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Abstract
An existing microsatellite genotype database has been used for several years in population genetic assignment analyses of boll weevils, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), captured in eradication zones. It is important to update it in case of changes in genotype frequency at any of the locations over time. Such changes at neutral loci could be caused by drift, immigration, or population bottlenecks. We examined allele frequency distribution for 10 microsatellite loci to determine genetic differentiation among 10 boll weevil populations sampled from Texas and Mexico in 2009. In addition, temporal changes in genetic composition were examined in the eight populations for which samples were available from previous years. Substantial levels of spatial genetic structure were observed, with the 10 populations clustering as four major groups. Pairwise F_{ST} estimates in 2009 samples ranged from 0.001 (College Station-Cameron) to 0.492 (College Station-Ojinaga). There was little change in genetic profiles over time at four of the eight locations. Thus, for those four locations, genotype and allele frequency data can be pooled over the two sample dates, which will provide greater statistical power in future population assignment tests. However, genetic profiles changed substantially at Ojinaga, and to a lesser extent at Uvalde, Cameron, and Rosales, so the 2009 genotype data should be substituted in future analyses. Finally, populations from two new locations, Brownsville and Lockhart, TX, were sampled, genotyped, and added to the database. The addition of Lockhart is particularly important given its surprisingly high differentiation from the relatively nearby populations of Cameron and Uvalde.

Keywords
Anthonomus grandis grandis, population genetics, population assignment, microsatellites, population structure

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Temporal Changes in Genetic Variation of Boll Weevil (Coleoptera: Curculionidae) Populations, and Implications for Population Assignment in Eradication Zones

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ABSTRACT An existing microsatellite genotype database has been used for several years in population genetic assignment analyses of boll weevils, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), captured in eradication zones. It is important to update it in case of changes in genotype frequency at any of the locations over time. Such changes at neutral loci could be caused by drift, immigration, or population bottlenecks. We examined allele frequency distribution for 10 microsatellite loci to determine genetic differentiation among 10 boll weevil populations sampled from Texas and Mexico in 2009. In addition, temporal changes in genetic composition were examined in the eight populations for which samples were available from previous years. Substantial levels of spatial genetic structure were observed, with the 10 populations clustering as four major groups. Pairwise F_{ST} estimates in 2009 samples ranged from 0.001 (College Station-Cameron) to 0.492 (College Station-Ojinaga). There was little change in genetic profiles over time at four of the eight locations. Thus, for those four locations, genotype and allele frequency data can be pooled over the two sample dates, which will provide greater statistical power in future population assignment tests. However, genetic profiles changed substantially at Ojinaga, and to a lesser extent at Uvalde, Cameron, and Rosales, so the 2009 genotype data should be substituted in future analyses. Finally, populations from two new locations, Brownsville and Lockhart, TX, were sampled, genotyped, and added to the database. The addition of Lockhart is particularly important given its surprisingly high differentiation from the relatively nearby populations of Cameron and Uvalde.

KEY WORDS *Anthonomus grandis grandis*, population genetics, population assignment, microsatellites, population structure

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), has been one of the most injurious pests of cultivated cotton, *Gossypium hirsutum* L., in North America for over a century (Allen 2008, Smith et al. 2010), and in South America for several decades (Stadler and Buteler 2007). Most boll weevil movement results in dispersal over relatively short distances (Johnson et al. 1975, Moody et al. 1993, Raulston et al. 1996), but long distance dispersal over several hundreds of kilometers is not unusual (Guerra 1988; Spurgeon et al. 1997; Kim and Sappington 2004a,b, 2006; Kim et al. 2006, 2010; Westbrook et al. 2010a). Reintroduction of weevils to eradication zones where populations have been eliminated or substantially suppressed is a perpetual concern because of the expense and difficulty involved in eradicating new infestations (Culin et al. 1990, Kim et al. 2010, Smith et al. 2010, Westbrook et al. 2010a). Knowledge of pathways and magnitude of immigration is also important for planning and implementing the most effective strategies for achieving final eradication in the Lower Rio Grande Valley and parts of the South Texas/Winter Garden zones, where elimination of endemic infestations has been especially difficult (Smith et al. 2010, Troxclair 2010, Westbrook et al. 2010b).

Genetic markers can be used to identify likely first-generation migrants and their possible origins (Rannala and Mountain 1997, Cornuet et al. 1999, Paetkau et al. 2004, Piry et al. 2004, Broquet and Petit 2009, Lowe and Allendorf 2010). Such population assign-

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Table 1. Location, years, and sample sizes (*N*) of boll weevils genotyped at 10 microsatellite loci

Sample location	2009 samples		Previous samples			
	Location designation	*N*	Sample location	Location designation	Yr sampled	*N*
Weslaco, TX	WTXn	50	Weslaco, TX	WTX	2000	54
Bishop and Rivera, TX	KTXn	44	Kingsville, TX	KTX	2002	54
Mumford, TX	CSTXn	50	College Station, TX	CSTX	2000	16
Rosales, Chihuahua, Mexico	MDRn	50	Rosales, Chihuahua	MDR	2004	52
Ojinaga, Chihuahua, Mexico	OJIn	30	Ojinaga, Chihuahua	OJI	2004	52
Gomez Palacio, Durango, Mexico	TLOn	50	Tlahualilo, Durango	TLO	2004	53
Uvalde, TX	Uvaln	50	Uvalde, TX	Uval	2007	40
Taylor, TX	Camn	50	Cameron, TX	Cam	2007	42
Lockhart, TX	LOKT	50	None			
Brownsville, TX	BROT	50	None			

ment analyses are widely used in conservation and fisheries management (Manel et al. 2005, Hauser et al. 2006), invasive species studies (Davies et al. 1999, Aketarawong et al. 2007, Ciosi et al. 2008), eradication contexts (Bonizzoni et al. 2004, Miller et al. 2009), and many other ecological situations (Broquet and Petit 2009, Mayer et al. 2009). Application of population genetic assignment methods using selectively neutral microsatellite markers has proven a useful and powerful tool in a number of contexts for elucidating likely origins of newly captured boll weevils in eradication zones (Kim and Sappington 2006; Kim et al. 2006, 2008, 2010). Identifying the geographic origin of weevils unexpectedly captured in an eradication zone helps program managers make the most appropriate immediate responses to prevent reestablishment, as well as to guide future monitoring strategies (Kim et al. 2006, 2010).

Changes in genotype frequency at neutral loci can be caused by drift, immigration, or population bottlenecks from recurring population extinction and recolonization. All of these processes are particularly likely in the case of boll weevil populations, which have suffered intense anthropogenic mortality under eradication regimes. Distinguishing between changes caused by drift or immigration after local eradication or near-eradication is difficult at any given location, and we have detected both kinds of situations in the past using population assignment strategies with these markers. For example, an infestation of boll weevils was discovered in 2004 in a noneradication area near Tlahualilo, Durango, just south of the South Central eradication zone in Chihuahua state, after a decade of apparent absence. By bringing genetic assignment and exclusion tests to bear, it was determined that the most likely source of this infestation was an endemic population that remained below detection thresholds rather than natural or human-transported immigrants from the north or east (Kim et al. 2006). In contrast, after Tropical Storm Erin passed through Texas in August 2007, monitoring traps began to capture large numbers of boll weevils in the Southern Rolling Plains eradication zone, which had been weevil-free since 2004. The most likely source was determined to be the still-infested Winter Garden area around Uvalde based on a combination of evidence from genetic assignment methods, pollen fingerprinting, and atmospheric trajectory analysis (Kim et al. 2010).

The genetic assignment methods used for boll weevils involve comparison of the genotypes of target individuals against genotypes in a reference database of potential source populations sampled over the last decade. To maintain its effectiveness, it is important to update the reference database in case there have been changes in genotype frequencies within any given population over time. The goal of this study was to determine genetic differentiation at microsatellite loci among 10 populations of boll weevil (*A. g. grandis*), collected from Texas and Mexico in 2009, and to examine temporal changes in genetic composition in eight of those populations which were sampled in previous years, from 2000 to 2007. Although causes for any temporal changes are inherently interesting, the null hypothesis we tested for each location was that there had been no significant change in genotypic profile between the time of initial sampling and new samples in 2009. Lack of genetic change justifies pooling the previous samples with those of 2009, because the indistinguishable genetic profiles indicate stasis over the sampling interval. Pooling is desirable, when justified, because it increases the power of future assignment tests. Conversely, significant change over time indicates that genotype data from the previous sample should be discarded in favor of the most recent data. We also expanded the database to include two new locations not previously sampled (Lockhart and Brownsville, TX).

Materials and Methods

Sample Collection. Boll weevils were collected from 10 locations in central and southern Texas, and northern Mexico (Table 1; Fig. 1). Eight of the locations, or equivalent nearby locations, had been sampled previously, and two (Lockhart and Brownsville, TX) represent new areas. The genetic equivalency of nearby locations is assumed from previous population genetics analyses indicating effective migrant exchange and lack of significant structuring at distances $<$300 km (Kim and Sappington 2006, and unpublished data). New collections from other locations in the United States previously sampled and genotyped (Kim and Sappington 2006, Kim et al. 2006) were not possible because eradication efforts had essentially driven those populations to or near extinction by 2009. Tampico, Mexico, was not be resampled for logistical rea-

Fig. 1. Locations of boll weevils sampled in 2009. All locations except Brownsville and Lockhart also were sampled previously (see Table 1 for sample years).

sons. Mixed sexes from each sampled location were collected by traps baited with aggregation pheromone during May–July 2009. A total of 474 individuals were genotyped, including 50 individuals per location when possible (Table 1). All samples were stored in ethanol until DNA extraction.

DNA Extraction and Microsatellites Analysis. Each boll weevil was ground with pestle in liquid nitrogen, and genomic DNA was extracted using the Gentra Puregene tissue kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Ten polymorphic dinucleotide-repeat microsatellite markers, AG-D1–7 and AG-D10–12, developed for *A. grandis* by Kim and Sappington (2004c), were amplified in two multiplexed polymerase chain reactions (PCRs) as described by Kim et al. (2006). Touchdown PCR was carried out under the following conditions: initial denaturation for 15 min at 94°C, followed by seven touchdown cycles starting at 94°C for 30 s, 67°C for 90 s, and 72°C for 60 s, with annealing temperature decreasing by 2°C per cycle to 53°C, followed by an additional 25 cycles at 94°C for 30 s, 53°C for 90 s, 72°C for 60 s, and final extension at 60°C for 30 min. Individuals were genotyped using a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA), as described by Kim and Sappington (2004c).

An allele at 232 bp for locus AG-D11 had been noticed at low frequency in previous studies but was not reported because we considered it to be a PCR artifact resulting from an adenylation event during PCR. We now conclude it is a true allele because it was frequently detected in our 2009 samples. Accordingly, we reexamined all data sets from earlier samples and restored the 232 allele in several weevils. All analyses reported herein were conducted with the 232 allele restored.

Data Analysis. The number of alleles, allelic richness per population and locus, observed heterozygosity (H_O), and unbiased estimates of expected heterozygosity (H_E) under the assumption of Hardy–Weinberg equilibrium (HWE) were calculated with the Microsatellite Toolkit (Park 2001) and FSTAT version 2.9.3 (Goudet 1995). Pairwise F_{ST} estimates and their significance were calculated using a permutation approach in FSTAT version 2.9.3. GENEPOP 4.0.6 (Raymond and Rousset 1995) was used to conduct exact probability tests (Guo and Thompson 1992) for linkage disequilibrium between locus pairs, and deviation from HWE for each locus and population. The sequential Bonferroni correction was applied in multiple comparisons to maintain the nominal significance level of $\alpha = 0.05$ (Rice 1989).

STRUCTURE 2.3.3 software (Pritchard et al. 2000) was used to reveal boll weevil population structure among geographic locations sampled in 2009, and among temporal samples within the same location, based on genetic similarity and clustering. Likelihood values, Ln P(D), were generated for each of five runs of STRUCTURE at K values of 1–10 for boll weevils at the 10 locations sampled in 2009. The initial burn-in period was 100,000, followed by 200,000 replications after burn-in. Temporal samples at the same location likewise were examined with STRUCTURE at K values of 1–5 to provide an indication of which locations harbor populations that have changed genetically between the two sample dates.

Changes in performance using genetic data from earlier samples in individual assignment (Paetkau et al. 1995, Rannala and Mountain 1997, Cornuet et al. 1999) and exclusion (Cornuet et al. 1999) tests were examined by including temporal samples from the same location. Both types of tests were carried out using the direct frequency-based and simulation approaches, respectively, implemented in the program GeneClass2 (Piry et al. 2004), as described by Kim et al. (2006). Using both helps in interpreting results and lends confidence to outcomes that are supported by multiple analyses. Individual assignment was conducted using the leave-one-out procedure (Paetkau et al. 1998, 2004; Cornuet et al. 1999), where the genotype of an individual to be assigned is not included in the population from which it was sampled. If the individual's allele was absent from one of the tested populations, the value was set to 0.01. For assignment tests, the mean value of individual assignment scores was used rather than the population score, because the leave-one-out procedure is not an option for the latter. This procedure is particularly useful for populations that are not at equilibrium, which can be caused by the presence of immigrants (Cornuet et al. 1999). To visualize the amount of genetic change over time within a location, the log likelihood values of individual genotypes being assigned to the earlier sample was plotted against its log likelihood value of being assigned to the later sample. The plotted points for each location are color-coded by whether the assigned individual was collected in the early or late sample. Lack of temporal change is evidenced by intermingling of values of the two colors. Conversely, temporal change results in clouds of values separated by color, i.e., separated by early and late origins.

Table 2. Comparison of genetic diversity estimates between previously collected (Prev.) and 2009 genotype data for boll weevils at 10 microsatellite loci

Location	Allelic diversity[a]		Allelic richness[a]		H_E		H_O	
	Prev.	2009	Prev.	2009	Prev.	2009	Prev.	2009
WTXn	4.8	4.8	3.913	3.959	0.5624	0.5458	0.5370	0.5500
KTXn	4.6	4.3	3.437	3.644	0.4800	0.4796	0.4519	0.4886
CSTXn	2.4	2.4	2.400	2.135	0.2065	0.1837	0.1875	0.1860
MDRn	2.5	2.6	2.428	2.499	0.4028	0.4435	0.3904	0.4520
OJIn	1.8	2.2	1.789	2.153	0.2913	0.3925	0.2923	0.3533
TLOn	2.5	2.9	2.377	2.572	0.3850	0.3842	0.3962	0.3260
Uvaln	3.9	4.2	3.532	3.722	0.5618	0.5398	0.4900	0.5200
Camn	2.3	2.3	2.029	2.117	0.1795	0.1924	0.1381	0.1620
LOKT		3.8		3.428		0.5369		0.5020
BROT		4.1		3.539		0.4903		0.4460
Mean	3.1	3.4	2.738	2.977	0.3837	0.4189	0.3604	0.3986

See Table 1 for collection years, sample sizes, and location designations.
[a] Allelic diversity, mean number of alleles per locus; allelic richness, mean allelic richness per locus per population.

Results

Genetic Diversity of Boll Weevils Collected in 2009. In total, 54 alleles were detected across the 10 boll weevil microsatellite loci analyzed in the 10 populations sampled in 2009. Of the alleles in the eight populations sampled in 2009 and previously, only one allele (108 bp for locus AG-D1 in Weslaco, TX) had not been observed in any of the 24 total populations sampled in our earlier studies, and seven that had been observed earlier were not observed in 2009. All of these alleles were at low frequencies (<0.05) when detected.

The number of alleles per locus ranged from 2 to 10 with a mean of 5.4. Allelic diversity and allelic richness ranged, respectively, from 4.8 and 4.0 in the sample from Weslaco to 2.2 and 2.2 in the sample from Ojinaga. H_O and H_E heterozygosity showed a trend similar to that of allelic diversity. The highest genetic diversity was observed in Weslaco (H_E = 0.546) and the lowest in College Station (H_E = 0.184; Table 2). There were no instances of linkage disequilibrium, nor did any individual loci or populations significantly deviate from HWE.

Spatial Population Differentiation and Genetic Structure. Genetic differentiation between each pair of populations (i.e., pairwise F_{ST}) and significance are shown in Table 3. Pairwise F_{ST} estimates among populations sampled in 2009 ranged from 0.001 (College Station versus Cameron) to 0.492 (College Station versus Ojinaga), and were significant in all but four of 45 comparisons, namely, between Cameron and College Station and between Weslaco, Kingsville, and Brownsville.

STRUCTURE 2.3.3 software was used to estimate population structure among the 10 populations sampled in 2009. The highest likelihood value in all runs was for K = 4 (Table 4), indicating that the boll weevils from the 10 locations can potentially be divided into four main populations. The bar plot for K = 4 of one of the five iterations with the highest likelihood value (Fig. 2) suggests that genotypes are best clustered as a Mexican population (Rosales, Ojinaga,

Table 3. Pairwise F_{ST} estimates (below diagonal) and their significance (above diagonal) among boll weevil populations over time and space

Location	2009										Pre-2009							
	WTXn	KTXn	CSTXn	MDRn	OJIn	TLOn	Uvaln	Camn	LOKT	BROT	WTX	KTX	CSTX	MDR	OJI	TLO	Uval	Cam
WTXn	—	NS	S	S	S	S	S	S	S	NS	***NS***	NS	S	S	S	S	S	S
KTXn	0.014	—	S	S	S	S	S	S	S	NS	NS	***NS***	S	S	S	S	S	S
CSTXn	0.174	0.176	—	S	S	S	S	NS	S	S	S	S	***NS***	S	S	S	S	S
MDRn	0.154	0.220	0.362	—	S	S	S	S	S	S	S	S	S	***S***	S	S	S	S
OJIn	0.255	0.309	0.492	0.138	—	S	S	S	S	S	S	S	S	S	***S***	S	S	S
TLOn	0.209	0.258	0.344	0.145	0.184	—	S	S	S	S	S	S	S	S	S	***NS***	S	S
Uvaln	0.034	0.041	0.249	0.193	0.258	0.264	—	S	S	S	NS	S	S	S	S	S	***S***	S
Camn	0.169	0.166	0.001	0.355	0.478	0.328	0.239	—	S	S	S	S	NS	S	S	S	S	***S***
LOKT	0.099	0.101	0.336	0.200	0.290	0.295	0.058	0.323	—	S	S	S	S	S	S	S	S	S
BROT	0.007	0.003	0.147	0.209	0.299	0.245	0.037	0.138	0.102	—	NS	NS	S	S	S	S	S	S
WTX	**0.019**	0.032	0.263	0.184	0.272	0.267	0.011	0.254	0.050	0.018	—	NS	S	S	S	S	NS	S
KTX	0.014	**0.010**	0.144	0.215	0.320	0.247	0.052	0.133	0.116	−0.005	0.026	—	S	S	S	S	S	S
CSTX	0.131	0.131	**0.024**	0.299	0.423	0.282	0.181	−0.002	0.256	0.103	0.187	0.095	—	S	S	S	S	NS
MDR	0.224	0.279	0.434	**0.044**	0.146	0.183	0.239	0.424	0.222	0.262	0.228	0.270	0.366	—	S	S	S	S
OJI	0.354	0.401	0.620	0.258	**0.331**	0.434	0.296	0.606	0.254	0.388	0.319	0.415	0.561	0.188	—	S	S	S
TLO	0.206	0.259	0.343	0.121	0.209	**0.013**	0.275	0.330	0.289	0.247	0.266	0.246	0.287	0.155	0.410	—	S	S
Uval	0.051	0.057	0.278	0.160	0.259	0.246	**0.037**	0.268	0.019	0.053	0.017	0.061	0.199	0.194	0.272	0.238	—	S
Cam	0.166	0.171	0.022	0.354	0.497	0.327	0.240	**0.024**	0.324	0.144	0.260	0.141	0.013	0.428	0.617	0.326	0.262	—

Bold, entries for temporal comparisons within a location. Location abbreviations are as in Table 1. Significance obtained after 3,060 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is 0.000327; S, significant; NS, nonsignificant.

Table 4. Likelihood values, Ln P(D), from STRUCTURE analyses (Pritchard et al. 2000) to determine the most likely number of genetically distinct populations among a total of 474 boll weevils sampled from 10 locations in Texas and Mexico in 2009, based on genotypes at 10 microsatellite loci

Run	K = 1	K = 2	K = 3	K = 4	K = 5	K = 6	K = 7	K = 8	K = 9	K = 10
1	−9165.6	−8162.9	−7639.8	−7575.4	−7657.5	−7880.3	−7811.2	−7697.2	−8341.8	−8191.9
2	−9165.6	−8164.6	−7639.5	−7579.6	−7650.6	−7826.9	−8029.6	−7721.1	−8227.0	−8394.8
3	−9165.6	−8164.3	−7639.7	−7573.5	−7664.2	−7841.6	−7934.4	−7654.4	−8270.0	−8113.1
4	−9165.6	−8165.6	−7637.9	−7568.4	−7663.0	−7906.4	−7923.1	−8071.6	−8506.2	−8189.8
5	−9165.6	−8164.1	−7640.3	−7572.7	−7698.4	−7867.6	−7869.7	−8171.9	−8321.4	−8192.4
Mean	−9165.6	−8164.3	−7639.4	−7573.9	−7666.7	−7864.6	−7913.6	−7863.2	−8333.3	−8216.4

The highest mean likelihood value among the number of putative populations (K) tested in each of five runs (200,000 replications per run) indicates the most likely number of populations represented by the genotype data.

and Tlahualilo), a South Texas population (Weslaco, Kingsville, Brownsville, and Uvalde), North Texas population (College Station and Cameron), and the Lockhart population. Qualitatively, Uvalde seems to most resemble other samples in the South Texas population based on the STRUCTURE results (Fig. 2), even though it is not geographically near the other three locations in that group (Figs. 1 and 2). This is consistent with a study to determine the likely sources of a large influx of boll weevils into the Southern Rolling Plains eradication zone in late summer 2007, a zone where this insect had been successfully eradicated (Kim et al. 2010). Population assignment and exclusion tests of captured weevils in that study, using the same markers and genotype information for all populations in the database, suggested the most likely source area was Uvalde, but with Weslaco and Kingsville as possibilities as well. It also is supported by evidence of extensive boll weevil movement on prevailing southeasterly winds from the Lower Rio Grande Valley, which includes Weslaco and Brownsville, to the Winter Garden area where Uvalde is located (Westbrook et al. 2010a). However, pairwise F_{ST} estimates for 2009 samples between Uvalde and the other three sample locations from the South Texas group are all significant (Table 3) and thus do not support its clustering with this group. Given its geographic proximity, the Lockhart population is surprisingly distinct from the Uvalde and Cameron populations, although it is less diverged from Uvalde than from any other population sampled (Table 3; Fig. 2).

Comparisons With Previous Population Data. Genetic diversity estimates as expressed by mean number of alleles, allelic richness, H_E and H_O per locus, are presented for the eight locations resampled in 2009 (Table 2). There was a trend toward greater genetic diversity in Ojinaga in 2009 than when measured in 2004 (Table 2), but none of the differences were significant according to Wilcoxon rank sum tests conducted on pairs of values for each microsatellite locus ($P = 0.08–0.13$). Likewise, none of the paired-comparisons differences were significant for any of the locations.

Genetic differentiation within each population sampled in different years, measured as pairwise F_{ST}s and associated P values, provides one avenue for examining genetic change over time (Table 3). Four of the eight locations—Weslaco, Kingsville, College Station, and Tlahualilo—showed no significant differentiation over time. Temporal differentiation was statistically significant for the remaining four locations, although F_{ST} values were low for Rosales, Uvalde, and Cameron, ranging from 0.024 to 0.044. Only Ojinaga showed a high and significant temporal F_{ST} value (0.331), indicating that the boll weevil population in that location underwent a major change in genetic composition between 2004 and 2009 (Table 3). Among different locations, F_{ST} values were not significant between Weslaco and Kingsville in 2009 and between Cameron and College Station in all years, implying that these paired regions represent panmictic populations.

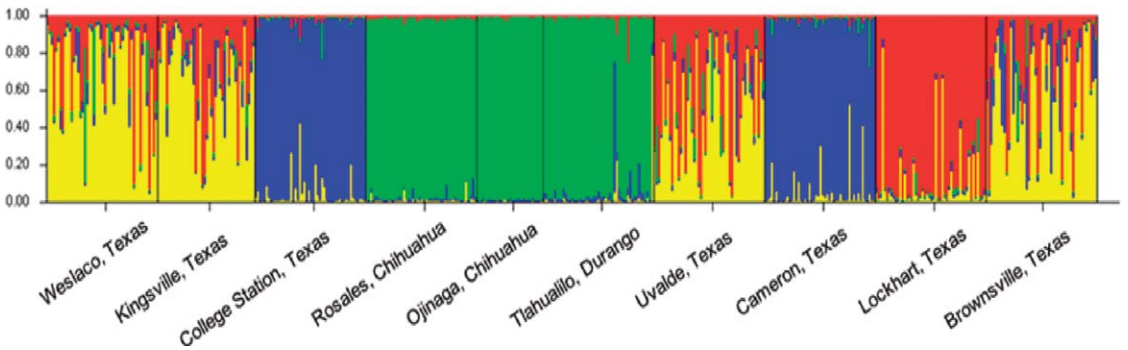

Fig. 2. Bar plot for population structure estimates of boll weevils collected in 2009, after five iterations of STRUCTURE at K = 1–10, based on highest observed likelihood value [K = 4, Ln P(D) = −7568.4]. All runs supported K = 4. The x-axis indicates the location of collection.

Table 5. Likelihood values, Ln P(D), from STRUCTURE analyses (Pritchard et al. 2000) to determine the most likely number of genetically distinct populations among all boll weevils sampled at each location pre-2009 and in 2009 (see Table 1 for sample years and sizes), based on genotypes at 10 microsatellite loci

Location	Run	K = 1	K = 2	K = 3	K = 4	K = 5
Weslaco, TX	1	−2,214.0	−2,232.6	−2,287.9	−2,273.1	−2,252.3
	2	−2,214.2	−2,221.0	−2,277.1	−2,253.6	−2,321.1
	3	−2,215.3	−2,233.1	−2,260.8	−2,235.2	−2,244.7
	4	−2,214.0	−2,226.4	−2,227.8	−2,271.3	−2,238.1
	5	−2,214.2	−2,222.6	−2,275.4	−2,232.4	−2,256.1
	Mean	−2,214.3	−2,227.1	−2,265.8	−2,253.1	−2,262.5
Kingsville, TX	1	−1,797.1	−1,799.1	−1,838.7	−1,888.1	−1,998.1
	2	−1,798.0	−1,805.7	−1,834.2	−1,888.6	−1,917.7
	3	−1,798.9	−1,806.5	−1,861.4	−1,926.6	−1,908.7
	4	−1,798.0	−1,801.9	−1,810.3	−1,873.4	−1,956.1
	5	−1,797.8	−1,804.7	−1,834.2	−1,877.0	−1,820.3
	Mean	−1,798.0	−1,803.6	−1,835.8	−1,890.7	−1,920.2
College Station, TX	1	−497.6	−502.0	−509.7	−504.3	−502.6
	2	−497.6	−497.4	−497.1	−501.3	−498.1
	3	−497.5	−497.8	−510.1	−499.9	−499.2
	4	−497.6	−502.3	−503.4	−515.8	−511.1
	5	−497.7	−499.3	−502.1	−497.0	−506.9
	Mean	−497.6	−499.8	−504.5	−503.7	−503.6
Rosales, Mexico	1	−1,448.9	−1,752.6	−1,693.5	−1,552.1	−1,705.4
	2	−1,449.1	−1,599.3	−1,503.9	−1,743.9	−1709.8
	3	−1,448.9	−1,729.6	−1,759.2	−1,783.9	−1,672.4
	4	−1,448.7	−1,572.6	−1,764.7	−1,705.6	−1,738.1
	5	−1,448.6	−1,479.2	−1,570.8	−1,592.2	−1,634.0
	Mean	−1,448.8	−1,626.7	−1,658.4	−1,675.5	−1,691.9
Ojinaga, Mexico	1	−1,056.1	−835.5	−829.0	−855.5	−896.6
	2	−1,056.1	−835.6	−829.3	−858.2	−887.5
	3	−1,056.1	−835.5	−828.8	−859.9	−900.9
	4	−1,056.2	−835.5	−829.3	−872.2	−907.6
	5	−1,055.9	−835.6	−828.8	−844.6	−904.3
	Mean	−1,056.1	−835.5	−829.0	−858.1	−899.4
Tlahualilo, Mexico	1	−1,335.8	−1,434.8	−1,439.1	−1,459.3	−1,798.7
	2	−1,335.6	−1,449.7	−1,513.4	−1,632.8	−1,337.6
	3	−1,335.9	−1,383.3	−1,503.2	−1,472.3	−1,792.3
	4	−1,335.9	−1,414.1	−1,513.6	−1,634.6	−1,883.1
	5	−1,335.6	−1,443.9	−1,467.0	−1,536.1	−1,765.0
	Mean	−1,335.8	−1,425.2	−1487.3	−1,547.0	−1,715.3
Uvalde, TX	1	−1,870.2	−1,849.3	−1,926.5	−1,872.4	−1,914.3
	2	−1,870.4	−1,848.4	−1,913.3	−1,885.1	−1,898.7
	3	−1,870.4	−2,323.3	−1,942.2	−1,866.9	−1,853.7
	4	−1,871.9	−1,875.2	−1,899.6	−1,889.6	−1,892.9
	5	−1,870.1	−1,846.6	−1,963.0	−1,878.6	−1,897.8
	Mean	−1,870.6	−1,948.6	−1,928.9	−1,878.5	−1,891.5
Cameron, TX	1	−684.8	−708.9	−844.2	−879.2	−838.3
	2	−684.9	−686.9	−1,063.7	−855.8	−837.5
	3	−684.8	−689.4	−828.0	−947.1	−1,017.2
	4	−683.6	−683.0	−812.6	−931.1	−907.4
	5	−685.2	−684.0	−800.4	−873.3	−928.2
	Mean	−684.7	−690.4	−869.8	−897.3	−905.7

The highest mean likelihood value among the number of putative populations (K) tested in each of five runs (200,000 replications per run) indicates the most likely number of populations represented by the pooled genotype data from the two sample years.

The mean pairwise F_{ST} across time within the same locations was 0.061, and was 0.243 among different locations. When Ojinaga was excluded, mean F_{ST} values were reduced to 0.023 for pairs from the same location and 0.200 among different locations, reflecting the high degree of genetic change that occurred in Ojinaga over time and its relatively high spatial isolation. Likewise, STRUCTURE analyses between old and new samples within each location support a single temporal population in all cases except Ojinaga (Table 5). The pairwise F_{ST}s between Ojinaga and the other populations did not change significantly over time as a group (paired comparisons *t*-test, mean difference new – old = −0.0203, $t_{df=7} = -0.53$, $P = 0.62$; Table 3).

Another approach to examine the extent of change in genetic profile in the same location over time is to conduct reciprocal assignment and exclusion tests on individuals between temporal samples. The best assignment value (i.e., lowest value in Table 6) or best nonexclusion value (i.e., highest percentage in Table 6) of boll weevils sampled in 2009 compared with possible source populations sampled pre-2009 do not always coincide with the same location, but in most cases, these scores are nevertheless close to the best. The same is true for assignment and exclusion values for boll weevils sampled in pre-2009 to possible source populations sampled in 2009. The exception is Ojinaga with poor reciprocal assignment scores relative to other putative source populations (Table 6). Most

Table 6. Summary of individual assignment and exclusion tests for 16 spatial and temporal populations

Pop	Test	Potential source pop															
		2009								Pre-2009							
		WTXn	KTXn	CSTXn	MDRn	OJIn	TLOn	Uvaln	Camn	WTX	KTX	CSTX	MDR	OJI	TLO	Uval	Cam
WTXn	Assign	7.57	7.95	12.66	15.07	18.19	15.45	8.43	12.84	7.79	7.67	12.21	16.35	23.63	16.15	9.3	13.46
	Not-E (%)	92.0	82.0	0.0	2.0	0.0	0.0	78.0	0.0	96.0	92.0	0.0	0.0	0.0	0.0	66.0	0.0
KTXn	Assign	6.8	6.62	10.56	15.23	18.4	15.1	7.56	11.06	7.05	6.72	10.41	16.37	23.25	15.92	7.69	11.8
	Not-E (%)	97.7	90.9	4.5	0.0	0.0	0.0	90.9	2.3	97.7	95.5	2.3	0.0	0.0	0.0	90.9	0.0
CSTXn	Assign	5.54	5.15	2.62	11.5	13.9	10.22	6.74	2.65	6.81	4.56	3.62	12.54	22.65	10.43	7.45	3.69
	Not-E (%)	100.0	100.0	98.0	2.0	0.0	4.0	100.0	98.0	100.0	100.0	88.0	0.0	0.0	2.0	94.0	74.0
MDRn	Assign	9.87	12.13	16.43	5.07	8	7.64	10.44	15.79	10.35	10.41	13.95	5.57	12.47	7.19	10.51	17.54
	Not-E (%)	70.0	8.0	0.0	98.0	24.0	50.0	52.0	0.0	70.0	50.0	0.0	88.0	0.0	44.0	42.0	0.0
OJIn	Assign	12.87	15.8	18.42	6.30	4.48	8.02	12.76	17.38	13.39	13.53	15.6	6.30	13.11	7.96	13.96	21.28
	Not-E (%)	10.0	0.0	0.0	80.0	90.0	43.3	16.7	0.0	10.0	6.7	0.0	70.0	3.3	30.0	0.0	0.0
TLOn	Assign	11.11	13.76	15.67	6.73	8.63	4.82	12.52	14.58	12.28	11.64	13.3	7.14	19.33	4.87	11.62	14.89
	Not-E (%)	32.0	12.0	0.0	76.0	16.0	94.0	22.0	2.0	26.0	24.0	2.0	34.0	0.0	94.0	28.0	2.0
Uvaln	Assign	7.9	8.44	14.5	15.21	17.9	16.06	7.17	14.83	7.77	8.16	12.64	16.19	20.32	17.45	8.9	15.16
	Not-E (%)	94.0	78.0	2.0	2.0	0.0	0.0	96.0	2.0	96.0	84.0	2.0	0.0	0.0	0.0	80.0	0.0
Camn	Assign	5.53	5.17	2.75	11.24	13.55	9.9	6.78	2.73	6.69	4.58	3.33	12.17	21.82	10.18	7.29	3.84
	Not-E (%)	100.0	100.0	96.0	2.0	0.0	6.0	98.0	96.0	98.0	100.0	88.0	2.0	0.0	6.0	94.0	72.0
WTX	Assign	8.17	8.52	14.71	16.84	19.5	17.27	8.59	14.81	7.85	8.44	13.19	17.59	23.15	18.3	8.89	15.32
	Not-E (%)	92.6	77.8	0.0	0.0	0.0	0.0	83.3	0.0	94.4	83.3	0.0	0.0	0.0	0.0	70.4	0.0
KTX	Assign	6.82	6.95	9.9	14.36	17.43	14.33	7.66	10	7.16	6.8	9.71	15.31	22.35	15.01	8.14	10.91
	Not-E (%)	100.0	90.7	9.3	1.9	0.0	1.9	87.0	9.3	98.1	94.4	9.3	1.9	0.0	1.9	81.5	5.6
CSTX	Assign	5.73	5.34	3.48	11.01	13.7	9.85	6.73	3.4	6.51	4.88	3.31	11.86	20.8	10.24	7.09	3.71
	Not-E (%)	100.0	100.0	87.5	0.0	0.0	12.5	93.8	75.0	100.0	100.0	87.5	0.0	0.0	6.3	100.0	68.8
MDR	Assign	11.03	13.21	17.41	5.16	7.88	7.41	10.96	16.68	10.9	11.3	14.87	4.71	9.7	6.89	10.63	18.85
	Not-E (%)	40.4	5.8	0.0	98.1	21.2	59.6	30.8	0.0	50.0	30.8	0.0	96.2	1.9	53.8	42.3	0.0
OJI	Assign	10.72	12.01	18.94	6.26	9.72	10.57	8.99	17.9	9.87	10.73	15.54	5.25	3.01	10.31	9.72	21.14
	Not-E (%)	46.2	15.4	0.0	78.8	3.8	1.9	84.6	0.0	82.7	30.8	0.0	88.5	98.1	0.0	61.5	0.0
TLO	Assign	10.39	12.73	15.07	6.16	9.24	4.65	12.22	14.06	11.49	10.85	13.33	6.48	18.39	4.42	10.83	14.57
	Not-E (%)	56.6	5.7	0.0	83.0	11.3	100.0	13.2	0.0	43.4	30.2	0.0	58.5	0.0	100.0	37.7	0.0
Uval	Assign	8.98	8.81	15.33	15.23	18.69	16.26	9.11	15.53	8.36	8.92	13.61	15.88	20.83	17.06	7.55	15.34
	Not-E	82.5	70.0	0.0	0.0	0.0	0.0	70.0	2.5	95.0	67.5	0.0	0.0	0.0	0.0	92.5	0.0
Cam	Assign	5.33	5.17	3.07	10.88	14.5	9.2	6.73	3.48	6.32	4.64	3.73	12.05	22.54	9.48	6.68	2.7
	Not-E (%)	100.0	97.6	88.1	2.4	0.0	2.4	97.6	88.1	100.0	97.6	83.3	0.0	0.0	2.4	97.6	88.1

For assignment test, mean value of individual assignment likelihood ($-\log L_{i\,to\,j}$) calculated using the Bayesian method with the leave-one-out option is listed. For exclusion test, percentage of individuals of each sample population that could not be statistically excluded (i.e., $P > 0.01$) as a potential source population is listed (Not-E).

Ojinaga weevils sampled in 2009 (96.7%) had genotype profiles that were statistically excluded as coming from the Ojinaga population sampled in 2004. Similarly, 96.3% of boll weevils sampled at Ojinaga in 2004 have genotype profiles that are statistically so dissimilar from those in the 2009 population that they were excluded. Plots of reciprocal assignment scores (Fig. 3) visually reveal the magnitude of similarity or difference of genotype profiles at a given location sampled pre-2009 versus 2009. The greatest change is easily visible in the case of Ojinaga, where the clouds of scores do not overlap.

Discussion

Maintaining the effectiveness of the microsatellite genotype database, on which population assignment methods rely, as a tool for boll weevil eradication and posteradication programs relies on its periodic updating and expansion when possible. In the case of Ojinaga, there clearly has been a substantial change in genetic composition since 2004, and the genotype data collected in 2009 should be substituted for the older data in future analyses. Given that measures of genetic diversity are greater in Rosales than Ojinaga, and that diversity in the latter increased between 2004 and 2009 (Table 2), it is possible that the change in genetic composition in Ojinaga was driven in part by immigration from Rosales. This inference is supported by results of the assignment and exclusion tests, which indicate the Ojinaga population of 2004 contributed little to the 2009 Ojinaga population (Table 6; Fig. 3). Only 3% of the 2009 Ojinaga weevils were genetically similar enough to profiles in the 2004 Ojinaga population not to be statistically excluded as being from the same population. Instead, both tests indicate the 2009 Ojinaga population is most closely related to the 2004 Rosales population, and, to a lesser extent, to the Tlahualilo 2004 population. Similarly, although high and significant, the lowest pairwise F_{ST}s involving the 2009 Ojinaga population are with the Rosales populations of 2004 and 2009, not the 2004 Ojinaga population. Migrant exchange between these locations was inferred from genetic evidence in an earlier study (Kim et al. 2006).

In addition to Ojinaga, we conclude that genotype data collected from Uvalde, Cameron, and Rosales in 2009 should replace the data from earlier samples. Though the temporal changes were not as dramatic in these locations as in Ojinaga, the evidence is nevertheless consistent that detectable change in genetic profiles has occurred. First, the temporal F_{ST}s are significant between new and old samples at these locations. And second, plots of reciprocal assignment values provide visual support for temporal genetic change in these populations (Fig. 3), though relatively slight in the cases of Cameron and Rosales.

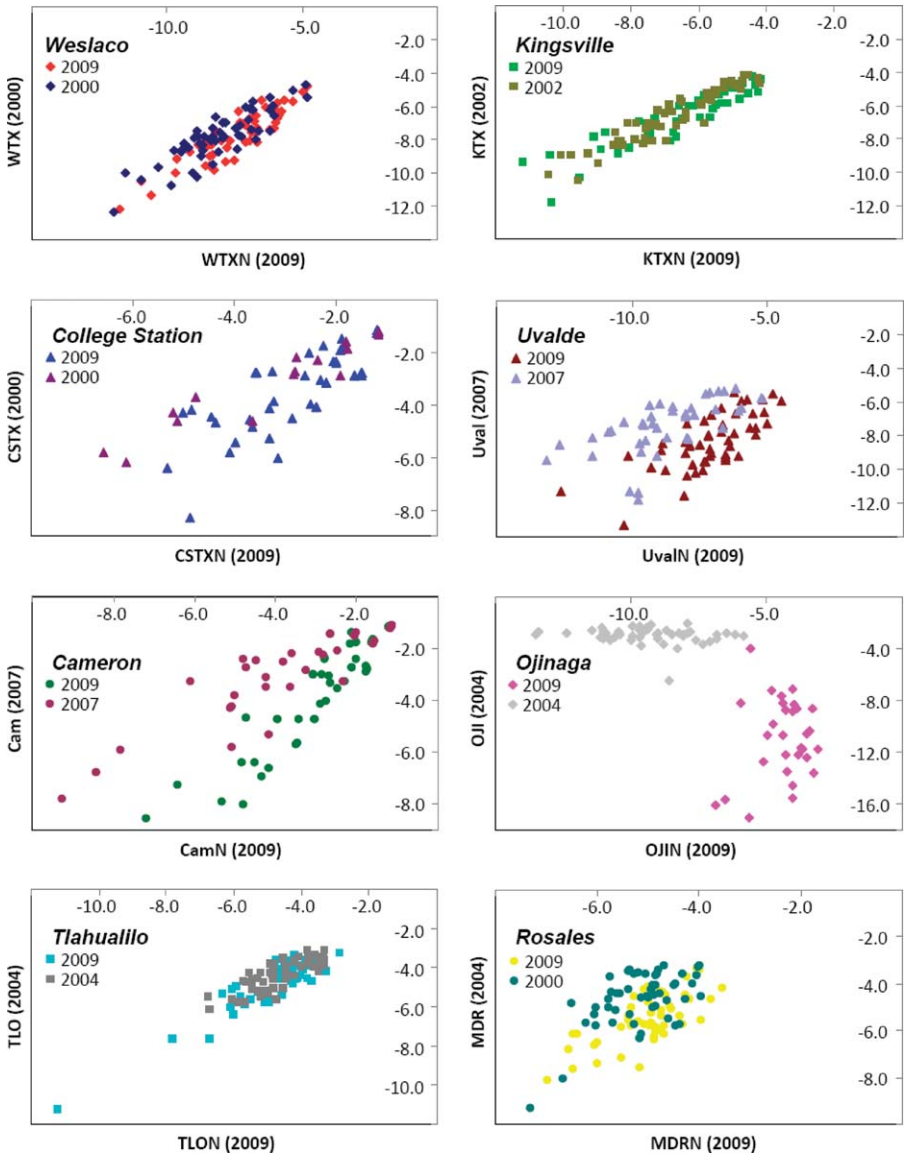

Fig. 3. Scatter plots of log likelihood values from reciprocal frequency-based assignment tests of individual boll weevils within locations sampled in 2009 and pre-2009, based on genotypes at 10 microsatellite loci. The higher the value on a given axis, the more likely the individual originated from the population associated with that axis.

We found no evidence for temporal change in genetic profiles at the remaining four of eight locations where boll weevils were sampled in both 2009 and a previous year. For those four locations—Weslaco, Kingsville, College Station, and Tlahualilo—genotype and allele frequency data can be pooled over the two sample dates, lending more statistical power to future population assignment tests involving those locations. The low magnitude of change in all but the Ojinaga population adds confidence to the future use of older data from populations for which new samples in 2009 could not be taken because of low population levels. However, the likelihood of change in those areas is greater than in the still-infested zones tested in this study because of probable genetic bottlenecks associated with severe population reductions resulting from eradication efforts, as well as increased scope for founder effects by recolonizing immigrants. Thus, results of future assignment tests using data from sites that could not be updated must be interpreted with due caution. Nevertheless, useful inferences can still be drawn from assignment and exclusion test results under such circumstances (e.g., Kim et al. 2006), especially if combined with evidence from other disciplines (Kim et al. 2010).

In addition to the temporal updates, two new locations, Brownsville and Lockhart, have been sampled, genotyped, and added to the database. The addition of

Lockhart is particularly important given its surprisingly high isolation from its nearest neighbors, Cameron and Uvalde.

The updated and expanded database recommended for future use in population genetic assignment applications is presented in Supp Table S1 [online only].

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