

Understanding a Plant Pathogen Through Genomics: the Interactions of Biogeography, Invasion, and Recombination in the Infection of Plants by *Xylella fastidiosa*

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My research is in population genetics and evolutionary genomics, with an emphasis on the application of evolutionary theory to practical problems. Current areas of research are: the evolutionary genomics of the plant pathogenic bacterium *Xylella fastidiosa*; the evolution of cancer suppression; and conservation genetics. The goal of the *Xylella* project is to understand host adaptation in order to control this bacterium that causes serious disease in a wide range of agricultural crops.

Xylella fastidiosa is a xylem-limited bacterium that causes leaf scorch and dwarfing diseases in a wide range of plant hosts (Hopkins & Purcell 2002), including more than 40 species of landscape trees (Sherald 2007). *X. fastidiosa* is transmitted between plant hosts by xylem-feeding insects, typically leafhoppers (Redak et al 2004). It is restricted to the Americas, and, based on DNA hybridization and sequence data, has been divided into four subspecies, *fastidiosa*, *multiplex*, *sandyi*, and *pauca* (Schaad et al 2004; Schuenzel et al 2005), with subsp. *pauca* unique to South America where it causes citrus variegated chlorosis (CVC) and coffee leaf scorch. The other subspecies are all found in North America.

To document genetic variation within and between subspecies in more detail we typed *X. fastidiosa* isolates using multilocus sequence typing (MLST). MLST is based on sequencing regions of (usually) seven housekeeping genes (Maiden et al 1998). Applied to *X. fastidiosa* it has confirmed the subspecific distinctions, and provided insight into their biogeography, host plant specialization, and the role of homologous recombination.

MLST of isolates has shown that there is very little overlap among the subspecies in the host plants that they naturally infect (see Nunney et al. 2013). Subsp. *fastidiosa* causes Pierce's disease (PD) of grapevine, although it also causes disease in elderberry, alfalfa, and almond. Subsp. *multiplex* is also found on almond, but otherwise infects hosts not infected by subsp. *fastidiosa*, including plum, peach, apricot, pecan, oak and sycamore. In contrast to the broad range of subsp. *multiplex*, subsp. *sandyi* was first discovered in the 1980s in southern California and it is almost entirely limited to oleander.

MLST data allow us to categorize isolates using a very simple system, and this remains useful even when we have whole genome data. *X. fastidiosa* subsp. *pauca* was the first plant bacterial pathogen to be fully sequenced by a Brazilian group (Simpson et al. 2000). We now have many more genome sequences; however, MLST allows a massive amount of sequence data to be summarized at a level that is useful for understanding important biological questions (Nunney et al 2012a).

Using MLST sequence we examined the phylogenetic relationships among the subspecies and their genetic diversity. Based on the rate of synonymous substitutions, a rough estimate of the age of the subspecies is a few tens of thousands of years (Figure 1), with the South American subsp. *pauca* being the most divergent.

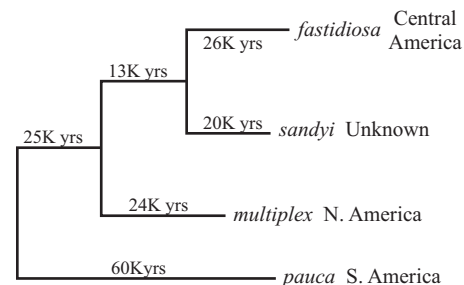


Figure 1. The relationships of the *X. fastidiosa* subspecies, showing their approximate times of divergence and the probable geographical region where they originated (redrawn from Nunney et al. 2012b).

Also shown in Figure 1 are the geographical regions in which we believe these subspecies originated. Based on the genetic data, we concluded that subsp. *fastidiosa* was native to Central America but that a single strain was introduced into the US just prior to the first recorded outbreak of PD in the late 1800s (Nunney et al. 2010). Subsp. *sandyi* is almost certainly a form recently introduced into the US, but its native range is unknown. One possibility is that this subspecies is native to southern Mexico.

An unexpected feature of the MLST data was the finding of extensive evidence of intersubspecific recombination. We have found intersubspecific genetic introgression in subsp. *fastidiosa* (Yuan et al. 2010) and in subsp. *multiplex* in the US (Nunney et al. 2013), and in subsp. *pauca* from Brazil (Nunney et al. 2012b). We have proposed that intersubspecific recombination can facilitate changes in host range. In particular, the data from subsp. *pauca* found in Brazil is consistent with the hypothesis that the recent invasion of citrus and coffee (crops cultivated in Brazil for several hundred years) became possible only after the introduction of new genetic variation via intersubspecific recombination.

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