THE UTILITY OF INTER-SIMPLE SEQUENCE REPEAT-POLYMERASE CHAIN REACTION (ISSR-PCR) TO DISTINGUISH GEOGRAPHIC POPULATIONS OF THE SMOKE-TREE SHARPSHOOTER AND EGG PARASITOID SPECIES OF THE GENUS *GONATOCERUS*

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ABSTRACT

In the current study, we tested the utility of the inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting method in distinguishing geographic populations of the smoke-tree sharpshooter (STSS) (*Homalodisca liturata* Ball) and in distinguishing several egg parasitoid species in the genus *Gonatocerus*. Four geographic populations of the STSS were analyzed and they included: near Central (Olgilby Rd.) CA, Riverside CA, Imperial CA, and Phoenix, AZ. Five unique or population-specific markers were identified in the population from Riverside with minor genetic variation within the population. Another five population-specific markers were identified in the rest of the STSS populations (near Central and Imperial CA and Phoenix AZ). Extensive genetic variation was detected in these STSS populations. Population-specific markers are an indication of subdivided populations and decreased gene flow. The following *Gonatocerus* ((Hymenotpera: Mymaridae) egg parasitoid species were analyzed: *G. triguttutas* (TX), *G. morrilli* (CA), *G. ashmeadi* (CA), *G. fasciatus* (LA) *G. metanotalis* (Argentina), near *G. ashmeadi* (Argentina), near *G. triguttatus* (Argentina), and *G. tuberculifemur* (Argentina). Each *Gonatocerus* species was associated with a unique ISSR-PCR banding pattern. In general, not much variation was seen within each species. Some variation was seen in *G. tuberculifemur*, while extensive variation was seen in *G. fasciatus*. The current results confirm the utility of using the sensitive ISSR-PCR method to distinguish geographic populations of the STSS and to distinguish several egg parasitoid species in the genus *Gonatocerus*. Rapid distinction of egg parasitoid species will speed up the identification process in a biological control program, thus saving time and cost.

INTRODUCTION

Homalodisca liturata Ball (Homoptera: Cicadellidae), the smoke-tree sharpshooter (STSS) is distributed in Arizona, southern California, Baja California, Mexico, Guatemala, and Costa Rica (Young 1958, 1968, Turner and Pollard 1959, Ball 1979). Prior to the arrival of the glassy-winged sharpshooter (GWSS) (*H. coagulata* Say) in California, one of the most common sharpshooter vectors of Pierce's disease in California were native sharpshooters, such as, the STSS (Varela et al. 2001, Redak et al. 2004). Both the GWSS and the STSS are xylem feeding leafhoppers that transmit a strain of *Xylella fastidiosa* Wells *et al*., a bacterium that causes Pierce's disease in grapevines (*Vitis vinifera* L. and *V. labrusca* L.), as well as diseases in many other plants (Hopkins and Mollenhauer 1973).

Mymarid wasps, on the other hand, are the best-known egg parasitoids for controlling populations of leafhoppers (Huber 1986, Döbel and Denno 1993). Detailed taxonomic and biological studies are crucial to biological controls programs (Logarzo et al. 2004, Virla et al. 2005). Release of unidentified and uncharacterized strains could make it impossible to document their establishment and disperal; therefore, genetic typing of strains prior to their release in the field is necessary. Accurate identification of natural enemies is critical to the success of classical biological control programs, as it is essential for 1) selecting the most suitable natural enemy, 2) evaluating establishment, dispersal, and efficacy of natural enemies, and 3) improving mass production. Lack of proper identification procedures has affected several projects (Messing and Aliniazee 1988, Löhr et al. 1990, Narang et al. 1993).

There is a need for molecular markers to provide new characters for studies of phylogenetic relatedness, for identification of cryptic species and biotypes, and for the assessment of heritable variation for population genetics and ecological investigations (Unruh and Woolley 1999). Studies of allele or marker frequencies in naturally occurring parasitoid populations are important, not only for identifying genetic variation of potential benefit, but also for the detection of genetic markers indicative of specific biological traits or geographic origins. Furthermore, the recognition of intraspecific variation can be as crucial for the success of biological control programs as is sound species determination (Powell and Walton 1989, Narang et al. 1993, Unruh and Woolley 1999).

Recently, we developed DNA markers for *H. coagulata* for the purpose of estimating genetic variation in natural populations (de León and Jones 2004). Inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) (Zietkiewicz et al. 1994) was shown to be a sensitive and efficient procedure with *H. coagulata* and several egg parasitoid species in the genus *Gonatocerus* (de León et al. 2004b, de León et al. 2005 submitted) This DNA fingerprinting procedure permits detection of DNA variation in simple sequence repeats (SSR) without the need to isolate and sequence specific DNA fragments.

OBJECTIVES

1. Determine if the inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting method was suitable or sensitive enough to detect genetic variation and distinguish geographic populations of the smoke-tree sharpshooter (*Homalodisca liturata*) and
2. Determine if ISSR-PCR was suitable enough to distinguish several *Gonatocerus* species of egg parasitoids that attack the glassy-winged sharpshooter (GWSS) (*Homalodisca coagulata*).

RESULTS AND CONCLUSIONS

ISSR-PCR DNA fingerprinting of smoke-tree sharpshooter geographic populations

Amplification reactions were performed with total genomic DNA from ten separate individuals per population with a 5'-anchored ISSR primer (Zietkiewicz et al. 1994, de León and Jones 2004, de León et al. 2004, de León and Jones 2005). The populations analyzed included: Riverside CA, Imperial CA, near Central (Olgilby Rd) CA, and Phoenix AZ. Five population-specific markers (indicated by the arrows) were identified in the STSS population from Riverside CA with minor genetic variation within the population (Figure 1). Population-specific markers are an indication of subdivided populations and decreased gene flow. Decreased gene flow leads to increased genetic differentiation among populations. The results also show some evidence of reproductive isolation. Another five population-specific markers were also identified within the rest of the STSS populations (near Central CA, Imperial CA, and Phoenix AZ). These populations shared several bands, indicating that there are genetic similarities among them. Extensive variation is also seen in these populations. In addition, these populations are located in an area closer to each other than to the Riverside CA population.

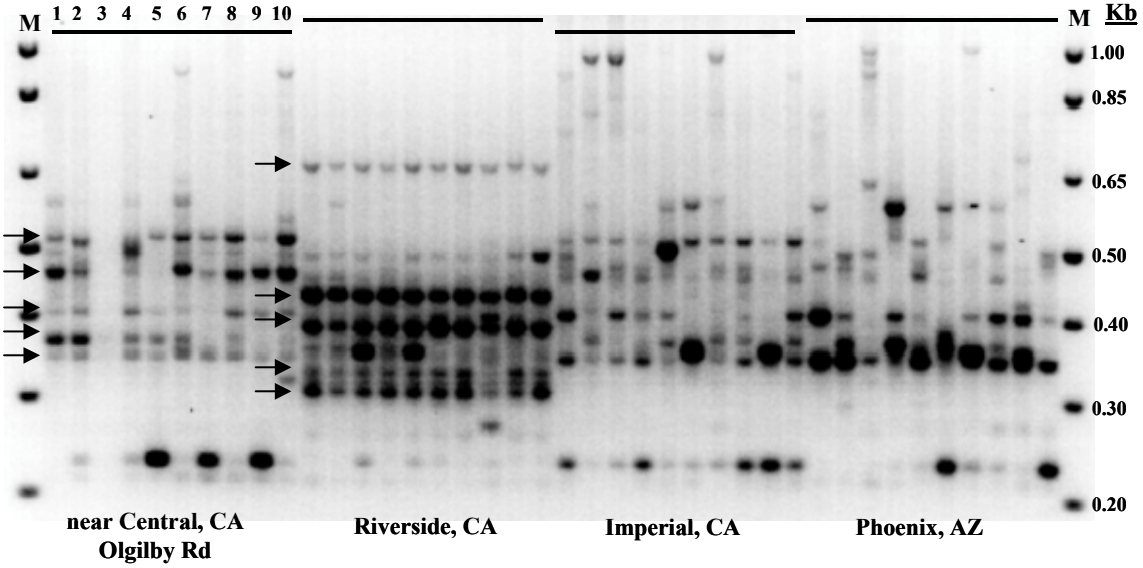

Figure 1. ISSR-PCR DNA fingerprinting of smoke-tree sharpshooter geographic populations from California and Arizona. Reactions were performed with total genomic DNA from 10 separate field collected individuals and a 5'-anchored ISSR primer (Zietkiewicz et al. 1994, de León et al. 2004). Arrows point out the unique markers identified in the populations. Smoke-tree sharpshooter geographic populations are indicated above. M**:** 1.0 Kb Plus DNA Ladder.

ISSR-PCR DNA fingerprinting of *Gonatocerus* egg parasitoid species

Amplification reactions were performed with total genomic DNA from five separate individuals per species with a 5'-anchored ISSR primer (Zietkiewicz et al. 1994, de León and Jones 2004, de León et al. 2004a, 2004b, de León and Jones 2005, de León et al. 2005 submitted). *Gonatocerus* species analyzed from both North and South America included: *G. triguttatus* Girault (TX), *G. morrilli* Howard (CA), *G. ashmeadi* Girault (CA), *G. fasciatus* Girault (LA), *G. metanotalis* Ogloblin (Argentina), near *G. ashmeadi* (Argentina), near *G. triguttatus* (Argentina), and *G. tuberculifemur* Ogloblin (Argentina). The results of this analysis are shown on Figure 2. As seen, each *Gonatocerus* species was associated with a unique ISSR-PCR banding pattern. In general, not much variation was seen within each species. Some variation was seen in *G. tuberculifemur*, while extensive variation was seen in *G. fasciatus*. The present results confirm that the ISSR-PCR DNA

fingerprinting method is an excellent method to distinguish halpodiploid egg parasitoid *Gontocerus* species and is also a good tool for distinguishing geographic populations of STSS.

Even though ISSR-PCR markers are scored as dominant, the ISSR-PCR technique using 5'-anchored or compound ISSR primers is still a very sensitive and useful technique because it targets random SSR or microsatellites (Zietkiewicz et al. 1994, de León and Jones 2004). An additional advantage is that the same ISSR primer can be rapidly applied across several different orders (e.g., insects, plants, fungi, bacteria) without prior knowledge of DNA sequences (de León, unpublished data), a capability not found with microsatellites. Banding patterns are consistent because the anchors serve to fix the annealing of the primer to a single position of the target site, thus resulting in a low level of slippage during amplification (Zietkiewicz et al. 1994, reviewed in Karp and Edwards 1997).

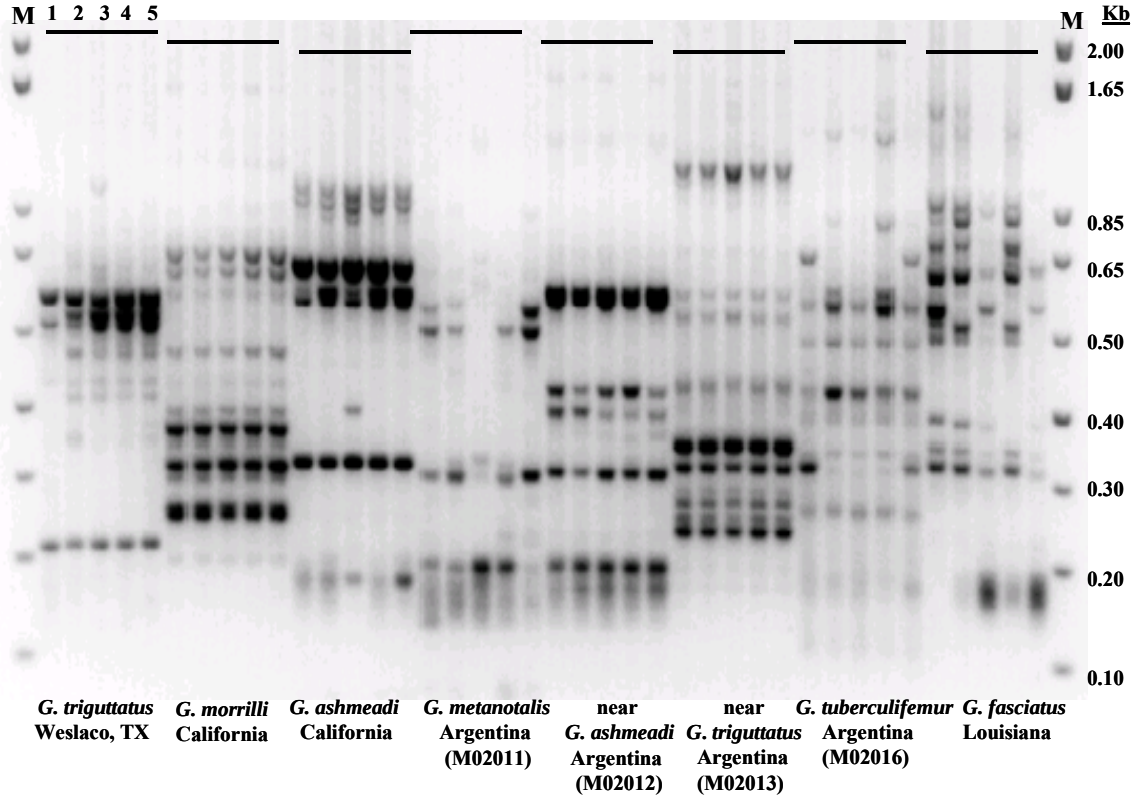

Figure 2. ISSR-PCR DNA fingerprinting of several *Gonatocerus* egg parasitoid species from both the U. S. and South America (Argentina). Reactions were performed with total genomic DNA from five separate field collected individuals and a 5'-anchored ISSR primer (Zietkiewicz et al. 1994; de León et al. 2004). *Gonatocerus* egg parasitoid species are indicated above. **M:** 1.0 Kb Plus DNA Ladder.

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