

Identification of microsatellites from *Geosmithia morbida*, the fungus associated with Thousand Canker Disease (TCD) of Walnut, and their use for characterizing its genetic diversity.

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Photo courtesy: Charles Leslie

Above: *Juglans hindsii* affected with Thousand Canker Disease
Corners: *Pityophthorus juglandis* (Tisserat et al., 2009)

ABSTRACT

Thousand Canker Disease (TCD) is a beetle/fungus complex that recently emerged as a serious threat to members of the Juglandaceae. The walnut twig beetle and *Geosmithia* fungus were once apparently restricted to a small area of the southwestern U.S., but in the past decade or so expanded rapidly across the entire western U.S. *Geosmithia* species are often associated with bark beetles, but *G. morbida* is a new species with no previous genetic or genomic information. In order to obtain this information, microsatellites were developed as tools to track the genetic identity and diversity of the *Geosmithia* associated with TCD in *Juglans* spp. Seventeen microsatellite regions were selected for preliminary screening against a panel of DNA from 12 highly diverse *G. morbida* isolates. Thirteen loci: GEO679, GEO3416, GEO3896, GEO12473, GEO7713, GEO6823, GEO194, GEO223, GEO1851, GEO4045, GEO2849, GEO12289, and GEO 2514 amplified consistently producing, in all, 45 alleles from 11 isolates. So far, the most polymorphic locus was GEO12473, producing at least 8 alleles. We observed an average of 4 alleles per locus. Of the 11 isolates for which we had clear results, each had a unique genotype (haplotype). This result was not surprising because the isolates had been selected previously for genetic diversity at their Internal Transcribed Spacer (ITS). Eventually we expect to genotype over 150 isolates of *G. morbida* and evaluate their spatial genetic structure in relation to host and disease virulence. These are the first microsatellites ever described for this important family of fungi.

Level: Undergraduate

Division: Research

Introduction

Thousand Canker Disease (TCD) affects trees of the genus *Juglans*. It is caused by a beetle/fungus symbiotic complex that can quickly lead to tree death. TCD has spread during the last decade through most of the western United States and was recently discovered in Tennessee. Many species of *Juglans* are valued for their wood products as well as their nuts, but *Juglans nigra*, which is highly susceptible to TCD, is especially important to forest landowners in the eastern U.S. TCD could have a devastating effect if allowed to spread throughout the range of *J. nigra*. The fungus associated with TCD is *Geosmithia morbida*, a newly discovered species that remains largely unstudied. Other than its relationship with TCD and the beetle *Pityophthorus juglandis*, not much is known about it. To better understand how TCD has emerged and spread, and how it affects infected trees, we have begun to explore the genome of *G. morbida*, beginning with a characterization of its genetic diversity.

Methods and Materials

Primers developed

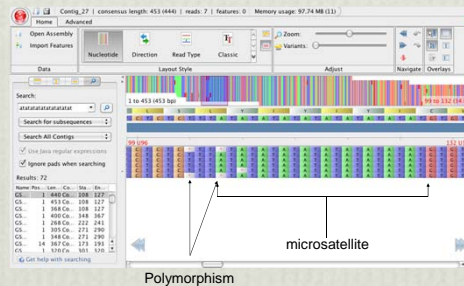
- DNA from 6 fungal isolates was enriched for microsatellites.
- Polymorphic microsatellites were identified by the use of Tablet and Mega 4 software. The sequence data was then used to design primers that would amplify polymorphic regions in the DNA.

PCR used to amplify desired regions

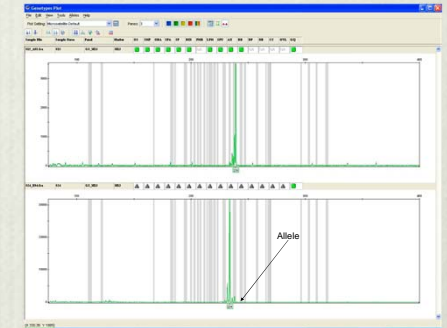
- Used designed primers in a three primer method that used forward, reverse and M13 primers simultaneously.
- This method produced a fluorescently tagged product for genotyping.

Genotype results analyzed

- The genotyped results were analyzed to identify alleles in the genome.
- These alleles, which were highly polymorphic, will help us identify the relationships among fungal isolates and relate isolate haplotypes to etiology of disease.



Above: Screenshot of Tablet software used to locate microsatellites. Over 13,000 contigs were examined for suitability for primer development.



Screenshot of results from the genomics center opened in GeneMapper

Note: One allele per locus due to haploid nature of the fungus

Results

Thirteen loci, GEO679, GEO3416, GEO3896, GEO12473, GEO7713, GEO6823, GEO194, GEO223, GEO1851, GEO4045, GEO2849, GEO12289, and GEO 2514, used in the PCR procedures amplified consistently. They produced 51 alleles from 11 *G. morbida* isolates. Each isolate had a unique haplotype, probably because the isolates had been selected previously to provide maximum genetic diversity at their Internal Transcribed Spacer (ITS). Of the thirteen loci, GEO12473 was the most polymorphic.

	Loci												
Isolates	679	3416	3896	12473	7713	6823	194	223	1851	4044	2849	12289	2514
1249	?	228	218	238	339	(3107)	?	186	205	184	310	212	271
1226	238	214	?	?	?	?	?	184	214	?	?	?	?
1505	238	?	208	268	?	?	?	187	203	184	?	?	267
1236	238	?	208	220	369	?	?	184	205	184	310	218	?
1245	?	228	218	207	?	134	112	(1867)	208	184	316	212	?
1239	234	228	?	206	297	319	270	184	214	184	?	224	?
1234	238	244	200	218	?	303	266	182	214	195	?	?	?
1222	238	?	(2087)	168	361	134	?	186	214	184	?	212	?
1218	238	234	208	(2427)	339	(3227)	?	186	205	184	(3117)	212	268
1217	238	214	200	247	296	310	269	184	214	184	?	212	?
1256	238	228	208	?	339	310	?	184	205	184	316	218	268
Alleles	2	4	4	97	4	5	37	4	4	2	3	3	3

Discussion and Conclusions

Any member in the genus *Juglans* can be affected by *G. morbida*; however, different members experience varying degrees of damage. *Juglans nigra*, a particularly valuable hardwood in the United States, is devastated by TCD. By exploring the associations between haplotypes of *G. morbida* and disease patterns such as virulence and host preference, insight can be gained into which haplotypes are most likely to affect *J. nigra*.

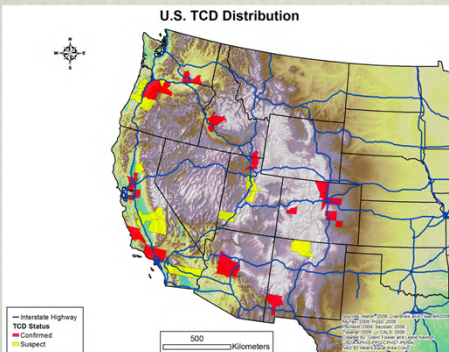
G. morbida is highly genetically diverse, indicating that its association with *P. juglandis* is probably longstanding, thus TCD is probably not a new disease. High levels of diversity in *G. morbida* indicate a diploid (sexual) stage of the fungus is present, though it has never been observed. This further indicates that the fungus is native and that it will adapt to changes in host and host environment. This will make control of *G. morbida* more difficult.

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References

"Pathway Assessment: *Geosmithia* sp. and *Pityophthorus juglandis* Blackman movement from the western into the eastern United States", USDA, 19 October 2009

Tisserat, Ned, et al. *Black Walnut Mortality in Colorado Caused by the Walnut Twig Beetle and Thousand Cankers Disease*. Plant Management Network, 11 August 2009



Above: Distribution of TCD in the western US. In summer, 2011, TCD was discovered in eastern Tennessee.