GENETIC STRUCTURE OF *XYLELLA FASTIDIOSA* WITHIN TWO IMPORTANT GRAPE GROWING REGIONS IN THE UNITED STATES: CALIFORNIA AND TEXAS

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ABSTRACT

Xylella fastidiosa (*Xf*) causes Pierce's disease (PD) in grapevine. Here, we report genetic diversity and population genetic structure of grape *Xf* strains between two important grape growing regions in the United States, California and Texas. Using multilocus microsatellite (also known as simple sequence repeat) markers, genetic diversity of *Xf* was measured in California and Texas populations with a grand mean haploid genetic diversity of 0.427. Partitioning of genetic diversity (heterozygosity) across 13 microsatellites (SSR) found high values within the two different grape growing regions with 0.460 within Californian isolates, and 0.452 within Texas isolates, respectively. Cluster analysis of Nei's genetic distances and hierarchical analysis of molecular variance separated Californian isolates from Texas regardless of host, and also showed significant genetic differentiations between the isolates collected from these two broad geographic regions. Pairwise (F_{ST}) comparisons of local level geographical structure within Californian populations found significant genetic differentiations among the isolates collected from Mendocino, Sonoma, Napa Kern and Riverside counties. However, some populations from the most genetically diverse Napa County differed from each other, and shared genetic similarities with Kern and Riverside separately. On the other hand, in Texas no geographic association was observed with grape or non-grape strains although host-associated structure was observed with these strains there. Bayesian modeling using the STRUCTURE software indicated that *Xf* in California and Texas may be derived from different origins regardless of host. However, the observation that some California counties had stains with up to 17% Texas origin leading us to hypothesize the introduction of Texas origin into California. The introduction of Texas *Xf* strains seems to have initiated in southern California (Temecula region) followed by range expansion throughout different regions in California.

LAYPERSON SUMMARY

Xylella fastidiosa (*Xf*) causes Pierce's disease in grapevine. In this study, we report genetic diversity and population genetic structure of grape *Xf* strains between two improtant grape growing regions in the United States, California and Texas. Using multiple sets of molecular markers, analysis showed genetic differentiations in both in California and Texas' populations. Further genetic analyses indicates local level geographical structure within Californian populations where significant genetic differentiations were found among the isolates collected from Mendocino, Sonoma, Napa, Kern and Riverside counties. However, some isolates from Napa County differed from each other and showed some genetic similarity with Kern and Riverside separately. On the other hand, no geographic association was observed in grape or non-grape strains in Texas. Sharing and distribution of 17% Texas origin in different regions (counties) in Californian implied the possibility of recent introduction of Texas strains into California.

INTRODUCTION

California and Texas are productive agricultural ecosystems, and are both important American viticultural regions in United States. California accounts for nearly 90 percent of the entire American wine production, and the Texas wine industry is continuing its steady pace of expansion and has gained a reputation as an established wine growing region in the United States. The winegrowers of Texas have dealt with Pierce's disease (PD) for over a century. PD has also been a serious but intermittent threat in the California grape-growing regions for more than 100 years (Purcell 1997). Limited genetic variations among crop cultivars and oftentimes mono-culture practices may impose directional selection on the pathogen (*Xylella fastidiosa (Xf)* populations. Changes in pathogen population structure or virulence can lead to resistance breakdown. Therefore, to understand about the epidemiology of the PD disease caused by *Xf*, it is critical to understand the genetic diversity, gene flow and genetic structure of this pathogen. Until now, no detailed genetic information at the population level is available for the *Xf* grape strains within the United States from Florida to California or outside the USA in Central and South America. Previous molecular genetics studies mostly by conserved genes were unable determine population differentiations of grape strains of *Xf* at the local level even at the wider geographical ranges of the United States (Hendson et al. 2001; Schuenzel et al. 2005; Yuan et al. 2010). PD strains have been present in the United States since at least since the

1880s (Pierce 1892), and the evolutionary process associated with the genetic variation was expected to be considerably more rapid at non-coding loci (e.g. microsatellite) than conserved gene regions (Yuan et al. 2010), therefore, here we have investiagated *Xf* grape strains using highly variable microsatellite markers. We also incorporated some non-grape strains (based on the availabily of samples), and comapreted their genetic diversity and structure with grape strains. In this study, we have employed the 13 most informative microsatellite markers (Lin et al. 2005), and used them to analyze *Xf* grape populations at the local as well as wider geographic level i.e., within and between two important grape growing regions in the United States i.e., California and Texas.

OBJECTIVES

1. To investigate genetic structure of *Xf* grape strains between two important grpae growing regions in the United States (California and Texas).
2. To analyze the genetic diversity and population structure of *Xf* grape populations at the local geographic areas within California and within Texas.
3. To investigate some non-grape strains (based on the availabily of samples) and compare their structre with grape strains.

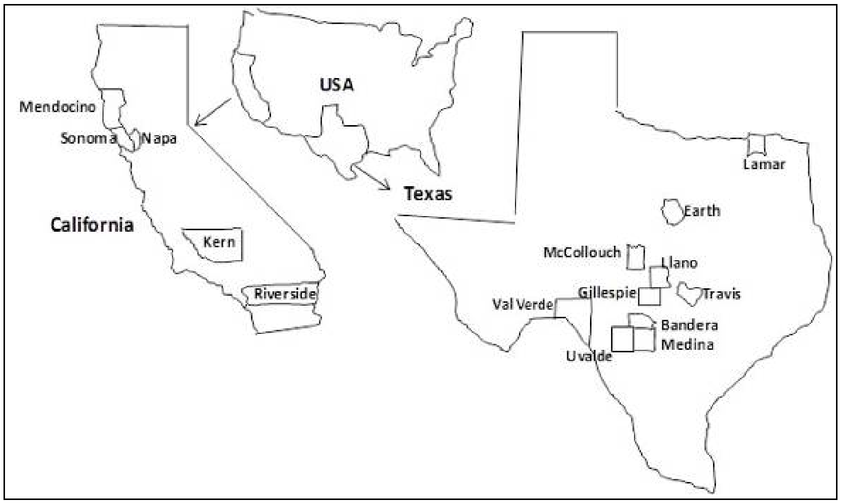

Figure 1. Sampling locations of *Xylella fastidiosa* populations across California and Texas.

RESULTS AND DISCUSSION

Within population genetic diversity in California and Texas

We investigated 12 *Xf* populations across five areas (counties) from California including some sub-populations from Sonoma and Napa countries based on the location of vineyard or type of cultivar. In Texas, 10 populations (both grape and non-grape strains) were investigated from ten different areas (counties) (**Figure 1**). Overall, strain diversity of *Xf* throughout California and Texas was found to be high in microsatellite DNA polymorphism analyses. The allelic diversity at the microsatellite loci analyzed where 7-13 alleles were detected per locus (data not shown). Grand mean genetic diversity (0.427) for overall *Xf* isolates at California and Texas was found to be high. Highly variable microsatellite markers were capable of distinguishing genetic diversities across the populations in different regions in California and Texas. Partitioning of genetic diversity in California showed that Napa county is the most diversified regions for *Xf* where heterozygosities ranged from 0.246 (Oakville) to 0.620 (north of the city of Napa). In Texas, the lowest diversity was found in non-grape population i.e, Medina (within oleander strains) (**Table 1**). Although the overall diversity was high, there were relatively lower levels of allelic diversities at individual population levels in California and Texas separately (**Table 1**). Np, or number of private alleles (alleles unique to a single population in the data set), were low overall and only slightly higher at a single locus in a single population of St. Helena Napa (variety: Cabernet Sauvignon) in California. This Np of 0.077 did not indicate strong distinction of this population from others. However, higher frequencies of rare alleles at two Texas populations (Medina at 0.308 and Val Verde at 0.231) indicate private distinction of these populations from others (**Table 1**). This distinction likely resulted from the strains variations based on the specific host plants as the respective private alleles were found within all Oleander strains in Medina and giant ragweed strains in Val Verde.

Table 1 Population information and descriptive statistics of microsatellite loci across different populations of *Xylella fastidiosa* in California and Texas, USA

Population ID	Counites	Vineyear or Location	Host	Cultivar	N	Na	Ne	Np	H
California									
CA-1	Mendocino	Hopland, southern	Grape	Sauvignon Musque	5	1.9	1.8	0.0	0.404
CA-2	Sonoma	Russian River, Healdsburg	Grape	Sauvignon blanc	10	2.4	2.0	0.0	0.461
CA-3	Sonoma	Northern Santa Rosa,	Grape	Pinot noir	9	2.6	1.8	0.0	0.356
CA-4	Sonoma	Dry Creek Valley,	Grape	Cabernet franc	3	2.2	2.0	0.0	0.466
CA-5	Sonoma	Alexander Valley,	Grape	Merlot	5	1.5	1.3	0.0	0.172
CA-6	Napa	Rutherford	Grape	Chardonnay	7	2.2	1.8	0.0	0.317
CA-7	Napa	St. Helena(1)	Grape	Chardonnay	6	2.1	1.6	0.0	0.325
CA-8	Napa	Oakville	Grape	Cabernet Sauvignon	5	1.8	1.6	0.0	0.246
CA-9	Napa	St. Helena(2)	Grape	Cabernet Sauvignon	8	2.5	2.0	0.1	0.438
CA-10	Napa	North of the city of Napa	Grape	Chardonnay	10	4.0	3.1	0.0	0.620
CA-11	Kern	Eastern Bakersfield	Grape	Colombard	10	3.8	2.6	0.0	0.517
CA-12	Riverside	Temecula	Grape	Chardonnay	5	2.8	2.5	0.0	0.550
Average					6.9	2.5	2.0	0.0	0.406
Texas									
TX-1	Gillespie		7 Grape ; 2 Ragweed		9	4.2	2.8	0.0	0.625
TX-2	Bandera		1 Grape; 1 Ragweed		2	1.7	1.7	0.0	0.346
TX-3	Earth		3 Grape, 2 Ragweed		5	4.0	3.8	0.0	0.695
TX-4	Lamar		Grape		3	1.9	1.8	0.0	0.380
TX-5	McCollouch		1 Grape; 1 Ragweed		2	1.5	1.5	0.0	0.231
TX-6	Llano		5 Grpae; 1 Ragweed		6	3.1	2.5	0.0	0.560
TX-7	Medina		Oleander		4	1.5	1.4	0.3	0.198
TX-8	Travis		Grape		4	2.2	2.0	0.1	0.424
TX-9	Uvalde		1 Grape, 2 ragweed		3	2.3	2.2	0.0	0.509
TX-10	Val Verde		4 Grape ; 1 Ragweed		5	2.7	2.5	0.2	0.549
Average					4.3	2.5	2.2	0.1	0.452

N, Number of individuals; Na, number of alleles per locus; Ne, Number of effective alleles per locus; Np, Number of private alleles per locus, H, Gene diversity.

Genetic differentiation and population genetic structure at the wider and local geographic level

Cluster analysis of Nei’s genetic distances (Nei 1978) with microsatellite loci separated Californian isolates from Texas isolates (**Figure 1A**). This was expected based on their geographical range due to geographic isolation or their sources of origin.

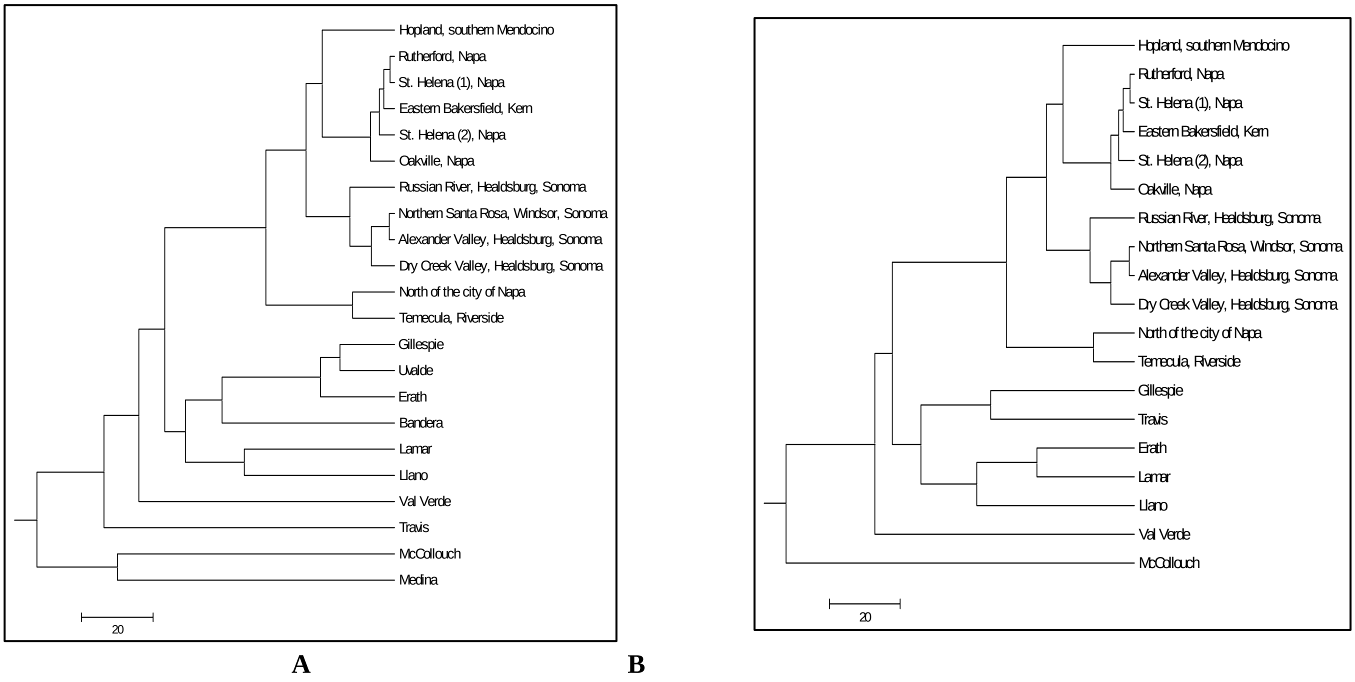

Figure 1 A. Dendrogram of genetic similarity of *Xf* within between grape growing regions in California (five regions with 12 grape populations) and Texas (10 regions with 10 populations contains grape and non- grape). **B.** Only grapes strains in California and Texas. We excluded Bandera and Uvalde from this analysis as they contained only one isolates when we excluded non-grape strains from these two populations.

Within California some local level population differentiations were identified. Different sub-populations at the most diversified wine grape producing regions in California i.e., Napa County produced two major groups. Each of the major genetic group of Napa mixed with the populations of two geographically distant counties i.e., Kern, Riverside (Temecula). However, in a broad sense, clear separation was observed among the isolates of Mendocino, Sonoma, Napa (except those isolates shared genetic similarity with Kern and Riverside), Kern and Riverside (**Figure 1 A**). Similar observations were found in the previous study though there was some relation between Kern and Riverside (Lin et al. 2005). No grouping was observed according to grape cultivar in California. In Texas, three smaller groupings were also formed but the remainder of the populations were found to be independent i.e., did not group with other populations. Though the genetic similarity of Gillespie-Uvalde–Earth is not recognized based on the geography, the host is likely influencing these on groupings, as these populations contain both grape and with few non-grape strains. When we excluded non-grape populations from those populations, Gillespie-Earth did not fall in one group (**Figure 1B**). Therefore, it can be inferred that in Texas genetic differentiations between the populations is influenced by the host i.e. grape and ragweed (subsp. *multiplex*) as described by (Morano et al. 2008).

Comparisons of pairwise F_{ST} values between the populations also evaluated the genetic differentiations among populations for short divergence time. Local level genetic variation within two important grape growing regions in the United States i.e., California and Texas are presented in **Table 2**.

Table 2 Pairwise F_{ST} comparison between *Xylella fastidiosa* grape populations in Californa and Texas

Californai-All Host	Hopland, southern Mendocino	Russian River, Healdsburg, Sonoma	Northern Santa Rosa, Windsor, Sonoma	Dry Creek Valley, Healdsburg, Sonoma	Alexander Valley, Healdsburg, Sonoma	Rutherford, Napa	St. Helena (1), Napa	Oakville, Napa	St. Helena (2), Napa	North of the city of Napa	Eastern Bakersfield, Kern
Russian River, Healdsburg, Sonoma	0.199										
Northern Santa Rosa, Windsor, Sonoma	0.352	0.172									
Dry Creek Valley, Healdsburg, Sonoma	0.113	0.029	0.010								
Alexander Valley, Healdsburg, Sonoma	0.458	0.259	-0.046	0.098							
Rutherford, Napa	0.331	0.196	0.373	0.295	0.527						
St. Helena (1), Napa	0.286	0.202	0.383	0.255	0.540	-0.045					
Oakville, Napa	0.333	0.224	0.422	0.327	0.597	0.114	0.020				
St. Helena (2), Napa	0.183	0.170	0.335	0.151	0.438	0.041	-0.003	0.074			
North of the city of Napa	0.205	0.177	0.306	0.122	0.378	0.168	0.155	0.178	0.149		
Eastern Bakersfield, Kern	0.205	0.158	0.286	0.115	0.384	0.019	0.032	0.163	0.051	0.102	
Temecula, Riverside	0.284	0.308	0.444	0.201	0.537	0.347	0.316	0.398	0.273	0.017	0.160

Texas-All Host	Gillespie	Bandera	Erath	Lamar	McCollouch	Llano	Medina	Travis	Uvalde
Bandera	0.112								
Erath	0.031	-0.109							
Lamar	0.286	0.203	0.095						
McCollouch	0.058	0.071	-0.215	0.229					
Llano	0.212	0.121	0.056	0.215	0.020				
Medina	0.450	0.493	0.325	0.576	0.503	0.472			
Travis	0.159	0.280	0.119	0.334	0.228	0.252	0.576		
Uvalde	-0.019	-0.025	-0.027	0.304	0.039	0.190	0.464	0.216	
Val Verde	0.236	0.036	0.071	0.242	0.034	0.162	0.417	0.247	0.198

TX-Grape Only in Texas	Gillespie	Erath	Lamar	McCollouch	Llano	Travis
Erath	0.086					
Lamar	0.262	0.054				
McCollouch	0.066	-0.150	0.205			
Llano	0.250	0.156	0.245	0.141		
Travis	0.153	0.085	0.315	0.205	0.304	
Val Verde	0.245	0.099	0.253	0.072	0.235	0.274

Gray highlighted pairs showed no significant genetic differentiation

Our microsatellite marker analysis showed significant genetic differentiations among the local geographic areas (Mendocino, Sonoma-Napa-Kern-Riverside) or even at the very local level i.e., within the counties (between some isolates of Napa and Sonoma County) in California. However, some populations of Napa County did not show significant genetic differentiations with geographically distant countries Kern and Riverside separately. These results suggest the evidence of the genetic similarity or the possibility of gene flow between Napa and Kern, and Napa and Riverside. In Texas, most of the populations (combining grape and non-grape strains) strains were not well differentiated according to the geography. Geographic structures were not observed as well from the few number of genetically differentiated population pairs when we excluded non-grape populations (**Table 2**). However, significant genetic differentiations were observed between the all populations pairs that were compared with Medina (all oleander strains), which indicates host associated genetic differentiations. Some clues about the influences of host on differentiating population were also noticed from the pairs compared with Lamer i.e., when we excluded non-grape (ragweed strains), genetic differentiations was not significant with Llano and Val Verde (**Table 2**).

In individual-based clustering analysis, Bayesian modeling approach within STRUCTURE predicted that *Xf* strains throughout the grape growing regions in California (grape), and Texas (grape and non-grape) derived from two different clusters i.e., two different origins/ancestors (**Figure 2**). Majority of the Californian strains (83%) fall into its own distinct origin i.e., cluster1 (Red). In Texas 98% individuals (both grape and non-grape strains) fall into another distinct cluster II (Yellow) with a pure ancestry. Seventeen % strains in California showed genetic similarity to Texas strains, and which are more or less distributed every region in California we studied. Though a very small percentage of Californian origin is found admixed within one population of Texas (Gillespie County, 11% individuals admixed with 27% Californian ancestry), it certainly indicates the evidence of appearing Californian origin in Texas. The diversity of Texas isolates from grape and non-grape hosts is consistent with the idea that there have been at least two different types of ancestor/ strains since at least the 1880s (Pierce 1892), a non-grape and a grape strain. This work also suggests that populations of the grapevines have evolved uniformly as a unique genetic structure over the past 100 plus years in their own geographical areas with strong selection pressure, and with perhaps one episode of gene flow between these two grape growing regions.

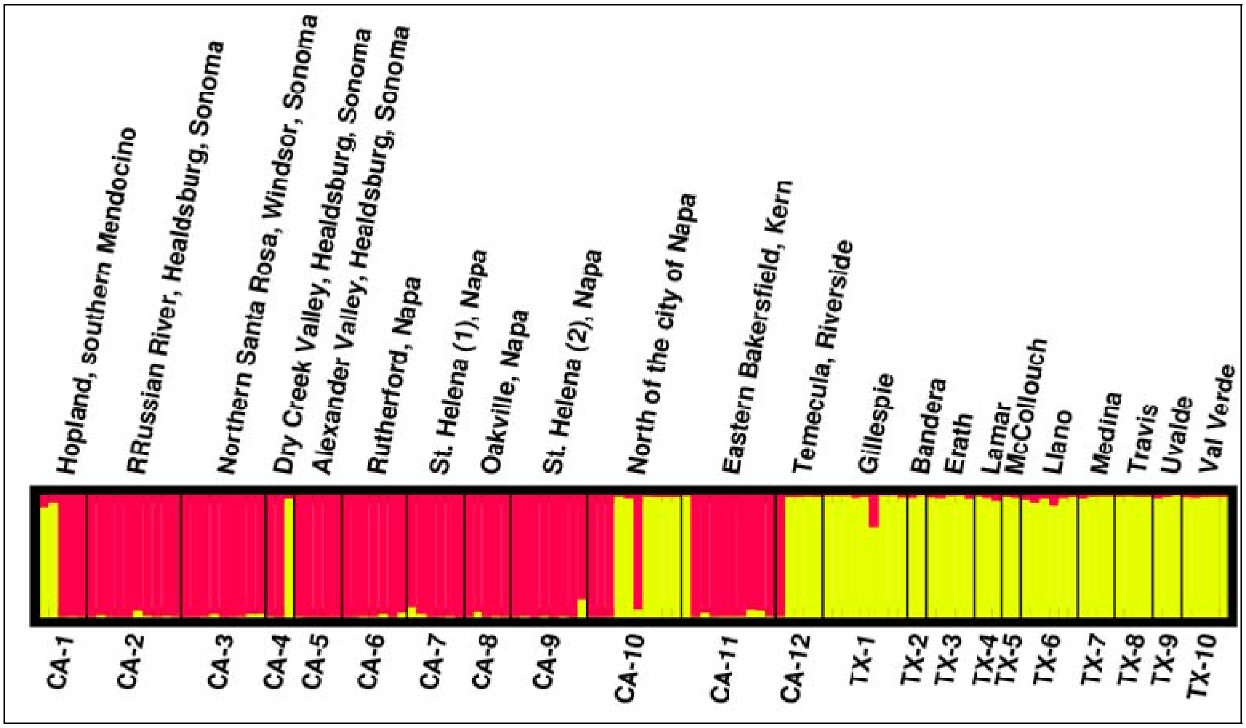

Figure 2 Individual assignment by STRUCTURE analysis; there were two clusters (K). Black lines within the squares distinguish populations.

However, sharing and distribution of 17% Texas origin in different regions (counties) in Californian let us propose a hypothesis of recent introduction of Texas strains into California (or less likely California strains back to Texas). This close genetic similarity of Texas and California grape strain populations could be explained by the introduction of the insects vectors, the glassy-winged sharpshooter (GWSS) (*Homalodisca vitripennis*), into the Southern California (Temecula region) in the early time, and its subsequent range expansion throughout California (De Leon et al. 2004; Morano et al. 2008). A phylogeny of GWSS has revealed that populations of this insect pest introduced into California likely originated from Texas (De Leon et al. 2004).

CONCLUSIONS

Xf genetic analysis clearly identified two different genetic structures of *Xf* grape strains in California and Texas, and are consistent with the introduction of Texas origin to California though the insects vectors, the GWSS. This multi-locus marker system is able to distinguish local level genetic differentiations based on the geography in California, and identified some clues on host associated genetic differentiations in Texas. These findings may provide necessary information to better understanding genetic diversity and evolutionary potential of *Xf* populations in California and Texas.

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