SMALL SCALE POST-RELEASE EVALUATION OF A *GONATOCERUS MORRILLI* PROGRAM IN CALIFORNIA AGAINST THE GLASSY-WINGED SHARPSHOOTER: UTILITY OF DEVELOPED MOLECULAR DIAGNOSTIC TOOLS

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ABSTRACT

Previously we discovered a cryptic species complex in *Gonatocerus morrilli* (Howard) and developed molecular diagnostic markers that distinguished the two cryptic species. In the current study we tested the utility of the two developed molecular diagnostic markers to evaluate the establishment of *G. morrilli* in California. In the two cryptic species, the size of the internal transcribed spacer 2 region (ITS2) varies by about 212 base pairs; the Texas *G. morrilli* species is associated with a size of about 851-853 base pairs and the California *G. morrilli* (nov.) species with a size of about 1063-1067 base pairs. Secondly, the two cryptic species do not share any inter-simple sequence-polymerase chain reaction (ISSR-PCR) bands or markers. Initially releases were made from what was thought to be a Mexico culture, but contamination was suspected to have occurred from a Texas culture and therefore, the culture was name 'TX/MX". Post-released collections from years 2002 and 2003 were made from the following locations: San Juan Capistrano, Glen Ivy, Pauma, Temecula, and San Marcos. Amplification of the ITS rDNA fragments demonstrated that all or 100% of the randomly chosen individuals (125 total) were of the California ITS2 genotype and none were of the Texas ITS2 genotype. ISSR-PCR DNA fingerprinting of the TX/MX colony along with native California and Texas *G. morrilli* species demonstrated that the TX/MX ISSR-PCR banding pattern was superimposable to that of the California *G. morrilli* (nov.) species. The results demonstrated that the TX/MX colony was contaminated with the California species, indicating that what was being released in California was California's own native species. Therefore, this is why screening with the ITS2 fragment detected only the California ITS2 genotype. The present results confirm the utility of the two developed molecular diagnostic methods in monitoring the success of the *G. morrilli* biological control program in California. In addition, this molecular technology will allow us to monitor egg parasitoid colonies to eliminate unwanted species.

INTRODUCTION

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 (Rosen 1977, Messing and Aliniazee 1988, Löhr et al. 1990, Narang et al. 1993, Miller and Rossman 1995, Schauff and LaSalle 1998, Gordh and Beardsley 1999, Unruh and Woolley 1999). Correct identification of the pest is also extremely important in biological control. Geographic populations of the same species may differ in relevant biological characteristics of importance to biological control. In addition, pin-pointing the native origin of an exotic pest is crucial for collection of natural enemies in the native range of the pest (Rosen 1977, Narang et al. 1993, Unruh and Woolley 1999, Brown 2004, Roderick 2004). Recently, we demonstrated that the glassy-winged sharpshooter (GWSS) (*Homalodisca coagulata* Say) (Homoptera: Cicadellidae) that invaded California (CA) is of Texas (TX) origin, but more than one founding event occurred (de León et al. 2004a). Our data also showed that GWSS populations in the U. S. were genetically distinct, clustering into two main groups or clades, a 'southeastern' and a 'southwestern and western' clade. Similarly, molecular studies of *Gonatocerus morrilli* Howard (Hymenoptera: Mymaridae) populations from CA and TX using inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting and amplification of the internal transcribed spacer region 2 (ITS2) showed that these populations were highly differentiated ($G_{ST} = 0.92$) with restricted gene flow (de León et al. 2004b). Additional studies indeed confirmed that *G. morrilli* exists in nature as a cryptic species complex (de León et al. 2005 submitted and accompanying report).

OBJECTIVES

To examine the utility of developed molecular diagnostic markers in evaluating the establishment of *Gonatocerus morrilli* after release in California. Two molecular methods were tested: ISSR-PCR DNA fingerprinting and amplification of the internal transcribed spacer region 2 (ITS2).

RESULTS AND CONCLUSIONS

The use of the ITS2 rDNA fragment as a molecular diagnostic tool to evaluate post-released populations

Initially releases were made from a Mexico culture but contamination was suspected to have occurred from a Texas culture and therefore, the culture was name 'TX/MX". Post-released collections were made from the following locations: San Juan Capistrano (Orange Co.); Glen Ivy (Riverside Co.); Pauma (San Diego Co.); Temecula (Riverside Co.); and San Marcos (San Diego Co.). The ITS2 rDNA fragment was amplified with standard primers from several individuals per population. A

representative example is shown on Fig. 1. The expected ITS2 fragment sizes are: TX = 1063-1067 bp and CA = 851-853 bp. A TX *G. morrilli* individual was included for comparison. The data showed that all or 100% of individuals within this population (San Juan Capistrano) were of the ITS2-CA genotype. Analyses of the rest of the populations shown the same trend, that is, 100% of populations were of the ITS2-CA genotype and none were of the ITS2-TX genotype (Table 1).

Molecular diagnostic analysis of the TX/MX culture by ISSR-PCR DNA fingerprinting

Since the post-release evaluation of *G. morrilli* populations showed that what was being recovered were individuals with the ITS2-CA genotype, the question was asked, 'were these egg parasitoids not establishing or could the initial release culture be contaminated with the TX culture as previously suspected'. Though, none of the individuals tested carried the ITS2-TX genotype, so it was possible that these egg parasitoids were not establishing. To answer this question we performed ISSR-PCR DNA fingerprinting with several individuals (7) per culture. Included for comparison were native *G. morrilli* species from Texas and California. Fig. 2 shows the results of this experiment. The ISSR-PCR banding pattern of the TX/MX culture was superimposable with that of the native California *G. morrilli* (nov.) species, demonstrating that the TX/MX individuals were not of the Texas *G. morrilli* species. These results were in accord with those seen with the amplification of the ITS2. This is confirmed by comparing the ISSR-PCR banding pattern of the native Texas species. Individuals of *G. morrilli* from Mexico were not available for analysis, but we have previously shown a powerful correlation between egg parasitoid species (*G. morrilli*) and ISSR-PCR banding patterns (de León et al. 2004b, de León et al. 2005 submitted). Furthermore, we present an accompanying proceeding/report demonstrating the correlation of ISSR-PCR banding patterns and egg parasitoid species in the genus *Gonatocerus*. Based on these results it is assumed that if individuals from Mexico were a different species, the ISSR-PCR method would have detected it. These results indicate that what was being released in California was the native California *G. morrilli* (nov.) species, and therefore, offers the explanation for the detection of only the ITS2-CA genotype as shown on Fig. 1 and Table 1. So the answer to our question was that contamination of the culture did in fact occur with the California *G. morrilli* (nov.) species and not with the Texas species as originally suspected. These results also indicate that the California species out competed the TX/MX individuals in the colony.

Figure 1

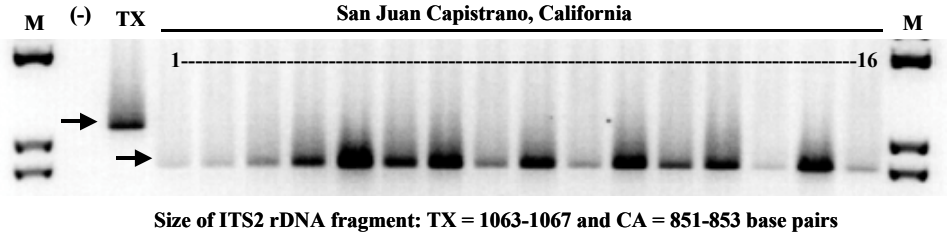

Figure. 1. Representative example of the use of the ITS2 rDNA fragment as a molecular diagnostic tool to evaluate post-released populations of *Gonatocerus morrilli* in California. The ITS2 rDNA fragment was amplified with standard primers from 16 individuals as previously demonstrated (de León et al. 2004b, de León et al. 2005 submitted). The size of the expected ITS2 amplification products are shown above and the arrows indicate the products for both Texas (TX) and the California population. A difference of about 212 base pairs is seen between TX and CA individuals. **M:** 1.0 Kb Plus DNA Ladder.

Table 1. Summary of populations from California evaluated by amplification of the ITS2 rDNA fragment. A total of 125 individuals were included from the various populations. No. ind., number of individuals; #ITS2-CA, number of individuals with the CA *G. morrilli* ITS2 genotype; #ITS2-TX; number of individuals with the TX *G. morrilli* ITS2 genotype. Populations were collected in years 2002 and 2003 and were randomly chosen for analysis.

Population	County	No. ind.	#ITS2-CA	#ITS2-TX
San Juan Capistrano	Orange	30	30	0
Glen Ivy	Riverside	17	17	0
Pauma	San Diego	30	30	0
Temecula	Riverside	14	14	0
San Marcos	San Diego	34	34	0

Figure 2

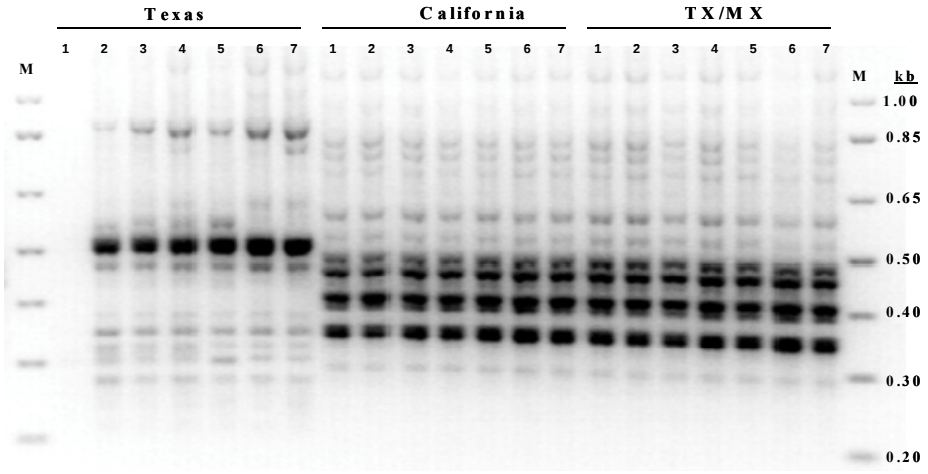

Figure 2. Evaluation of the *G. morrilli* (TX/MX) culture that was used for release in California. Inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) was utilized as a molecular diagnostic tool to evaluate or monitor the *G. morrilli* (TX/MX) culture. Releases were made from a Mexico culture but contamination was suspected to have occurred from a Texas culture. For comparison, individuals or species native from Texas and California were included. DNA fingerprinting was performed as previously described (Zietkiewicz et al. 1994, de León et al. 2004, de León et al. 2005 submitted). M: 1.0 Kb Plus DNA Ladder.

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