Title: Developing AFLP markers to study genetic differentiation of the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae)

Discipline: Arthropod Management

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Developing AFLP markers to study genetic differentiation of the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae)

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Abstract:

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) is one of the most important pests of cotton in the US. The cotton fleahopper attacks cotton in the early squaring stage causing flower buds (square) to abort. It is believed that fleahoppers migrate to cotton from other wild weed hosts which are abundant around cotton fields in early spring. However, movement of fleahoppers between hosts is less understood at their population level. Molecular markers such as amplified fragment length polymorphisms (AFLP) are useful to identify genetic differences between populations without a priori genetic information. In this study we report low DNA concentrations per individual in the cotton fleahopper. Instead of pooling several individuals together, we concentrated our DNA samples obtaining adequate markers per individual. AFLP markers obtained in our laboratory are currently being used to test if cotton fleahopper populations occurring in cotton and in the adjacent wild host plants are reproductively isolated.

Keywords: AFLP, fleahopper, host plant

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Introduction:

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) is one of the most important pests of cotton and causes considerable losses during the early stages of the crop (Williams, 2004). It infests cotton in the early squaring stage and causes squares to drop off thus, decreasing fruit setting. Prevalence of cotton fleahoppers is mostly recorded from the southwestern US cotton growing belt (i.e., Texas, Oklahoma, Arizona and Kansas). In 2005, about 2.8 million acres of cotton crop was infested by fleahoppers in Texas with a resulting loss of 32 thousand bales of lint (Williams, 2006). Previous researchers have reported that cotton fleahoppers initiate their first generation in early spring hosts and then sequentially move to other available weed hosts before reaching cotton fields (Reinhard, 1926; 1927; Almand et al., 1976; Schuster et al., 1969; Snodgrass et al., 1984). However, it is not clear which weed hosts contribute fleahoppers to cotton. Evidence of genetic differences exists in other agricultural pest systems where populations from the same pest species feeding on different hosts plant species are reproductively isolated (Feder, 1998, Via et al., 2000; Vialatte et al., 2005). Amplified fragment length polymorphisms (AFLP) markers can be used to assess reproductive isolation among fleahopper populations and to estimate gene flow among populations in cotton and adjacent or distant wild host plants. Such capabilities may prove useful for identifying the source(s) of fleahoppers in cotton. Studies were initiated in 2006 to examine genomic differences among fleahoppers collected from weed hosts and cotton. We found that DNA concentrations from individual fleahoppers were too low to analyze. The present paper reports a method to obtain adequate DNA concentrations without the need of pooling individuals together.

Materials and Methods:

The cotton fleahoppers were collected from horsemint, *Monarda punctata* L. (Laminales: Lamiaceae) and cotton, *Gossypium hirsutum* L. (Malvales: Malvaceae) during 2006 in Brazos County, Texas. The sampled sites were more than 15 km apart. Collected specimens were held in 70% EtOH and stored in a refrigerator. Genomic DNA was extracted from randomly selected individuals using Qiagen ® DNeasy kit (Valencia, CA) following the manufacturer recommended protocol for animal tissue (Quiagen 2002). Final dilution was in 100 µL of buffer AE. DNA concentrations were measured using a NanoDrop-1000 spectrophotometer (NanoDrop, Wilmington, DE). Since low DNA concentrations (≈ 30-40 ng/µL) were found per individual, DNA samples were concentrated using an Eppendorf vacufuge^{tm}. The final concentration was obtained by evaporating all the water in the buffer AE elution and by adding the necessary volume of ultra-pure water (18.2 mega-ohms/cm) to achieve a final DNA concentration of 150 ng/ µL. DNA concentration and quality was assessed after this process using the optical density 260/280 ratio. Samples yielding ratios between 1.8 and 2 were considered good quality DNA samples.

AFLP were developed by digesting the fleahopper genomic DNA by the restriction enzymes EcoRI and MseI and by ligating oligonucleotide adaptors compatible with these endonucleases. The digestion/ligation reactions were accomplished in a single reaction mixture of 11 µL. Each reaction consisted of 1.1 µL of 10X T4 DNA ligase buffer (50 mM Tris-HCL (pH 7.5), 10 mM $MgCl_2$, 10mM dithiothreitol, 1mM ATP, 25.5 µg/ml bovine serum albumin), 1.1 µL of 0.5M NaCL, 0.55 µL of diluted bovine serum albumin (1mg/ml), 0.05 µL of MseI (NEB R0525M), 0.05 µL of EcoRI (NEB R0101T), 0.03 µL of T4 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). Each 11-µL reaction aliquot contained approximately 700 ng of template DNA. Prior to each use, the adaptor pairs were preheated to 95 °C for 5 min, then allowed to cool over a 10 min period to room temperature. The mixture was incubated overnight at room temperature so that template DNA was completely digested. Each reaction was diluted to 1:18 (11 µL + 189 µL) with buffer TE_{thin} (15 mM Tris (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 of 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 was: 95°C for 1 min followed by 20 repetitive cycles of 95°C for 10 s, 56°C for 30 s, and 72°C for 90 s with a final hold at 75°C for 5 min. All samples were stored at 4°C following amplification on a GeneAmp 9700 PCR system (Applied Biosystems, Forest City, CA). The amplified product was diluted 20-fold adding 190 µL of buffer TE_{thin} to each reaction.

For selective PCR amplification of restriction fragments, 3 µL of the diluted pre-selective PCR product were mixed with 15 µL platinum super mix (Invitrogen 11306016), 1 µL of EcoRI-ACT FAM (ABI 402045) and 1 µL of MseI-CAT (ABI 402018). The PCR protocol for the selected amplification consisted of 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 90 s, followed by 35 cycles of 95°C for 11 s, 56°C for 40 seconds, 72°C for 90 s and finally a hold of 75°C for 5 min before storing the samples at 4°C.

To prepare DNA fragments for separation by capillary electrophoresis, sample loading solution was prepared with 0.5 µL of 400 HD-ROX-size standard (ABI 402985), 9 µL of HiDi

Formamide, and 1 μL of the selective PCR amplification product. Samples were analyzed in an ABI 3130 genetic analyzer (Applied Biosystems, Forest City, CA)

Results and Discussion:

In our initial effort to develop robust AFLP markers, we encountered low DNA concentrations (≈ 30-40 ng/μL) per individual cotton fleahoppers. Pooling individual insects from selected populations conveys missing information on within population genetic variability. Thus, we decided to concentrate the cotton fleahopper DNA (to ≈ 150 ng/μL) so it could be used for AFLP analyses. Final DNA concentration was verified. The new DNA concentration mean was 126.77 ng/ μL (n= 10 individuals), and DNA quality after concentrating the samples was good (Optical Density 260/280 = 2.08). AFLP were successfully obtained using MseI-CAT/EcoRI-ACT primer combination (Figure 1). An average of 50.4 bands with an average intensity of 528.44 Relative Fluorescent Units (RFU) was found per individual. It is anticipated that the use of AFLP markers will improve our understanding of the behavioral ecology and population genetics of the cotton fleahopper. We are currently assessing host switching behavior of cotton fleahopper between wild weed hosts and cultivated cotton.

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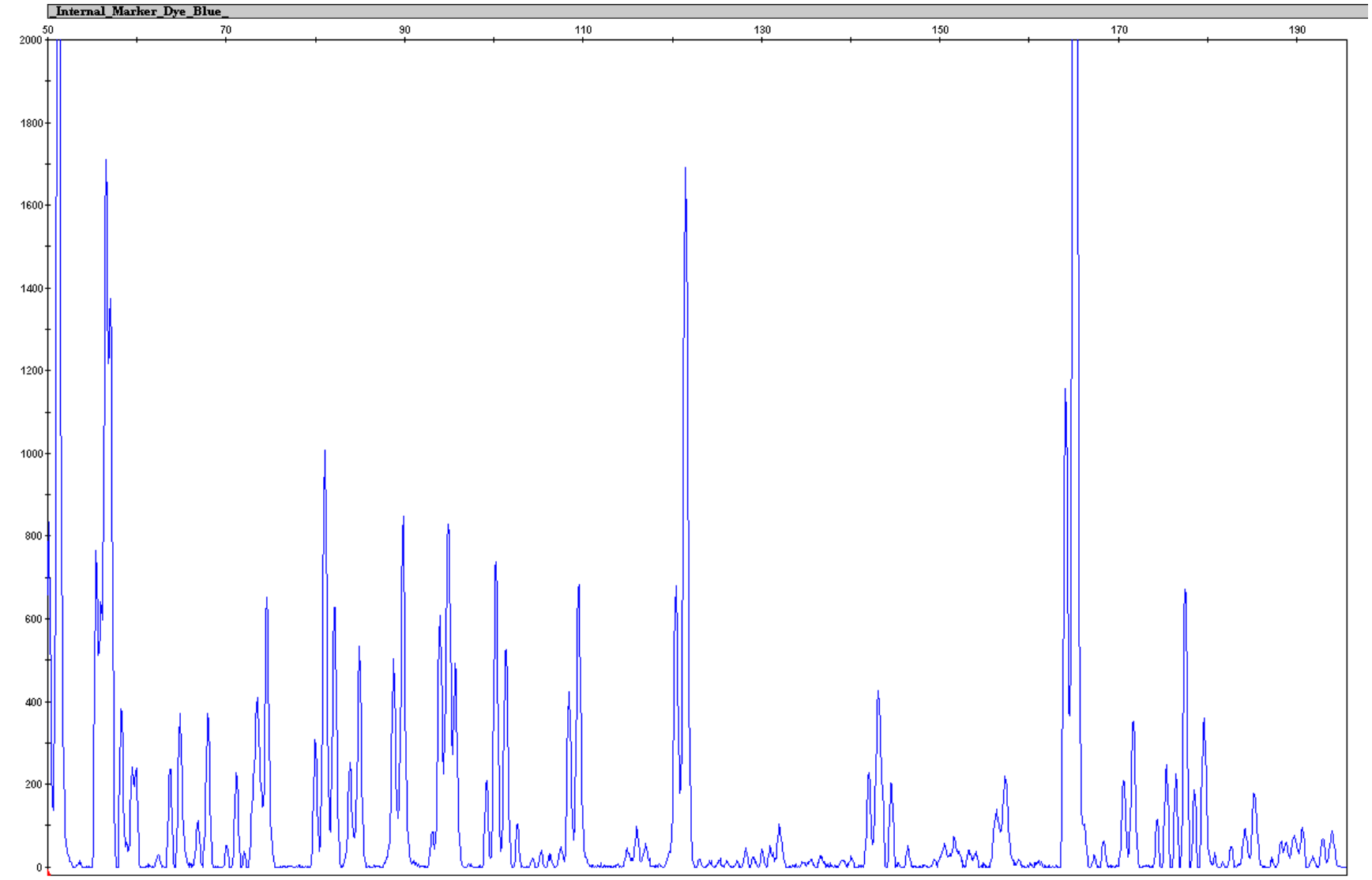

Fig 1. AFLP DNA fingerprint of *Pseudatomoscelis seriatus* (Reuter) showing peaks obtained using EcoR1-ACT and MseI-CAT (Generated from Genemapper 4.0 software).