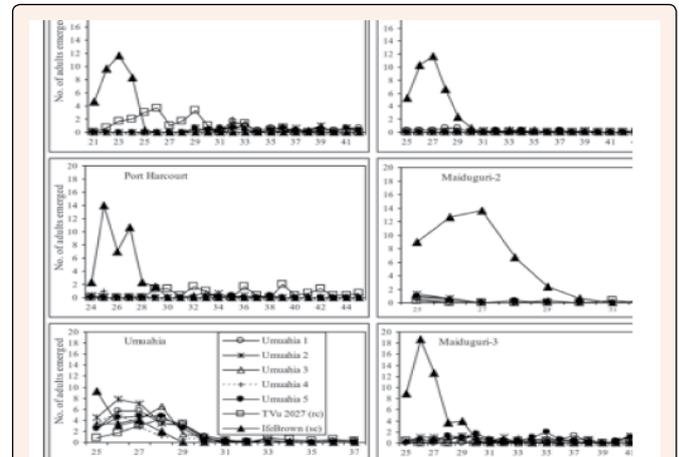

Figure 2: Daily emergence patterns of different populations of *Callosobruchus maculatus* on African yam bean (AYB) (Umuahia 1-5) and cowpea (rc and sc). The AYB cultivars [Umuahia 1-5] were from local markets in Umuahia; rc = resistant control, sc = susceptible control.

Beetle progeny performance

With a few exceptions, progeny from reciprocal crosses showed greater fitness than their parents on both S. *stenocarpa* and TVu 2027 for all variables recorded, with the exception of development time where progeny values were equal to, or higher than, those of the parents (Figure 3). Fitness of the progeny (percentage adult emergence and growth index) seemed to increase irrespective of the direction of the cross; also, the MD-1 population that produced no offspring from TVu 2027 (Table 3) did so when crossed with other beetle populations (Figure 3). Crosses involving females of PH and IT produced more adults than was the case from other crosses. The susceptible cultivar, Ife Brown, was suitable for the growth and development of the hybrids as well as the parents. The data are therefore not presented here.

PCR-RAPD analysis

Of the 60 random oligonucleotide primers (Operon Technologies sets H, I and Q) evaluated for their ability to prime PCR amplification of beetle genomic DNA, 50 resulted in only limited amplifications which were visualized in the gels as faint bands or smears. The remaining 10 primers adequately amplified the genomic DNA, giving reproducible RAPD amplification patterns with individual fragments that stained intensely (Figure 4). These primers were useful for detecting polymorphism among the beetle populations.

Table 3: Mean percentage emergence of adult *Callosobruchus maculatus* from different localities in Nigeria+ kept on seeds of *S. stenocarpa* and *V. unguiculata* cultivars. (n = 80).

Cultivar	**Callosobruchus Maculatus Population**					
	UM	PH	IT	MD-1	MD-2	MD-3
S. stenocarpa						
um1	75.4cE	0.5aA	11.9aD	4.1aBC	1.2aAB	10.6abCD
um2	66.6bcD	1.5aA	10.9aB	2.3aA	3.9aA	20.3bC
um3	44.0bcC	2.8aA	16.6aB	0.5aA	3.4aA	16.2abB
um4	78.3cD	4.3aAB	14.8aBC	0.9aA	0.5aA	19.3bC
um5	66.2bcD	1.0aA	12.8aBC	3.7aAB	2.9aA	22.9bC
V. unguiculata						
TVu 2027 (rc)	4.4aB	29.7bC	35.8bC	0.0aA	1.6aAB	4.0aB
Ife Brown (sc)	71.8cA	84.5cA	70.1cA	78.1bA	78.9bA	77.0cA
Mean (across all cultivars)	58.1C	17.7AB	24.7B	12.8A	13.2A	24.3B

rc = resistant control; sc = susceptible control.; +see map of Nigeria: UM = Umuahia; PH = Port Harcourt; IT = IITA Ibadan; MD1,2,3 = Maiduguri. Values followed by the same lowercase (columns) or uppercase (rows) letters are not significantly different at P ≤ 0.05 (Fisher's Protected LSD).
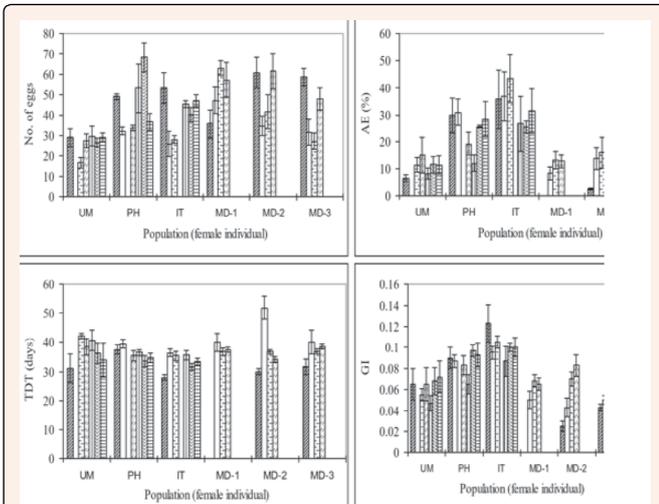
Figure 3: Egg deposition, development time, adult emergence and suitability index of progeny from reciprocal crosses between different populations of *Callosobruchus maculatus* kept on cowpea cultivar TVu 2027 (resistant to the IITA population). (IT = IITA Ibadan; PH = Port Harcourt; UM = Umuahia; MD-1, 2, 3 = Maiduguri).
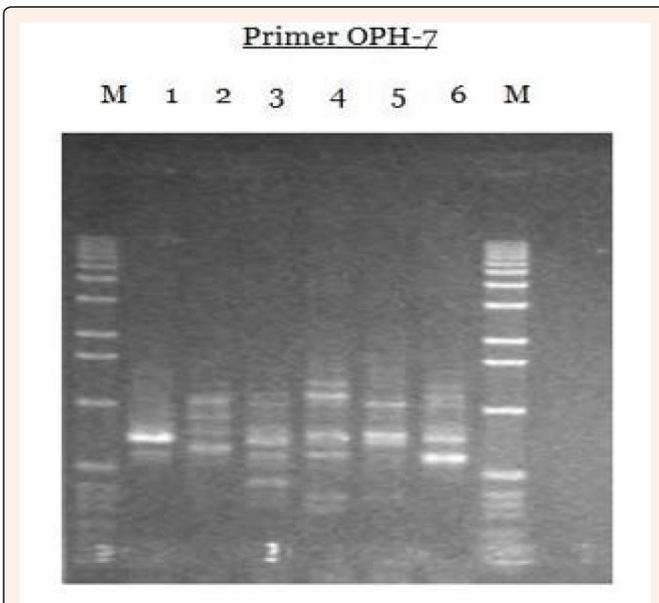

Figure 4: RAPD electrophoretic profiles of genomic DNA from eight beetle populations of *Callosobruchus maculatus* amplified with primer OPH-7. Lane M = 1 kb DNA size marker. 1 = IT, 2 = PH, 3 = UM, 4=MD(1), 5 = MD(2), 6=MD(3).

Based on cluster analysis, the overall genetic relationship among the investigated beetles representing different populations is illustrated (Figure 5). Based on this, the insects were categorized into 6 different lineages with the IT and MD2 populations showing the closest relationship (50% similarity) and PH being the most distant from the Ibadan (IT) population. The results also show close relationship between the three MD populations, as well as between PH and UM populations.

Discussion

This study suggests that discrete populations of *C. maculatus* are distinguishable from the study localities used in Nigeria. Based on the demographic responses, particularly in beetle development and survival on *S. stenocarpa* and a contrasting variety of *V. unguiculata* (TVu 2027), of these populations it appears as though "ecotypes" or ecologically discrete populations of this insect occur in Nigeria but were not reproductively isolated. The differences between the bruchid populations were obvious in some cases. For example,

the UM beetles developed well on *S. stenocarpa* while others did rather poorly. Along with all three MD populations, UM did not find the resistant control, TVu 2027, suitable for development. The rather impressive performance of UM beetles on *S. stenocarpa* could be the result of co-evolution. The African yam bean is cultivated for human consumption in Umuahia and other parts of southeastern Nigeria. It is therefore conceivable that *C. maculatus* whose principal host is cowpea, fortuitously colonized *S. stenocarpa* and over time disarmed the seed lectins [23, 24, 38], in the new host in the "UM" region of Nigeria. A great deal of variation in the response of *C. maculatus* to feeding on *S. stenocarpa* cultivars has been reported elsewhere, where it develops well on some [30]and not at all on others [23]. The assumption has always been that the same insect genotype is the same was used in these tests. The possibility of polymorphism in this bruchid had not been previously considered or investigated. *Sphenostylis stenocarpa* is not a common host of *C. maculatus*, except possibly in the eastern and 'middle' belt of Nigeria where production of this crop seems to be concentrated [39, 25]. It is likely that genes for vigour or fitness exist at a rather high frequency within the insect population found associated with the crop. It is also possible that the UM and PH ecotypes had somehow encountered the resistant genes of TVu 2027 (originally from Sokoto in the northwest of Nigeria) [9], which may have spread throughout the cowpea belt of northern Nigeria. Although not grown in the eastern part of Nigeria, TVu 2027 is probably marketed and stored in the southern parts of the country along with other varieties of cowpea. Although there are established trade routes, movement of food commodities within Nigeria can sometimes be unpredictable. Therefore, although more cowpea may be moved annually from northeast to southeast than from northwest to southeast, it is likely that some TVu 2027 found its way to the PH area through the vibrant commercial traffic of western dwellers and other traders who visit or live in Port Harcourt and its vicinity. Even so, this would, in part, only explain in part the relatively good performance of PH on this cultivar (adult emergence approximately 30 %), and not explain the poor performance of the UM beetle population (adult emergence 6.7 %). The possible pre-adaptive occurrence of genes for vigor within the PH population, or inadvertent introductions of IT genes from the western part of the country to the Port Harcourt area, cannot be discounted. The ability of PH and UM beetles to survive on *S. stenocarpa* seemed to be crucial in their genetic separation from the other beetle populations. Poor performance of the MD populations on *S. stenocarpa* and cowpea cultivar TVu 2027 can be explained by their relative isolation. Despite what appears to be a rather high fecundity, the actual adult recruitment into the population reared on these accessions was extremely low, especially in MD-1 and MD-2. This may be explained by the geographic isolation of these insects for long periods from eastern Nigeria where *S. stenocarpa* is grown, and from the northwest of the country where TVu 2027 is grown. These two sections of Nigeria are separated from each other and the western part by extensive stretches of unstable savanna grasslands and transition forests. Alternatively, it may be assumed that there has been little gene flow (movement of resistance genes) to these areas. Consequently, the already isolated *C. maculatus* populations have not encountered the genes in TVu 2027 and *S. stenocarpa*. The performance of IT and PH populations has a different significance. Although development time on TVu 2027 was comparable among all populations, percentage adult emergence and growth index values were several-fold
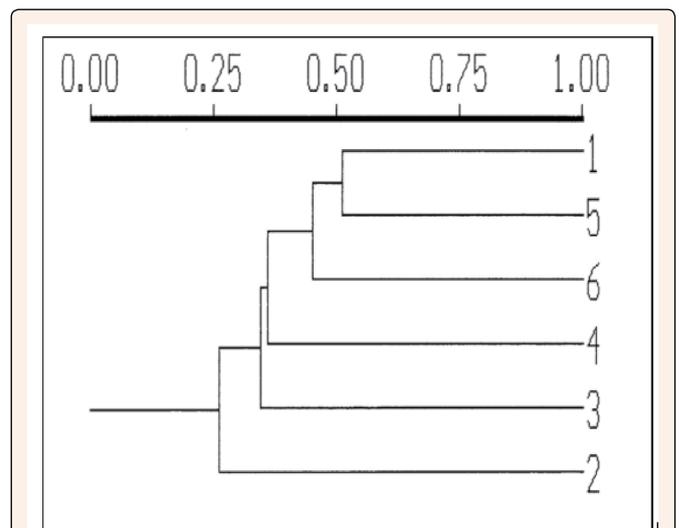

Figure 5: Genetic relationship of representative individuals of populations of *Callosobruchus maculatus* from different parts of Nigeria. (1 = IT [IITA Ibadan]; 2 = PH [Port Harcourt]; 3 = UM [Umuahia]; 4-6 = MD-1, 2, 3 [Maiduguri]).

higher in the IT and PH populations. In agreement with previous workers [12], of all parameters studied, percentage adult emergence gave the best discrimination between populations [12]. we have reported much higher emergence values for the IT population, but this difference could be as a result of the constant out-crossing of the IITA (IT) (stock) population with "wild" insects. This process would likely reduce the gene frequency for fitness in the resident population. However, despite this out-crossing, the population has recently shown increased fitness on TVu 2027 [23].

The possibility of ecotype or biotype formation (i. e. increased frequency of genes for virulence on TVu 2027) upon the application of intense selection pressure has been long suspected [40, 15, 41, 42]. In addition, studies at Purdue University using beetle populations from different parts of the world have confirmed this [27. Differences among the populations are further supported by the PCR-RAPD analysis which shows that the IITA ecotype (IT-1) had the least similarity to the PH and UM ecotypes, a fact clearly demonstrated in their better survival on S. stenocarpa. The PH ecotype stood clearly different from all other groups to which it had only 25% similarity. The MD ecotypes were intermediate, and although closely related, the MD populations appear to be a highly heterogeneous group. This may be explained in part by the possible fortuitous contamination by beetles from neighbouring Cameroon, which has an active cowpea trade with Nigeria. At 50 % level of similarity, all three MD populations are in different lineages. Whether this explains the extreme sensitivity of the MD-1 ecotype to TVu 2027 or not remains to be verified. Since all populations cross-mated readily, we assume that they are the same species. Performance of the beetle progeny was indicative of hybrid vigor with potential to overcome resistance barriers in cowpea. Unfortunately, logistic difficulties precluded including S. stenocarpa in this aspect of our study. Nevertheless, better progeny performance on TVu 2027, especially with regard to adult emergence, is of ecological interest. Firstly, it is quite possible for the different beetle populations to be inadvertently interbred through the activity of traders from different parts of the country or from neighboring countries such as Cameroon. Once this happens, there would occur an increase in the frequency of the genes for counteracting the resistance of TVu 2027 in the population [40]. This is indicated by the superior performance of the hybrids on TVu 2027 (Figure 3), and should indeed be cause for concern to cowpea breeders in the region. Given the foregoing scenario, a different set of resistance genes (possibly a cocktail of genes) would be required to protect resistant varieties derived from either TVu 2027 or S. stenocarpa. The causes of poor development of this insect on S. stenocarpa are unknown, although preliminary results from past work at IITA suggest that both primary and secondary compounds may be involved. Our findings are indicative of the existence of beetle ecotypes with genes for virulence to S. stenocarpa and to a lesser extent cowpea cultivar TVu 2027. Since both inter-, and intra-, specific variation in the genus Callosobruchus exist within and outside Nigeria, a systematic study of bruchid populations is necessary in order to streamline the deployment of resistant cowpea cultivars in the region. This would help to monitor and map the occurrence of genes enabling bruchids to overcome resistance mechanisms, and accordingly guide breeding efforts [43-62].

Acknowledgments

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