POPULATION GENETIC STRUCTURE OF THE GLASSY-WINGED SHARPSHOOTER DETERMINED BY ISSR-PCR DNA FINGERPRINTING

Project Leader:
Jesse H. de León
USDA- ARS
Beneficial Insects Research Unit
Weslaco, Texas 78596

Cooperators:
Walker A. Jones
USDA- ARS
Beneficial Insects Research Unit
Weslaco, Texas 78596

David J. W. Morgan
Cal. Dept. Food and Agriculture
Mount Rubidoux Field Station
Riverside, California 92501

Reporting Period: The results presented here are from work conducted from FY2002 to FY2003.

ABSTRACT
In the present study compound Inter-Simple Sequence Repeat (ISSR) primers containing CA/GT-repeat motifs in their sequences were utilized to estimate the population genetic structure of *Homalodisca coagulata* (Say). Eighteen populations from throughout the U. S. and a population from Tahiti, French Polynesia were analyzed. The eighteen U. S. populations were arbitrarily assigned to three regions- southeastern (SE), southwestern (SW) (Texas), and western (W) (California) regions. A total of 62 and 91 neutral polymorphic markers were identified with p-15 and p-13, respectively. Exact tests for population differentiation indicated significant differences in marker frequencies among the 18 populations; in addition, significant differences were also observed within each region. Analyses of molecular variance (AMOVA) showed a significant partitioning of gene diversity among regions, 11% with p-15 and a lower value of 3% with p-13. The majority of the variance, however, was distributed within populations, 83% and 88% with p-15 and p-13, respectively. Values of G_{ST} (8-11%) and θ (7-10%) for among region variation were of comparable magnitudes to the AMOVA results. A dendrogram based on Reynolds coancestry distance performed with p-15 clustered the U. S. populations into two main groups, with the southeastern populations in one cluster and the southwestern and western populations in another cluster. Within the western region, dendrograms performed with p-13 and p-15 showed in both cases that the Edison and Bakersfield populations clustered as outliers. The present results estimate, for the first time, the population genetic structure of *H. coagulata* and suggest that a subset of insects in California may have their origins in the southwestern region (Texas); furthermore, these results are suggestive of more than one founding event in California.

INTRODUCTION
The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), is a large xylem feeding leafhopper that is a serious pest because it vectors a strain of *Xylella fastidiosa*, a bacterium that causes Pierce's disease in grapevines (*Vitis vinifera* and *V. labrusca*) (Hopkins and Mollenbauer 1973). *H. coagulata* are native to the southern United States, from Florida to Texas and they are also distributed in Northern Mexico (Turner and Pollard 1959, Nielsen 1968, Brlansky et al. 1983). Within the last 10 years, *H. coagulata* have established in southern California where they pose a serious threat to the wine and table grape industry (Sorensen and Gill 1996). Recently, we developed DNA markers for *H. coagulata* for the purpose of estimating genetic variation in natural populations (de León and Walker 2003). These DNA fingerprinting procedures permit detection of DNA variation in Simple Sequence Repeats (SSR) without the need to isolate and sequence specific DNA fragments (reviewed in Karp and Edwards 1997). Many classes of microsatellite repeat motifs have been identified, though the class most abundant in eukaryotic genomes is the CA-repeat. The presence of these repeat motifs in high copy number and their dispersion throughout the genome of all eukaryotes tested has been demonstrated by earlier studies (Tóth et al. 2000). Therefore, because of their high density, oligonucleotides complementary to these CA-repeat motifs can be used as single primers to target a significant portion of the genome and reveal highly polymorphic banding patterns (Zietkiewicz et al. 1994).

OBJECTIVES
The objectives of the present study were to:
1. Estimate genetic variation within and among populations
2. Estimate the population genetic structure
3. Ascertain if our method was sensitive enough to determine the origin of *H. coagulata* present in California.

RESULTS AND CONCLUSIONS
ISSR-PCR was utilized and we demonstrated and compared the ability of two compound ISSR primers p-13, A(CA)7(TA)2T and p-15, T(GT)7(AT)2 to generate polymorphic markers and to estimate geographic variation in 18 natural populations (529 individuals) of *H. coagulata* from the U. S. and a population from Tahiti, French Polynesia (15 individuals). Different approaches to estimate population differentiation- Exact tests (Raymond and Rousset 1995), ΦST (Excoffier at al. 1992), GST (Nei 1987), θ (Weir 1990, 1996), and dendrograms based on genetic distance (Reynolds et al. 1983) (UPGMA method) were than applied and compared.

A total of 62 and 91 neutral polymorphic markers were identified with compound ISSR primers p-15 and p-13, respectively in the 19 populations of *H. coagulata* (Table 1). Within the U. S., percentage polymorphic loci was 100% for each region with both ISSR primers and the highest polymorphic ratio (number of polymorphic loci per number of insects) was seen in the southwestern populations. Exact tests for population differentiation indicated that significant differences in marker frequencies existed among the 18 populations from the U. S. (χ^2 = 664; df = 138; P = 0.0000; p-15 and 1,279; df = 202; P = 0.0000; p-13) and in addition, exact tests showed marker frequency differences within regions, with the western populations showing the highest values with both primers.

Tables 2 and 3 present results from different approaches used to apportion variation into within- and among-population levels. AMOVA analyses showed a statistically significant partitioning of gene diversity among regions, 11% (Φ_{CT} = 0.114; df = 2; P = 0.001) with p-15 and a lower value of 3% (Φ_{CT} = 0.027; df = 2; P = 0.001) with p-13 (Table 2). Significant differentiation was also distributed among populations within regions, 6% (Φ_{SC} = 0.064; df = 15; P = 0.001) with p-15 and 9% (Φ_{SC} = 0.088; df = 15; P = 0.001) with p-13. The majority of the variance, however, was distributed within populations, 83% (Φ_{ST} = 0.171; df = 511; P = 0.001) with p-15 and 88% (Φ_{ST} = 0.113; df = 511; P = 0.001) with p-13. Table 3 shows a comparison of other genetic differentiation estimates, G_{ST} and θ. Excellent agreement was seen between G_{ST} and θ values for the within population variances, 89.0 and 90.1% and 92.0 and 93.0% with p-15 and p-13, respectively. Within each region, little genetic differentiation was seen with G_{ST} and θ results with either primer, though the western region populations demonstrated slightly higher G_{ST} (0.0492; p-15 and 0.0843; p-13) and θ (0.0403; p-15 and 0.0631; p-13) values; furthermore, the indirect estimate of gene flow, Nm based on G_{ST}, demonstrated slightly lower values (9.66; p-15 and 5.43; p-13) with both primers, indicating that the western region was slightly more differentiated, in accord with the Exact tests above. Overall, gene flow was greater among populations within regions than among regions. Taken together though, these overall results indicate moderate genetic differentiation of *H. coagulata* populations from the U. S., but the fact that most of the genetic variation is distributed within populations may be an indication of strong microgeographical differentiation.

A dendrogram based on Reynolds et al. (1983) coancestry distance performed with p-15 is shown on Fig. 1A. Two main clusters were formed with the southeastern region populations (cluster B) separated from the southwestern and western region populations (cluster A). Within cluster A, clusters or subgroups are formed with Edison and Bakersfield populations forming the second separate cluster (d). Two more clusters are seen within cluster c. Western or California populations are distributed within three separate clusters within the main cluster A. Southeastern populations formed two clusters within cluster B, with Tifton and Cairo, GA residing in one subgroup. Results performed with p-13 showed a similar pattern of clustering but with some variation, in that case Weslaco and Monte Alto, TX populations were clustered within the southeastern populations, though the western and southeastern region populations were still clustered separately (data not shown). In order to see a clearer picture of the western region populations, analyses were performed separately from the rest of the U. S. populations with p-15 and p-13 and are demonstrated on Figs. 1B and 1C, respectively. With both ISSR primers two main clusters (A and B) were formed. Some variation in clustering of the populations is seen within cluster A between the two primers; however, results show that in both cases, the Edison and Bakersfield populations were clustered (B) as outliers from the rest of the California populations. In the western region, Edison and Bakersfield are more geographically isolated from the rest of the western populations and appear to be more differentiated.

Table 1. Analyses of H. coagulata from the U. S. analyzed by region.
No. P, number of polymorphic loci; Polymorphic ratio (number of polymorphic loci per number of insects); %P, percent polymorphic loci (POPGENE program); Exact tests (χ^2) (results over loci) (TPFGA program) for population differentiation (Raymond and Rousset 1995). Marker frequencies were based on Lynch and Milligan's (1994) Taylor expansion estimate. df = degrees of freedom (for Exact tests); ***, P = 0.0000 (overall P-value).

Region	No. Insects	No. P	Polym. ratio	%P	χ^2		df
p-15:							
SE	169	34	0.20	100	201.90	***	72
SW	120	54	0.45	100	178.83	***	112
W	240	46	0.19	100	311.76	***	102
All	529	62	0.12	100	664.39	***	138
p-13:							
SE	169	67	0.40	100	415.92	***	126
SW	120	84	0.70	100	403.77	***	178
W	240	83	0.35	100	640.83	***	174
All	529	91	0.17	100	1,279.04	***	202

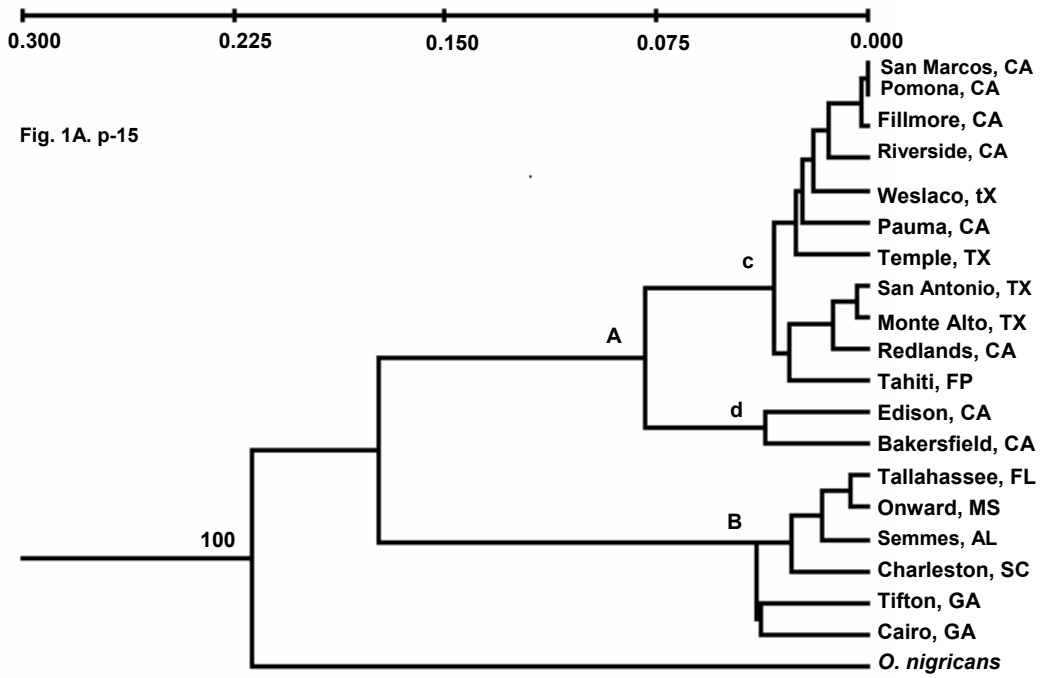
Table 2. Analyses of molecular variance (AMOVA) (GenAlEx program) for *H. coagulata* populations from the U. S. Statistics include: df, degrees of freedom; SS, sum of squares; MS, mean squares; Est. var., estimated variance; and %D, distribution of total variance. **, $P = 0.001$.

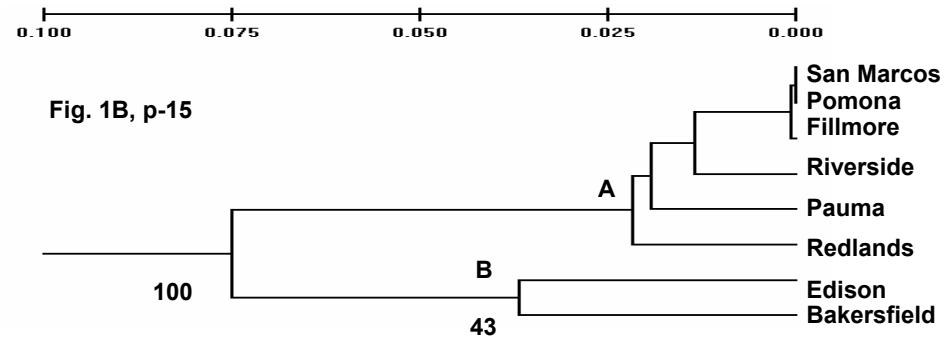
Source	df	SS	MS	Est. var.	Φ- statistics	%D
p-15:						
amg regions	2	93.43	46.71	0.244	$\Phi_{CT} = 0.114$ **	11
amg pops./regions	15	80.24	5.35	0.122	$\Phi_{SC} = 0.064$ **	6
within pops	511	904.0	1.77	1.769	$\Phi_{ST} = 0.171$ **	83
p-13:						
amg regions	2	75.28	37.64	0.128	$\Phi_{CT} = 0.027$ **	3
amg pops./regions	15	239.7	15.98	0.403	$\Phi_{SC} = 0.088$ **	9
within pops	511	2124.3	4.16	4.157	$\Phi_{ST} = 0.113$ **	88

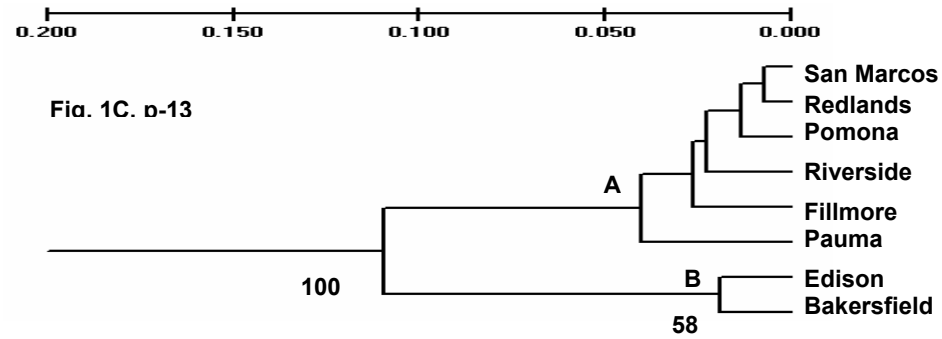
Table 3. Estimates and comparison of G_{ST} (POPGENE) and θ (TFPGA) values for *H. coagulata* populations from the three regions of the U. S. G_{ST} (mean), coefficient of gene differentiation; θ (mean), theta is analogous to F_{ST}; and Nm, gene flow (POPGENE).

Region	G_{ST}	θ (SD)	Nm	Region	G_{ST}	θ (SD)	Nm
p-15:				**p-13:**			
SE	0.0426	0.0321 (0.008)	11.24	SE	0.0615	0.0551 (0.008)	7.63
SW	0.0426	0.0376 (0.014)	11.22	SW	0.0465	0.0449 (0.010)	10.25
W	0.0492	0.0403 (0.016)	9.66	W	0.0843	0.0631 (0.026)	5.43
All	0.1101	0.0989 (0.048)	4.04	All	0.0799	0.0668 (0.012)	5.75

Figure 1. Dendrograms based on Reynolds coancestry distance (TFPGA). Relationships showing the 19 geographic populations of *H. coagulata* performed with p-15 (A). *Oncometopia nigricans* (23) are included as an outgroup. Western region (California) populations were analyzed separately with p-15 (B) and p-13 (C). Genetic distances are indicated above the dendrograms and bootstrap support values (greater than 40%) are indicated at the nodes.







REFERENCES

Brlansky, R.H., L.W. Timmer, W.J. French, and R.E. McCoy. 1983. Colonization of the sharpshooter vectors, *Oncometopia nigricans* and *Homalodisca coagulata*, by xylem-limited bacteria carriers of xylem-limited bacterial disease. Phytopathology 73: 530-535.

de León, J.H., and W.A. Jones. 2003. Detection of DNA polymorphisms in *Homalodisca coagulata* (Homoptera: Cicadellidae) by PCR-based DNA fingerprinting methods. Ann. Entomol. Soc. Am. (in press).

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. Genetics 131: 479-491.

Hopkins, D.L., and H.H. Mollenhauer. 1973. Rickettsia-like bacterium associated with Pierce's disease of grapes. Science 179: 298-300.

Karp, A., and J. Edwards. 1997. DNA markers: a global overview, pp. 1-13 in DNA markers- protocols, applications, and overviews, edited by G. Caetano-Anolles and P. M. Gresshoff. Wiley-Liss, Inc., New York, NY.

Lynch, M., and B.G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3: 91-99.

Nei, M. 1987. Molecular evolutionary genetics / Masatoshi Nei. New York : Columbia University Press, pp. 176-187.

Nielson, M.W. 1968. The leafhopper vectors of phytopathogenic viruses (Homoptera, Cicadellidae) taxonomy, biology, and virus transmission. USDA Tech. Bull. 1382: 81-84.

Raymond, M.L., and F. Rousset. 1995. An exact test for population differentiation. Evolution 49: 1280-1283.

Reynolds, J., B.S. Weir, and C.C. Cockerham. 1983. Estimation of the coancestry coeffient: basis for a short-term genetic distance. Genetics 105: 767-779.

Sorensen, J.T., and R.J. Gill. 1996. A range extension of *Homalodisca coagulata (Say)* (Hemiptera: Clypeorrhyncha: Cicadellidae) to southern California. Pan-Pacific Entomol. 72: 160-161.

Tóth, G., Z. Gáspári, and J. Jurka. 2000. Microsatellites in different eukaryotic genomes: Survey and Analysis. Genome Res. 10: 967-981.

Turner, W.F., and H.N. Pollard. 1959. Life histories and behavior of five insect vectors of phony peach disease. USDA Tech. Bull. 1188: 28 pp.

Weir, B.S. 1990. Genetic Data Analysis: Methods for discrete population genetic data. Sinauer Associates, Inc. Sunderland, Massachusetts. 377 pp.

Weir, B.S. 1996. Genetic Data Analysis II: Methods for discrete population genetic data. Sinauer Associates, Inc. Sunderland, Massachusetts. 445 pp.

Zietkiewicz, E., A. Rafalski., and D. Labuda. 1994. Genomic fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 20: 176-183.

FUNDING AGENCIES

Funding for this project was provided by the USDA Agricultural Research Service.