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Analysis of Genetic Relationships Between Potato Psyllid (Bactericera cockerelli) Populations in the United States, Mexico and Guatemala Using ITS2 and Inter Simple Sequence Repeat (ISSR) Data

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ABSTRACT

The potato psyllid, *Bactericera cockerelli* (Sulc) is an important factor in the Zebra Complex (ZC), a disease that causes economic loss on potato crops. Although the exact cause of ZC is not yet known, it may be related to the toxicity of psyllid saliva, pathogens transmitted by this insect, or a combination of factors. Different combinations of these factors have produced ZC-like symptoms in preliminary laboratory settings. Two putative biotypes of *B. cockerelli* exist, which may have different salivary toxin profiles or may differ in their ability to transmit associated pathogens. The identification of potato psyllids is further confounded by migration of populations over long distances. In this study, ITS2 sequence data and ISSR markers were used to compare populations of *B. cockerelli* samples from geographically isolated populations from southern United States, central Mexico, and Guatemala. Understanding population movement should help growers effectively develop management practices that target psyllid populations that are involved in the disease system.

Additional Index Words: *Bactericera cockerelli*, zebra chip, zebra complex, vector

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Zebra Complex (ZC), a disease of potatoes, was first documented in 1994 in potato fields in the Saltillo, Mexico area. The defining characteristic of zebra complex is the defect causing discoloration of the medullary rays of tubers, resulting in a streaked or zebra-like appearance when sliced and fried. In addition, ZC infected plants show a wide range of other symptoms, including stunting, chlorosis, swollen internodes of the upper growth, aerial tubers, browning of the vascular system in belowground portions of stems, leaf scorching, and early plant decline (Munyaneza 2007). Many of these foliar symptoms resemble those of potato psyllid feeding damage (called "psyllid yellows") and potato purple top wilt syndrome which is the result of phytoplasma infection. Zebra Complex is becoming increasingly more economically important, as the severity and range of the disease increases in the United States, Mexico, and Central America.

At the present time, the causal agent of ZC is unknown, although several hypotheses have been proposed. Researchers have shown an association of the *Bactericera cockerelli* with zebra complex (Munyaneza 2007, Goolsby et al. 2008, Goolsby et al. in press). *Bactericera cockerelli* is alternately known as the potato psyllid or tomato psyllid based on association with potato and tomato damage (Arslan 1985, Blood 1933). The potato psyllid has also been associated with recent damages to pepper crops, although it has long been known to be a host to psyllids. The potato psyllid is associated with psyllid yellows, caused by an unknown salivary toxin injected upon nymph feeding. Potato psyllid damage has been well documented over a long period of time, however ZC symptoms are a recent phenomenon. One hypothesis is that potato psyllid populations have changed in some way facilitating the spread of ZC.

Potato psyllids are migratory, and recently, the emergence of two separate biotypes of the potato psyllid have been elucidated: one primarily in central

North America, from central Mexico up through the central United States, and a new western North American biotype that is adapted to the coastal region (Liu 2006). Because potato psyllids are associated with ZC, either as a vector or as the causal agent themselves, it is important to know which biotypes and which populations are in a given region at a given time, so they can be further correlated with incidences of ZC. In this study, ITS2 sequences were used to reliably show that insects were from the same species; further, we have differentiated potato psyllid populations using ISSR techniques to better understand the movement of populations between major potato-growing regions of North America.

MATERIALS AND METHODS

Sample collection. Bactericera cockerelli samples were collected from yellow sticky traps (Trecé Inc., Adair, OK) in potato fields between October 2004 and September 2006 in Guatemala, in September 2006 in Saltillo and La Solidad, Mexico, and February to May 2007, in McAllen, Texas, United States. Samples were placed in 95% ethanol and shipped overnight to Tyler, TX. Upon arrival they were frozen at -20C until processing.

DNA processing. DNA was extracted from insect samples by crushing individual insects in PBS buffer followed by extraction of DNA using the DNeasy tissue kit (Qiagen, Valencia, CA). For DNA sequencing, PCR amplification of the ITS2 region of rDNA was performed using the primers (5′-GATCGATGAAGACCGCAGC) and (5′-TCCTCCGCTTATTGATATGC) (Liu 2006). PCR amplification was carried out in 20ul reactions containing 1uM forward and reverse primers, 2ul extracted DNA, and IQ Supermix (Biorad, Hercules, CA) containing dNTPs, MgCl and Taq polymerase. PCR cycling conditions were as follows: 95° C for 4 min, followed by 36 cycles of 95° C for 45 s, 53° C for 1 min, and 72° C for 1.5 min, then a final extension cycle of 72°C for 10 min. PCR products were separated on 1% agarose gels, then the 807 bp band was extracted using the "freeze-squeeze" method (Islam 2002) and purified using the QiaQuick PCR cleanup kit (Qiagen, Valencia, CA). DNA concentration was determined spectrophotometrically. The DNA sequencing reaction was carried out in 20ul reactions using 3.2 pmol of single primers and 50ng DNA, followed by standard protocols using the DTCS start mix (Beckman Coulter, Fullerton, CA). Sequencing reactions were desalted by ethanol precipitation then read using a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). Sequences were aligned using BioEdit and compared to the GenBank database.

For ISSR samples, PCR amplification was performed using the primer ISSR847 (Liu 2006), labeled with WellRed dye. PCR amplification was carried out in 20ul reactions containing 200 nM primer, 2ul extracted DNA, and IQ Supermix (Biorad, Hercules, CA) containing dNTPs, MgCl and Taq polymerase. PCR conditions were as follows: initial denature of 95^{o}C for 3m followed by 50 cycles of 95^{o}C for 30s, 59.5^{o}C for 45s, 72^{o}C for 90s followed by a final extension of 72^{o}C for 20m. PCR products were purified using QiaQuick PCR cleanup kit (Qiagen, Valencia, CA), then desalted using an isopropanol precipitation. Fragment analysis was carried out using microcapillary electrophoresis on a CEQ8000 (Beckman Coulter, Fullerton, CA) using the following run conditions: run temperature 55^{o}C, run voltage 3.0V, runtime 180m, inject voltage 2.0V, and inject time 30s.

Data Analysis. Fragments were scored for presence or absence by analyzing a densitogram of the capillary electrophoresis using LabImage 1D (Kapelan GmbH, Halle, Germany). This binary matrix was analyzed using the unweighted pair group method with arithmetic averages (UPGMA) using PHYLIP v3.67 (Felsenstein 1989). Gene diversity, gene flow estimates, and UPGMA cluster analysis of populations and groups were obtained using POPGENE (Yeh, 1997). To obtain a consensus tree based on 10,000 bootstrap iterations of the data, allele frequency data from the PopGene output was used with the PHYLIP package (programs seqboot, genedist, neighbor, consense) to obtain UPGMA bootstrap branch values. Analysis of molecular variance (AMOVA) and pairwise comparisons of genetic distances (Fst) were performed using ARLEQUIN to determine if there were significant differences between populations, and to differentiate variation within and among populations. Discriminant analysis with jackknifing of the dataset was performed using SYSTAT 12.

RESULTS

ITS2 sequencing data. Five individuals from each population (Guatemala, Mexcio and McAllen) were selected for sequencing; however, of the McAllen samples, only one gave a useable sequence. Of an 807 bp long fragment amplified by the ITS2 primers, after alignment, trimming and consensus, we obtained a sequence length of 791 bp; of these, 779 (98.5%) were identical. A total of 11 sequences were submitted to GenBank (accession numbers GQ249858 - GQ249868).

ISSR fragment data. The single ISSR primer used (ISSR847) produced 132 fragments across 75

individuals sampled, including 21 from Guatemala, 31 from Mexico and 23 from McAllen. Bands from 270 – 1320 bp were considered, based on the range of sensitivity of detection using the CEQ 8000 for fragment detection (Table 1).

The unweighted pair group method with arithmetic averages (UPGMA) was used to create a dendrogram based on pairwise genetic differences between populations, using POPGENE (Fig. 1). Based on the clustering pattern, analyses were conducted with 2 groups, the first consisting of the Guatemala population (Group 1) and the second consisting of the Mexico and McAllen populations (Group 2). In 10,000 bootstrap replicates, these clusters were found 9997 times (99.97%).

Gene diversity and gene flow estimates of the populations are as follows: the Gene diversity (H), Shannon's information index (I), percentage of polymorphic loci (P) for Guatemala are (H = 0.3413, I = 0.5106, P = 97.7), for Mexico are (H = 0.3080, I = 0.4708, P = 97.0), and for McAllen are (H = 0.2952, I = 0.4571, P = 95.5). Group 1 includes Guatemala only and the statistics for Group 2 (Mexico and McAllen) are (H = 0.3108, I = 0.41819, P = 99.2). The coefficient of gene differentiation (Gst), and gene flow (Nm) are Overall (Gst = 0.0513, Nm = 20.4559), and Group 2 (Gst = 0.0369, Nm = 13.0644) (Table 2).

Analysis of molecular variance (AMOVA) indicated that the source of 91.7% of the total variance was within populations, 5.3% of the variation was

Table 1. Classification matrix and Jackknifed classification matrix of a discriminant analysis of ISSR fragment data.

Group	GUA[1]	MCA[2]	% Correct
Classification Matrix			
Group 1 (GUA)	13	5	72
Group 2 (Mex / McA)	9	44	83
Total	22	49	80
Jackknifed Classification Matrix			
Group 1 (GUA)	14	4	78
Group 2 (MEX / McA)	9	44	83
Total	23	48	82

[1] Guatemala population.
[2] McAllen, Texas population.

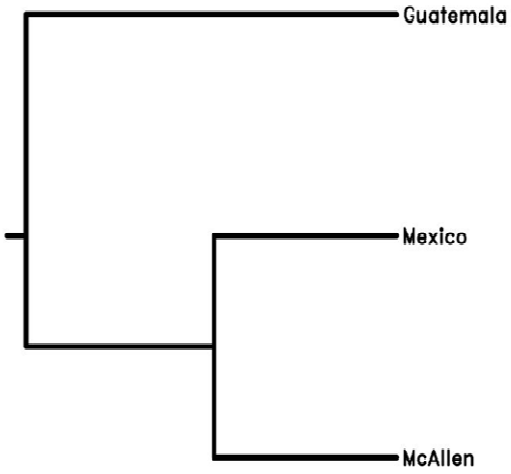

Fig 1. UPGMA dendrogram based on pairwise genetic differences between populations using ISSR data.

Table 2. Gene diversity and gene flow estimates including gene diversity (H), Shannon's information index (I), percentage of polymorphic loci (P), coefficient of gene differentiation (Gst) and gene flow (Nm), for individual populations and groups. Group 1 consists of Guatemala only and Group 2 consists of Mexico and McAllen.

Group	H	I	P	Gst	Nm
Guatemala	0.3413	0.5106	97.7		
Mexico	0.3080	0.4708	97.0		
McAllen	0.2952	0.4571	95.5		
Group 2	0.3108	0.41819	99.2	0.0369	13.0644
Overall				0.0513	20.4559

among groups, and 3.0% of the variance was among populations within groups. Pairwise comparisons of genetic distances (Fst) showed significant genetic difference ($p < 0.001$) between all populations. The pairwise distance between Guatemala and Mexico was 0.07637, between Guatemala and McAllen 0.08567, and between Mexico and McAllen, 0.03386. Discriminant analysis of ISSR fragment data revealed that using the two-group classification scheme allowed correct classification in approximately 80% of samples (Table 2). Multivariate means differed significantly between groups (Wilk's lambda = 0.034, df = 41, 29 F = 19.883, $p < 0.001$).

DISCUSSION

ITS2 sequences of all individuals sampled were very similar (98.5% identity) indicating that all psyllids were of the same species (*Bactericera cockerelli*), regardless of geographic location. However, given the highly variable nature of microsatellite sites, the ISSR data showed far more polymorphism (99%), and were more useful in population comparisons.

Analysis of ISSR data indicates that the populations from Guatemala, central Mexico, and McAllen, Texas (United States) show small, but statistically significant differences. Although migration probably plays a role, especially between central Mexico and McAllen, these data suggests that there are likely stable populations in each of these regions.

Data also indicate that Mexican and McAllen populations are more closely related to each other than to Guatemalan populations. The pairwise distances between Guatemalan populations and Mexico or McAllen populations was more than double the distance between Mexico and McAllen populations. Gene flow between McAllen and Mexico (13.1) accounts for 64% of gene flow between all groups (20.5), indicating more gene flow within Group 2 than between Groups 1 and 2. This is not surprising because there is evidence that psyllids migrate northward in the spring on the warm monsoon winds from the Gulf of Mexico (Pletsch 1947; Liu 2006). There may also be other geographic barriers between Guatemala and Mexico that impede northward migration.

Temperature is critical for psyllid survival and reproduction, as temperatures below 15.5C or above 32.2C result in low survival rates and impede development (List 1939a; 1939b), and it has been suggested that temperature fluctuations play a major role in the abundance or lack of potato psyllids in a region during a given year. In addition, all three sites considered in this study have conditions favorable for overwintering.

It is apparent that both stable populations and gene flow into McAllen, Texas exist, and not simply populations breeding in the south and migrating northward. Genetically distinguishable populations exist in central Mexico and South Texas, as well as significant gene flow between the two populations, and it is possible that cryptic incidence of ZC may be tied to different populations of *B. cockerelli*; it is also possible that a current ZC-causing biotype is displacing a previous non-ZC causing biotype. Future studies which define populations in central Mexico and the United States further by date and correlate them with ZC incidence will be vital to better understanding and predicting disease patterns.

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