# The spatial genetic differentiation of the legume pod borer, *Maruca vitrata* F. (Lepidoptera: Crambidae) populations in West Africa

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## ABSTRACT

We applied a set of microsatellite markers to assess the population structure of *Maruca vitrata* collected at five sites from Burkina Faso, Niger and Nigeria. Observed polymorphism ranged from 1 to 8 alleles per locus, and observed heterozygosities were from 0.0 to 0.8. Analysis of molecular variance (AMOVA) indicated that 67.4% level of the genetic variation was within individuals compared to 17.3% among populations. A global estimate of $F_{ST}$ = 0.1 (ENA corrected $F_{ST}$ = 0.1) was significant ($P \leq 0.05$) and corroborated by pairwise $F_{ST}$ estimates that were significant among all possible comparisons ($P \leq 0.005$). Cluster analysis by the program STRUCTURE predicted that co-ancestry of genotypes were indicative of three distinct populations. The spatial genetic variance among *M. vitrata* in West Africa may be due to limited gene flow, north-south seasonal movement pattern or other reproductive barriers. This information is important for the cultural, chemical, and biological control strategies for managing *M. vitrata*.

## INTRODUCTION

Cowpea production in West Africa accounts for more than 80% of the world's production, but typical infestations by *Maruca vitrata* can cause yield reductions of 20 to 80%. Efforts to enhance the effectiveness of biological control agents that attack *M. vitrata* has been hindered by the lack of population genetic data and information regarding the structure of *M. vitrata* populations in West Africa. Although significant advances have been made to understand the life-history and distribution patterns of *M. vitrata* using light trap and field studies, extensive population-level data are still needed for deployment of biocontrol agents to be effective.

Prior studies in sub-Saharan Africa, including Burkina Faso, have suggested that seasonal flowering patterns of the different host plants on a north-south gradient may influence the migration of *M. vitrata*. This seasonal movement occurs from temperate conditions along the coast into the Savannas of West Africa as the rainy season progresses. Despite the results of previous studies, many questions remain regarding the timing and spatial scale of *M. vitrata* migration patterns.

We developed and applied a set of microsatellite markers for the estimation of genetic variability, population structure, and gene flow among *M. vitrata* in the West African countries of Niger, Nigeria and Burkina Faso. The main objective of this study is to assess the genetic variability in the *M. vitrata* populations across West Africa, where these data will be useful for (i) determining effective areas for the release of biocontrol agents and, (ii) the recommendation of insect resistance management (IRM) protocols aimed at minimizing the threat of selection for insecticide resistance alleles in the major cowpea producing areas in West Africa.

## METHODS

*Maruca vitrata* adults and immature stages were collected from different locations within Burkina Faso, Niger and Nigeria. Total genomic DNA was extracted from the insect samples, and was used to develop microsatellite markers by enrichment with biotinylated $(CA)_{15}$ and $(GA)_{15}$ probes followed by cloning and sequencing of individual microsatellite loci. The original *M. vitrata* genomic DNA samples were also genotyped using polymerase chain reaction (PCR) markers that amplified the microsatellite loci. The mean number of alleles per locus, observed heterozygosity and expected heterozygosity was used to estimate within population genetic variability. Analysis of molecular variance (AMOVA), variance component to compute hierarchical *F*-statistics and their significance were tested at 1000 permutations. The program STRUCTURE 2.3.3. was used to estimate the co-ancestry among *M. vitrata* genotypes and to estimate the number of distinct populations (*K*) present in the set of samples.

## RESULTS

The 6 microsatellite loci used to screen *M. vitrata* samples collected at 5 West African sites showed significant deviation from HWE at 13 instances following 30 locus-by-site calculation. The mean number of alleles per locus was similar across all sample sites and the overall observed heterozygosity was less than expected ($F_{IS} \geq 0.1$) for all populations except at Fada, Burkina Faso (Table 1).

Table 1. Characteristics of the *M. vitrata* individuals across West Africa showing sample size (N), number of alleles (Na), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), and fixation index ($F_{IS}$) per sample site

| Location | Na (Mean per locus) | Ho | $H_E$ | Loci not in HWE |
|---|---|---|---|---|
| Niger | 25 (4.2) | 0.2 | 0.2 | 0 |
| Nigeria | 27 (4.5) | 0.2 | 0.3 | 4 |
| Fada | 18 (3.0) | 0.3 | 0.3 | 3 |
| Farakoba | 23 (3.8) | 0.2 | 0.3 | 3 |
| Kamboinse | 18 (3.0) | 0.2 | 0.3 | 3 |

Table 2. Analysis of Molecular Variance (AMOVA) for Maradi, Niger, Zaria Nigeria and Fada, Farakoba, and Kamboinse locations in Burkina Faso.

| Source of Variation | df | SS | % of Variation |
|---|---|---|---|
| Among Population | 4 | 56.1 | 17.3 |
| Among Individuals | 295 | 190.5 | 15.4 |
| Within Individuals | 300 | 133.0 | 67.4 |
| Total | 599 | 379.6 | 100.0 |

Table 3. Pairwise comparisons of *M. vitrata* samples showing the corrected $F_{ST}$ estimates (below diagonal) and significance of corresponding comparisons (*P*-values) as indicated above the diagonal. Significance thresholds were evaluated using a Benjamini and Yekutieli (B-Y) adusted α = 0.017.

| | Niger | Nigeria | Fada | Farakoba | Kamboinse |
|---|---|---|---|---|---|
| Niger | - | 0.005* | < 0.001* | <0.001* | 0.001* |
| Nigeria | 0.2 | - | < 0.001* | < 0.001* | 0.004* |
| Fada | 0.2 | 0.2 | - | < 0.001* | 0.002* |
| Farakoba | 0.2 | 0.2 | 0.1 | - | 0.001* |
| Kamboinse | 0.2 | 0.2 | 0.2 | 0.2 | - |

The partitioning of population genetic variance from AMOVA results indicated that ≥ 67.4% resides within individuals, and correspondingly 17.3% and 15.4% of the total genetic variation was among populations and among individuals (Table 2). Using a Benjamini-Yekutieli (B-Y) adjusted significance threshold, all comparisons of pairwise population ENA corrected $F_{ST}$ estimates across all loci showed significant differentiation (Table 3). Specifically, a critical significance level was achieved at 0.05/2.929 = 0.017. Results indicated that the *P*-values obtained from pairwise $F_{ST}$ estimates (≤ 0.005) were all statistically significant at the B-Y adjusted thresholds (Table 3).

Regression of uncorrected $F_{ST}$ estimates and geographic distance (km) among West African sample sites showed a significant dependence of genetic variation on geographic distance ($R^2$ = 0.6, Mantel *P* = 0.04), and showed the relative genetic similarity ($F_{ST}$ estimates) of genotypes at Niger and Nigeria, and among Burkina Faso samples (Figure 1). The "real" number of populations (*K*) estimated from the microsatellite-defined *M. vitrata* genotypes from the mL"(*K*)/sL(*K*) statistic calculated from STRUCTURE 2.3.3 output achieved a maximum value of 14.5 at *K* = 3 and suggested that three genetically-distinct *M. vitrata* ancestries exist in West Africa. The three genetically distinct ancestries across collection sites were represented in Figure 3 as vertical bars with Niger, Nigeria and Fada, Burkina Faso primarily red, Farakoba, Burkina Faso primarily green, Kamboinse, Burkina Faso primarily green and red and a minor cluster represented by yellow across all sites.

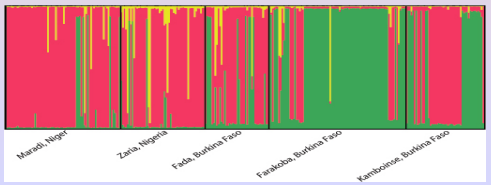


Figure 1. Partitioning of the co-ancestry among microsatellite-defined *M. vitrata* genotypes generated from STURUTURE using the LOCPRIOR command for Maradi, Niger, Zaria, Nigeria and Fada, Farakoba and Kamboinse locations in Burkina Faso. For each, the estimated co-ancestry was derived from the *Q*-matrix for each individual and represented as vertical lines showing the proportion of the $K^{th}$ segments that made up the individual genotype.

## DISCUSSION

Our study indicated some level of genetic differentiation in *M. vitrata* populations in West Africa and that there may be 3 distinct subpopulations of *M. vitrata* in West Africa. This evidence suggest that although *M. vitrata* has a seasonal north-south migration, there appears to be evidence of reduced gene flow that results in population differentiation.

Our results and previous ecological studies have implications for IRM strategies involving Bt-cowpea in West Africa. The implication is that in the north resistance will spread only slowly among *M. vitrata* populations because the populations eventually die out during the dry season; in other words the southern populations in endemic zones act as a source population. Also, the long-distance migration from the south to the north might be a source of susceptible populations into the northern part, which can slow down the evolution of resistance (if there are pockets of *M. vitrata* populations that survive in the north throughout the year). In the more humid south, *M. vitrata* can be found on different host plants throughout the year. The results of this study also have implications for the implementation of control strategies involving the release of biocontrol agents against *M. vitrata*. In keeping with an endemic zone to migratory zone hypothesis, the conclusions of this study agree with the conclusions by Margam et al. (2011) which suggested that the deployment of biocontrol agents (for classical biological control) would be most logical in the endemic zone directly south of migratory regions where *M. vitrata* is a significant pest during the cowpea growing season.

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