POPULATION LEVEL GENETIC VARIABILITY OF COTTON FLEAHOPPER IN THE UNITED STATES

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Abstract

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) is an economically important insect pest of cotton. Higher level infestations and resultant crop loss are primarily observed in southern parts of Texas. Although cotton fleahopper is not reported as an economically important pest of cotton from other cotton growing regions in the United States, cotton fleahopper has been personally observed and reported in cotton fields across the US cotton belt. The current study was conducted to examine the genetic variability of cotton fleahopper populations associated with cultivated cotton. It was hypothesized that cotton fleahopper populations could be genetically differentiated according to their geographic locations due to several factors; such as reproductive isolation due to geographic distance, and differential selection pressures at multiple locations. Cotton fleahopper samples were collected from cotton fields from the most intensive cotton growing areas in 11 different states (Arizona, Texas, Oklahoma, Arkansas, Mississippi, Louisiana, Alabama, Florida, Georgia, South Carolina, and North Carolina) and northern Mexico during the summers of 2009 and 2010. Amplified fragment length polymorphism (AFLP) markers were developed to evaluate genetic variation and genetic structure of cotton fleahopper populations. There was no observed evidence for geographic population structure in the cotton fleahopper. Thus, for management purposes, this pest species can be considered as a panmictic population in the US cotton belt.

Introduction

The cotton fleahopper, *Pseudatomoscelis seriatus* is a pest of cotton in the United States, especially in the southern regions of Texas. Occasionally, cotton fleahoppers have also been reported to cause yield loss in other cotton growing states, such as Oklahoma and Arkansas (Luttrell *et al.* 2002). Damage due to the cotton fleahopper is pronounced in the early growth stages of cotton, when pinhead squares (small flower buds) shed off and plants loose apical dominance (Reinhard 1926). At later growth stages, although cotton fleahoppers may be present, their infestations have almost no impact on cotton due to their inability to infest fruits or larger flower buds. Regardless of their ability to infest cotton, there is a clear regional distinction of cotton fleahopper abundance, which may be due to differential prevalence of preferred alternate wild host plant species such as horsemint, *Monarda punctata* and woolly croton, *Croton capitatus,* at different geographic locations.

The distribution range of cotton fleahopper in the United States extends from Baja California in the west to North Carolina in the east (Henry 1991; Knutson *et. al.* 2002). Cotton fleahopper has more than 160 reported host plant species belonging to 35 different families (Esquivel and Esquivel 2009). Considering their wide distribution range and polyphagous nature, one would expect cotton fleahoppers to be genetically distinct at different geographic locations. From a pest management perspective, the important question is whether cotton fleahopper populations are genetically similar or not. Differences in genetic make-up among cotton fleahopper populations might indicate limited migration of individuals among populations, selection and adaptation to particular environments and finally differences in behavior (e.g., host preference, feeding) and physiology (e.g., resistance to insecticides) that may impact its control.

There are several different molecular markers available to study genetic variation in natural population. The choice of molecular marker depends on several factors such as ease of development, cost, levels of marker polymorphism,

repeatability, and prior genetic information on the study organism (Behura 2006). AFLP provides a large number of markers in organisms whose genetic information is not known a priori. We used AFLP markers to study genetic variation of cotton fleahopper populations associated with cultivated cotton collected throughout the US cotton belt.

Materials and Methods

Sampling

Cotton fleahoppers were collected during the summer of 2009 and 2010 by using a standard sweep net and a motorized blower also known as keep-it-simple-sampler (Beerwinkle *et al.* 1997). Samples were collected from 1-5 localities in each of 11 States (Arizona, Texas, Oklahoma, Arkansas, Mississippi, Louisiana, Alabama, Florida, Georgia, South Carolina, and North Carolina). Cotton fleahopper sample collected from cotton was also obtained from Tamaulipas, Mexico. An effort was made to collect approximately 30-40 adults from each locality within a particular state. Samples were preserved in 85% ethanol at 4°C until used for DNA extractions. 24-30 individual cotton fleahoppers were randomly selected from each state for DNA extraction.

Genetic Methods

Genomic DNA was extracted from each randomly chosen individual insect using Qiagen ® DNeasy kit (Valencia, CA) following the manufacturer's recommended protocol for animal tissue. DNA concentration and quality were measured for each specimen using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). The final DNA concentration of each sample was adjusted using vaccufuge procedure (Barman *et al*. 2007) to around 150-200 ng/ul.

Amplified fragment length polymorphism (AFLP) markers were generated using the protocol proposed by Vos *et al.*(1995) with slight modifications. Samples were randomly arranged in 96-well plates for AFLP analyses. Two to three samples were repeated within each plate and these same samples were repeated in all the plates used in order to check the reproducibility of the analyses. Restriction digestion and ligation steps were performed by adding 5.5 µl of genomic DNA to 5.5 µl of master mix, which contained 1.1 µL of 10x T4 DNA ligase buffer, 1.1 µL of 0.5M NaCl, 0.55 µL of diluted bovine serum albumin (1 mg/mL), 0.05 µL of MseI (NEB R0525M), 0.05 µL of EcoRI (NEB R0101T), 0.03 µL of T4 DNA ligase (NEB M0202M), 1 µL of MseI and 1 µL of EcoRI adaptors (ABI 403077) and 0.61 µL of ultra pure water (18.2 mega-ohm/cm). The entire reaction was left overnight at room temperature for adequate digestion. The next morning, each reaction was diluted to 1:18 (11 µL + 189 µL) ratio with buffer TE_{thin} (15 mM Tris of pH 8.0, 0.1 mM EDTA). Preselective PCR amplification was performed in a 20 µL reaction containing 4 µL of the diluted restricted/ligated DNA and 16 µL of a mixture containing 1 µL of EcoRI and MseI AFLP pre-selective primers mix (ABI 403078) with 15 µL of AFLP core mix (ABI 402005). The PCR protocol for the pre-selective amplification consisted of: 95°C for 1 min followed by 20 repetitive cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 90 s with a final hold at 75°C for 5 min followed by storing temperature of 4°C until subsequent procedure. The amplified product was diluted 20-fold by adding 190 µL of buffer TE_{thin} to each reaction. For selective PCR amplification of restriction fragments, 4 µL of the diluted pre-selective PCR product was added with 15 µL platinum super mix (Invitrogen 11306016), 1 µL of primers EcoRI-AAC (ABI 4303053) and 1 µL of MseI-CTC (ABI 402016). The PCR parameters were an initial warm-up at 95°C for 30 s, 12 cycles of 95°C for 10 s, 65°C for 40 s with a lowering of 0.7°C per cycle, 72°C for 5 min, followed by 35 cycles of 95°C for 11 s, 56°C for 30 s, 72°C for 2 min and finally a hold of 75°C for 5 min before storing the samples at 4°C.

Samples were analyzed using capillary electrophoresis. Each reaction for electrophoresis was prepared by adding 0.5 µL of 400 HD-ROX-size standard (ABI 402985), 9 µL of HiDi formamide, and 1 µL of selective PCR amplification product. Samples were analyzed in an ABI 3130 genetic analyzer (Applied Biosystems, Forest City, CA). Results from capillary electrophoresis were analyzed by GeneMapper® 4.0 (Applied Biosystems, Forest City, CA) which provides a presence (1) absence (0) matrix for each individual. Electrophenograms in GeneMapper were evaluated using a 1 bp bin width. Only fragments between 50 and 400 bp were analyzed. The threshold for peak detection was set at 100. Thus, only fragments with relative florescent unit (RFU) of 100 or more were considered.

Statistical Analysis

The SESim statistic (Medina *et al.* 2006) was used to assess the adequateness of the number of individuals and AFLP markers used to detect genetic population structure. A SESim value lower than 0.05 indicates that a given combination of markers and individuals is sufficient to detect the pattern of genetic structuring at the geographic scale considered. Data obtained from one primer pair (E/AAC-M/CTC) were analyzed as a single matrix. Bayesian

clustering of individual genotypes was performed in STRUCTURE 2.3.1 (Falush *et al.* 2007; Pritchard *et al.* 2000). The STRUCTURE® run followed an admixture model, with 20 replicates for each *K*, assuming *K*= 1 to 12, with 100,000 burn-in and 50,000 replications. The best estimate of *K* was determined by the method described by Evanno *et al.* (2005) which takes into account the rate of change in the probability of data between successive *K* [Ln Pr(X|K)] values and graphically finds the uppermost hierarchical level of population structure for the tested scenario. Analysis of molecular variance (AMOVA) was carried out using GenAlEx 6.3 (Peakall & Smouse 2006) to partition the genotypic variation among and within populations as grouped by geographic locations.

Results and Discussion

A total of 188 individuals from 12 locations were included in our analysis and 112 AFLP loci were generated by using one primer pair (E/AAC-M/CTC). The SESim statistic (Medina *et al.* 2006) was lower than 0.05, which indicates that the pattern of genetic structure observed with the current number of individuals and loci is sufficient.

Genetic Variation

The genetic variability within each geographic population, as indicated by percent polymorphic loci and expected heterozygosity (H_e), is shown in Table 1. Overall, the polymorphic loci ranged between 7.1 and 22.3 %. The heterozygosity is similar in all the populations except Florida. This could be due to the relatively low number of samples (6 specimens) collected from Florida.

Table 1. Genetic variability indices (polymorphism and gene diversity) of cotton fleahopper populations.

Populations by geographic location	Number of individuals	Number of loci	Polymorphic loci (%)	H_e (Expected heterozygosity)
Alabama (AL)	23	112	9.8	0.04
Arkansas (AR)	8	112	22.3	0.06
Arizona (AZ)	16	112	10.7	0.04
Florida (FL)	6	112	20.5	0.07
Georgia (GA)	20	112	7.1	0.04
Louisiana (LA)	18	112	13.4	0.04
Mississippi (MS)	16	112	12.5	0.05
Mexico	14	112	11.6	0.05
North Carolina (NC)	17	112	17.0	0.05
Oklahoma (OK)	12	112	8.9	0.05
South Carolina (SC)	17	112	11.6	0.05
Texas (TX)	21	112	10.7	0.04

The molecular analysis of variance shows that most (97%) of the genetic variation in the cotton fleahopper is attributed to individuals within populations, whereas only 3% of genetic variation is attributed to populations grouped by geographic locations (Table 2).

Table 2. Molecular analysis of variance (AMOVA).

Source	df	SS	Estimated variance	% of variation
Among geographic populations	11	53.56	0.096	3.0
Within population	176	592.98	3.37	97.0

Population Genetic Structure

The overall F_{ST} value was 0.017, which indicates negligible genetic differentiation between subpopulations. The Bayesian analysis with STRUCTURE® also shows a similar result where no genetically different subpopulations were found (Fig. 1). The inferred number of populations (*K*) according to the method described by Evanno *et al.* (2005) was 2. However, these two genetically distinct populations did not correlate with geographic location and most of the individuals belonged to one population (shown in red). Few individuals were randomly assigned to the second population (shown in green) (Figure. 1).

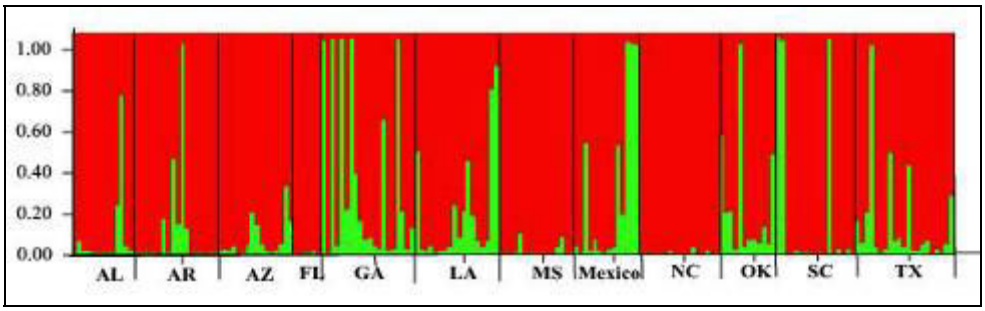

Figure 1. STRUCTURE® analysis of 12 cotton fleahopper populations inferred from AFLP markers. A black vertical bar separates individuals by location (see Table 1 for details regarding location). Two colors (red and green) represent two hypothetical gene pools based on allele frequency. A single genotype (red color) is predominant in all the geographic populations indicating a lack of population structure.

Summary

The origin of cotton fleahopper is believed to be the southern United States and northern Mexico. Current cultivation of cotton overlaps with the native range of the cotton fleahopper. Cotton fleahoppers collected from cotton show no evidence of geographic population structure. Insect pest populations can be differentiated according to geographical location or in contrast, their populations may be panmictic (belong to a single population) in spite being distributed over wide and diverse geographic regions. Structured populations may differ in traits relevant to their control. Therefore, it is necessary to evaluate the pest population structure and genetic variation of insect pests on a case-by-case basis. Genetic differentiation among geographic populations of insect pests depend on the pest history and pest behaviors such as dispersal ability and host plant preference; as well as on host plant range, strength of natural selection, and anthropogenic factors such as cultivation practices, insecticide usage, etc. (Lavandero *et al.* 2009; Timm *et al.* 2008). This study shows that cotton fleahoppers associated with cotton in the US cotton belt and northern Mexico mostly belong to a single genotype and there are no distinct genetic populations associated with any particular geographic region. This finding suggests that from a genetic stand point, no area-specific control measures are necessary to manage cotton fleahopper in the US cotton belt.

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