

AFLP Analysis of *Phytophthora nemorosa* and *P. pseudosyringae* Genetic Structure in North America¹

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Introduction

In California and Oregon, *Phytophthora ramorum* has an overlapping host and geographic range with two newly described homothallic *Phytophthora* species, *P. nemorosa* and *P. pseudosyringae*. *P. nemorosa* alone causes symptoms similar to those of *P. ramorum*, including lethal tanoak cankers, and *P. pseudosyringae* is associated with oak decline in Europe. However, epidemiological observations, namely broader geographic distribution and reduced virulence, suggest *P. nemorosa* and *P. pseudosyringae* are endemic in this region, while *P. ramorum* is hypothesized to have been introduced. Though molecular evidence suggests that *P. nemorosa* and *P. pseudosyringae* are each other's closest known relatives, both are rather distantly related to *P. ramorum*. Little is known about the characteristics of these newly described forest pathogens; however, the two putative endemic *Phytophthora* species can apparently share the same niche as *P. ramorum* and may affect *P. ramorum* disease epidemiology. Understanding their genetic structure, then, may contribute to understanding the range of potential interactions and outcomes during infection by these *Phytophthora* species. Our aim is a preliminary assessment of the genetic structure of *P. nemorosa* and *P. pseudosyringae* in western North America using Amplified Fragment Length Polymorphism (AFLP) genetic markers.

Materials and Methods

We analyzed 38 *P. nemorosa* and 29 *P. pseudosyringae* isolates selected from throughout most of their known host and geographic ranges in the western United States and included one *P. ilicis* isolate for use as an outgroup. Distribution of the isolates was rather continuous

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through the west coast of California and Oregon, excepting one *P. nemorosa* and two *P. pseudosyringae* isolates from Mariposa County, CA. For each isolate, total genomic DNA was extracted from 10 to 20 mg freeze-dried mycelium using the Puregene DNA extraction kit (Gentra, Minneapolis, MN). AFLP reactions were performed as in Ivors and others. (2004). We screened a total of 18 primer pairs for each species, selecting from among them six informative primer pairs for *P. nemorosa* and four for *P. pseudosyringae*. As there was only partial overlap between the informative primer pairs for each species, we included two isolates of *P. nemorosa* in the analysis of the full *P. pseudosyringae* dataset, along with *P. ilicis*, to function as outgroups, and *vice versa* for the full *P. nemorosa* analysis. The AFLP procedure was replicated for 10 percent of isolates in this preliminary work. Data analysis was performed as in Ivors and others (2004). To assess the degree of genetic similarity between each isolate and all of the others, we calculated a distance matrix for each species using the Jaccard coefficient of similarity, which measures the proportion of shared AFLP markers in a pairwise fashion, while correcting for the dominant nature of the data. We then constructed a neighbor-joining dendrogram for *P. nemorosa* and one for *P. pseudosyringae*; outgroups used in the dendrograms were as above.

Results

We scored 214 AFLP bands for *P. nemorosa*, 20 of which (10.7 percent) were polymorphic, and identified a total of 12 distinct AFLP genotypes among the 38 *P. nemorosa* isolates. Our measure of degree of genetic similarity, Jaccard similarity coefficients (S_j), fell between 0.941 and 1.000 (0.990 ± 0.011 ; mean $\pm$ SD) for *P. nemorosa* isolates – a score of 1.0 is equivalent to 100 percent identity. Over 50 percent of the pairwise comparisons showed 100 percent genetic similarity between isolates. There was no evidence of partitioning by host or by area of origin of the isolates based upon the branching pattern of the neighbor-joining dendrogram (tree not shown). We scored 198 AFLP bands for *P. pseudosyringae*, 20 of which (9.9 percent) were polymorphic, and identified 13 distinct AFLP genotypes among the 29 *P. pseudosyringae* isolates. The range of S_j values was 0.953 to 1.000 (0.987 ± 0.011 ; mean $\pm$ SD), with only about 20 percent of the pairwise comparisons yielding 100 percent similarity values within *P. pseudosyringae* isolates. As with *P. nemorosa*, the neighbor-joining dendrogram yielded no evidence of partitioning of the genetic variation by host or area of isolate origin (tree not shown).

Discussion

In our preliminary assessment of the genetic structure and diversity of both *P. nemorosa* and *P. pseudosyringae* from the west coast of the United States, we found extremely high levels of within-species genetic similarity and no evidence of partitioning of genetic diversity based upon the host or site from which study isolates were collected, including those from the isolated site in Mariposa county. The degree of genetic similarity is comparable to that found

in a similar study carried out for *P. ramorum*, in which the North American population is dominated by a single clone genotype with a few rare, but highly genetically similar, variants (Ivors and others 2004). While this lack of genetic diversity is unsurprising for the putatively non-native *P. ramorum*, as exotic species often undergo a population genetic bottleneck upon introduction to a new area, it is somewhat unexpected for a native organism. Therefore, our results for *P. nemorosa* and *P. pseudosyringae* are consistent with the hypothesis of recent, or relatively recent in the context of evolutionary time, introductions. However, there is a scarcity of studies on the genetic structure and diversity of homothallic, endemic *Phytophthora* species outside of the intense, artificial selection regimen of agriculture. These *Phytophthora* species may be endemic, and the low amount of genetic variation and lack of structure in the variation present may be due to self-compatible sexual reproduction. Also, the AFLP primers chosen may have had insufficient resolution to detect existing genetic variation. To assist us in distinguishing between these hypotheses, we will repeat and fully replicate this preliminary study using more isolates, expanding the host and geographic ranges covered, and including multiple European *P. pseudosyringae* isolates.

References

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