AFLP™ Analysis of the Fruit Fly Ceratitis capitata

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While Chile was declared free of the med fly in 1995, native populations of *Ceratitis capitata* remain in neighboring countries, so surveillance of over 2,000 miles of border is needed. Identification of the genetic origin of accidentally introduced individuals would help to localize control and facilitate the surveillance.

Amplified Fragment Length Polymorphism (AFLP) analysis is an efficient DNA fingerprinting method based on selective amplification using PCR of restriction fragments from a total digest of genomic DNA. The AFLP technique was developed primarily to reveal the differences between cultivars of plant species (1) and has been applied to several crops. AFLP analysis has identified a larger number of molecular markers than RAPDs and RFLP in soybean (2) and cotton (3), among other crops. The AFLP technique has been used to analyze genetic polymorphism of nematodes (4–5), fungi (6), corals (7), fish (8), and humans (9). One report related to AFLP analysis of arthropod species has been published (10). Polymorphism of wild populations of *C. capitata* has been studied using multilocus enzyme electrophoresis and RAPDs (11–13). In this study, the AFLP technique was used to detect genetic polymorphism in *C. capitata* (Wiedemann) (Diptera: Tephritidae).

METHODS

MATERIALS. *C. capitata* from strains Seibersdorf-6096 (S-6), Vienna-60 (a white pupae temperature-sensitive mutant of S-6), Toliman, and Lluta were obtained from Centro de Producción de Insectos Estériles, C.P.I.E. from the Servicio Agrícola y Ganadero, SAG) located in Lluta, Chile. Toliman is a cross between a native strain from Guatemala (Toliman) and the temperature-sensitive strain Vienna-42 that has been reared at C.P.I.E. since 1996. Lluta strain is a cross of native flies collected in valleys of southern Peru and Azapa (northern Chile) and has been reared at C.P.I.E. since 1993.

DNA EXTRACTION. Genomic DNA was prepared from individual larvae or adult specimens. Each individual conserved in 95% ethanol at −20°C was dried under vacuum in a 1.5-ml tube and ground at room temperature in 200 µl of lysis buffer [50 mM Tris-HCl (pH 8.0), 50 mM EDTA, 3% w/v SDS, 0.1 M 2-mercaptoethanol] with 25 µl proteinase K (10 mg/ml) until the solution turned a reddish color. Another 200 µl of lysis buffer were added, and the mixture incubated for 10 min at 65°C. 100 µl of 5 M potassium acetate were added, and the mixture was incubated on ice for 10 min. After centrifugation for 10 min at 12,000 × *g*, the supernatant was extracted with 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1). 0.6 volumes of cold isopropanol was added to the supernatant and incubated at −20°C for 20 min. The DNA was collected by centrifugation, and the pellet was washed with 70% ethanol, dried, and dissolved in 50 µl TE buffer [10 mM Tris-HCl, 1 mM EDTA (pH 8.0)]. DNA was incubated with 10 µl RNase A (10 mg/ml) for 45 min at 37°C and then treated with 10 µl of proteinase K (10 mg/ml) for 15 min at 50°C. TE was added to 400 µl, and the sample was extracted with 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1). The DNA precipitated with 1/10 volume of 3 M sodium acetate and 2 volumes of cold ethanol. After centrifugation, the DNA pellet was washed in 70% ethanol, dried, and dissolved in 40 µl of TE buffer.

AFLP REACTIONS. AFLP products were generated using the GIBCO BRL® AFLP Analysis System I according to the manufacturer's instructions. GIBCO BRL *Taq* DNA Polymerase was used for PCR. The AFLP products (5 µl) were separated on 5.8% or 6.0% (w/v) acrylamide gels at 70 to 80 W (1,700 −1,800 V) in 1X TBE on a Model S2001 gel electrophoresis system. After electrophoresis, the gel was dried and exposed to a Kodak X-Omat AR film.

ISOLATION AND CLONING OF POLYMORPHIC GENETIC MARKERS. The selected bands were cut directly from the dry gel, submerged in TE buffer containing 1 M NaCl, and incubated overnight at 37°C. The solution was collected and ethanol precipitated at −20°C for 30 min. After centrifugation at 12,000 × *g* for 20 min, the DNA was vacuum dried and dissolved in 20 µl of sterile double-distilled water. The sample was amplified using the same pair of primers selected from the AFLP reaction. PCR was 35 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 1 min, and a final soak at 4°C. The amplified DNA was visualized on a 3% agarose gel stained with ethidium bromide. The DNA was ethanol precipitated, and the pellet was washed with 70% ethanol, dried, and dissolved in sterile water. The DNA was ligated to a vector and transformed into DH5αF′™ competent cells. Both strands of the clones were sequenced with the GIBCO BRL dsDNA Cycle Sequencing System. Sequences were analyzed using the BLAST program from NCBI.

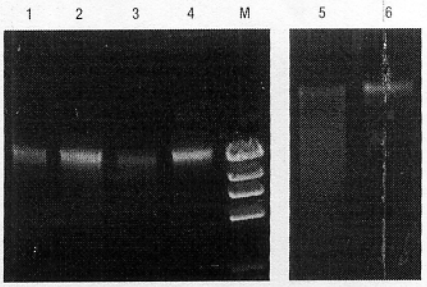

FIGURE 1. Purified genomic DNA. DNA was extracted from individual adult flies or larvae from different strains, and 1/4 of the samples were electrophoresed on 0.8% agarose TBE gels. *Lanes 1–3.* Strain Lluta (male, female, and larva, respectively). *Lane 4.* Strain S-6 (larva). *Lanes 5 and 6.* Strain Toliman (males), digested with EcoR I *(lane 5)* or undigested *(lane 6). Lane M.* λ DNA/*Hin*d III fragments.

RESULTS AND DISCUSSION

Purified genomic DNA from an adult fly or larvae yielded 4 to 5 µg of unsheared DNA with a 260/280 ratio of 1.6 to 1.8 *(figure 1)*. In initial AFLP experiments, 2 similar strains were screened with 64 primer combinations for at least one differentiating band *(data not shown)*. Generally, primer pairs produced 35 to 50 bands from 75 to 500 bp, but few primer pairs produced distinctive patterns.

Based on the initial screening, 8 primer pairs were chosen to analyze both sexes of 4 laboratory strains. The primer pair E-AAG/M-CAT identified specific markers of Lluta, Toliman, and Vienna-60 in both male and female individuals *(figure 2)*. S-6 presented a characteristic marker with E-AGG/M-CAG primers *(data not shown)*.

The AFLP patterns from several DNA samples of the same individual were reproducible *(data not shown)*. However, flies belonging to the same strain exhibited a number of bands that varied between individuals. Therefore, our analysis was focused on identifying marker bands that were common for all individuals of a specific strain. For example, comparing individuals from the Lluta strain, the expected common marker band was seen with both primer pairs, as well as a similarity in the banding pattern *(figure 3)*. However, a number of bands reflecting individual differences (and high genetic diversity) were observed, even though this strain has been reared in the laboratory for >30 generations. These assays were performed for the other strains, and the presence of the respective marker bands was detected for Toliman and Vienna-60 *(data not shown)*.

To use the marker bands as specific probes, we have isolated and cloned the genetic marker bands from Vienna-60, Lluta, and Toliman. The fragments were 280 bp (Lluta), 360 bp (Vienna 60), and 210 bp (Toliman). All fragments were A/T rich *(data not shown)*. A search of GENBANK for these fragments did not match with sequenced genes from *Drosophila* or *Ceratitis*. PCR and Southern hybridization are in progress to verify that these marker bands can be used as specific probes.

In summary, we have applied the AFLP technique to identify laboratory strains of med flies. Our goal is to map the genetic diversity of native wild med fly populations from different geographical locations surrounding the Chilean borders. Preliminary analysis of wild populations from different geographical locations suggests that local patterns can be obtained. The feasibility of isolating specific markers for each strain could eventually facilitate the analysis and monitoring of fly populations.

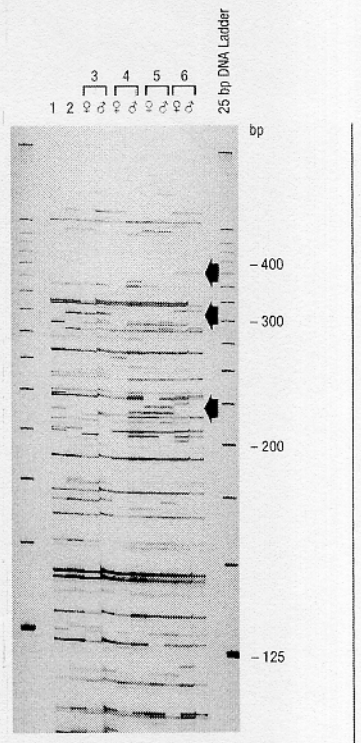

FIGURE 2. Comparison of AFLP patterns. Strains were analyzed using the selective primer pair E-AAG/M-CAT. *Lanes 1 and 2* correspond to the analysis of larval tissue from strains S-6 and Lluta, respectively. All other lanes show DNA extracted from adult flies. *Lane 3*. Strain Lluta. *Lane 4*. Strain S-6. *Lane 5*. Strain Toliman. *Lane 6*. Strain Vienna 60. For each strain, AFLP patterns from one male (♂) and one female (♀) are shown. Arrows indicate characteristic marker bands for each strain using this primer pair.

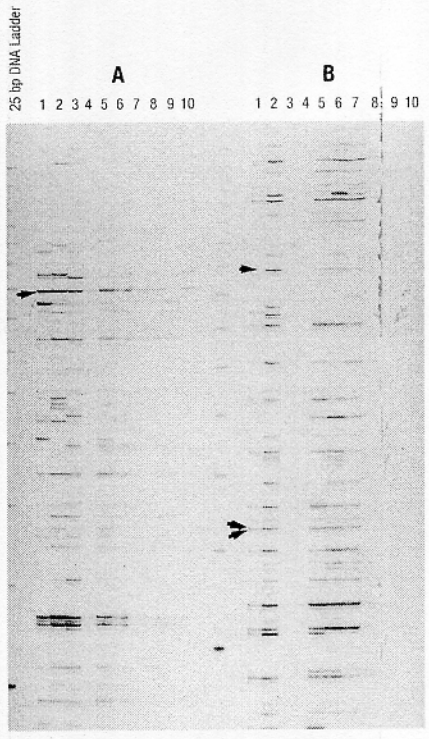

FIGURE 3. Presence of marker band in strain Lluta individuals. 5 males *(lanes 1–5)* and 5 females *(lanes 6–10)* were analyzed with E-AAG/M-CAT *(panel A)* and E-AAC/M-CAA *(panel B)*. Arrows indicate the presence of the characteristic marker band.

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