

Host-Induced Genome Alterations in *Phytophthora ramorum*

I. NA1 Lineage on Coast Live Oak in California

II. EU1 Lineage on *Chamaecyparis lawsoniana* in UK¹

Takao Kasuga,² Mai Bui,² Elizabeth Bernhardt,³ Tedmund Swiecki,³ Kamyar Aram,⁴ Lien Bertier,⁴ Jennifer Yuzon,⁴ Liliana M. Cano,⁵ Joan Webber,⁶ Clive Brasier,⁶ Caroline Press,⁷ Niklaus Grünwald,⁷ David Rizzo,⁴ and Matteo Garbelotto⁸

Abstract

Rapid phenotypic diversification in clonal invasive populations is often observed, although the underlying genetic mechanisms remain elusive. Lineages of the sudden oak death pathogen *Phytophthora ramorum* are exclusively clonal, yet isolates of the NA1 lineage from oak (*Quercus* spp.) frequently exhibit host-dependent, unstable colony phenotypes called non-wild type (*nwt*, fig. 1) (Brasier and others 2006).

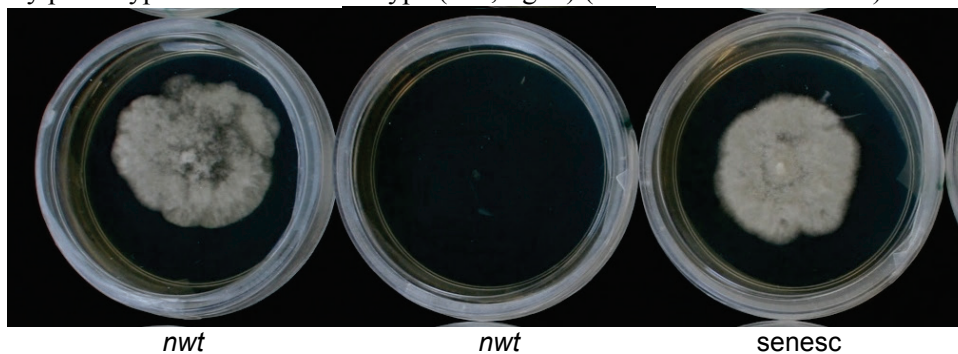


Figure 1—Colony types of NA1 lineage isolates of Californian *P. ramorum*. Note the variation in colony patterns and growth rates. *wt*: wild type, *nwt*: non-wild type. Sometimes *P. ramorum* isolates stop growing upon subculturing, which is termed senescence (*senesc*).

This phenotypic variation is seen despite the fact that population genetic and host-specificity studies negate any host-driven population subdivision. We also found comparable *nwt* phenotypes in EU1 isolates from Lawson cypress (*Chamaecyparis lawsoniana*) in the UK (fig. 2): isolates from the middle of a large ~4m long lesion on a mature tree were normal wild type (*wt*), while those from the extremities were variable *nwt* types. Previously only *wt* isolates were known in the EU1 lineage, including those from larch (*Larix*). Based on a large survey of genotypes from different hosts, we hypothesize that the

¹ A version of this paper was presented at the Sixth Sudden Oak Death Science Symposium, June 20-23, 2016, San Francisco, California.

² USDA ARS, Davis, CA.

³ Phytosphere Research, Vacaville, CA

⁴ Department of Plant Pathology, University of California, Davis, CA.

⁵ Department of Plant Pathology, University of Florida.

⁶ Forest Research, Surrey, UK.

⁷ USDA-ARS, Corvallis, OR.

⁸ Department of Environmental Science Policy & Management, University of California, Berkeley, CA.

Corresponding author: tkasuga@ucdavis.edu.

environment in the bark of oak and Lawson cypress is responsible for the unusual phenotypic diversification in *P. ramorum*.

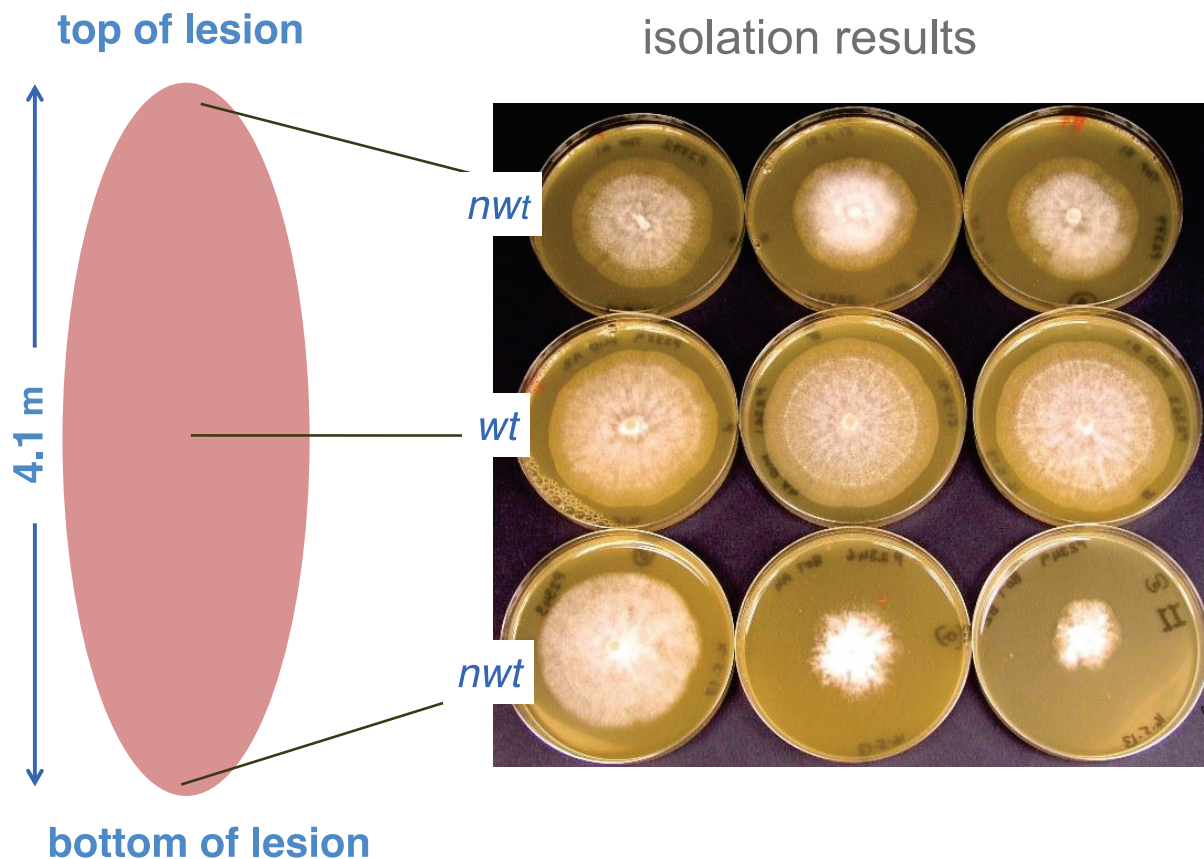


Figure 2—A single EU1 *P. ramorum* lesion developed in the stem of a 24m Lawson cypress yielded *nwt* colonies (top and bottom of lesion) as well as *wt* colonies (middle of lesion).

We conducted a series of passage experiments to test this hypothesis (Kasuga and others 2016). When *P. ramorum* NA1 isolates from foliar host California bay laurel (*Umbellularia californica*) were inoculated and re-isolated from mature canyon live oaks (*Q. chrysolepis*), both *wt* and *nwt* phenotypes were obtained. In the first inoculation experiment, 40%-60% of re-isolates displayed *nwt* phenotypes. In the second inoculation experiment, all re-isolates initially showed *wt* phenotype; however, some *wt* re-isolates from oak converted to *nwt* phenotypes during *in vitro* growth. In contrast, no such phenotypic changes were observed when isolates from California bay laurel and *Camellia* were inoculated and re-isolated from the foliar hosts California bay laurel, *Viburnum*, and *Rhododendron*.

High-throughput sequencing-based analyses identified major genomic alterations in NA1 *nwt* isolates from oaks included partial aneuploidy and copy-neutral loss of heterozygosity. Chromosomal breakpoints were found to be located at or near transposons, linking transposon de-repression caused by the chemical environment of oaks to structural genomic changes. Similarly, two EU1 *nwt* isolates and one *wt* isolate from *C. lawsoniana* exhibited large chromosomal regions with copy number variations and loss of heterozygosity, consistent with the isolates being copy number heterokaryons. Such genomic alterations were not identified in NA1 isolates from foliar hosts. In support of the sequence-based analysis, flow cytometry showed that in contrast to foliar isolates, nuclear contents of NA1 *nwt* isolates from oaks were variable and unstable.

To conclude, this work demonstrated that major genome alterations of *P. ramorum* could be induced in a host species-dependent manner (Fig. 3).

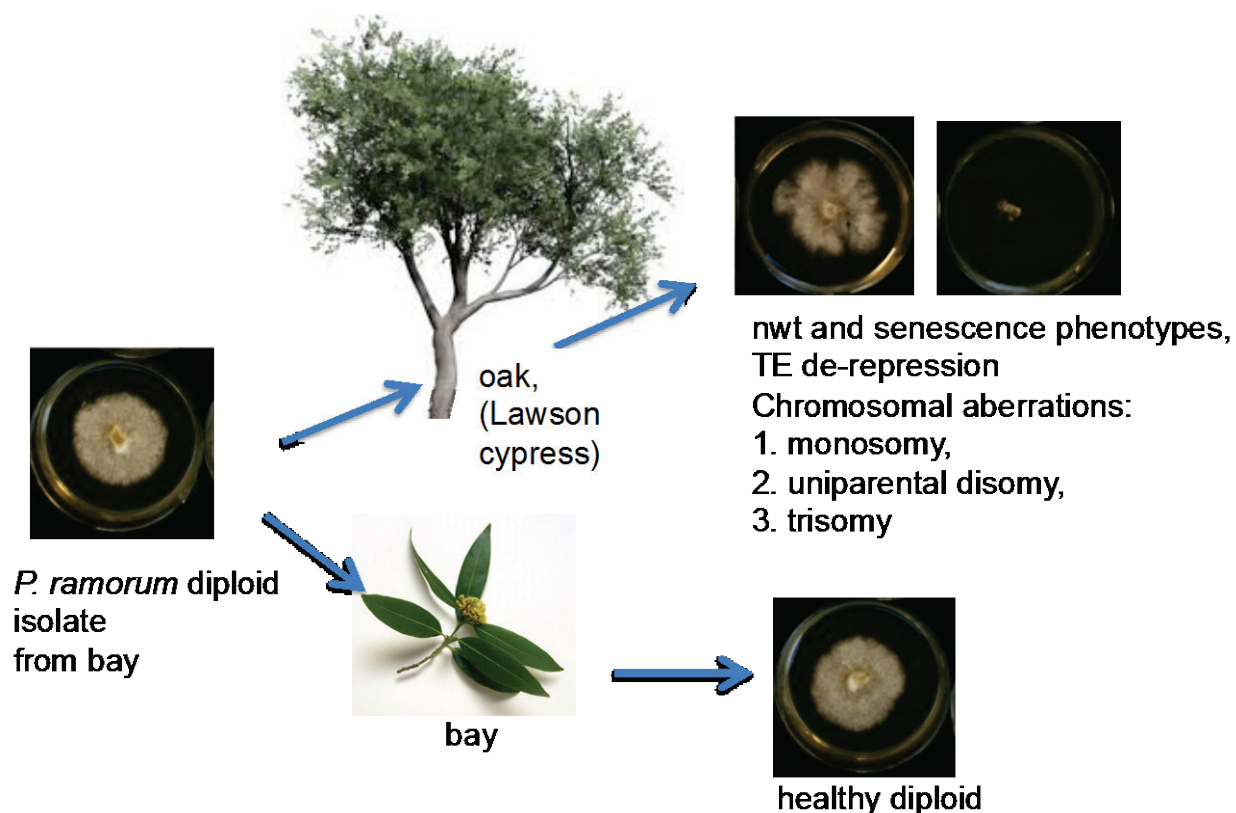


Figure 3—*P. ramorum* isolates from oaks are more likely to display the *nwt* colony phenotype than those from California bay laurel. We found that *P. ramorum* in oaks, and probably also in Lawson cypress, undergoes genome alterations that result in the *nwt* colony phenotype. *Nwt* phenotype is associated with de-repression of transposable elements.

Literature Cited

- Brasier, C.; Kirk, S.; Rose, J. 2006. Differences in phenotypic stability and adaptive variation between the main European and American lineages of *Phytophthora ramorum*. 3rd Int. IUFRO Work, Friesing, Germany. pp. 166-173.
- Kasuga, T.; Bui, M.; Bernhardt, E.; Swiecki, T.; Aram, K.; Cano, L.M.; Webber, J.; Brasier, C.; Press, C.; Grünwald, N.J.; Rizzo, D.M.; Garbelotto, M. 2016. Host-induced aneuploidy and phenotypic diversification in the Sudden Oak Death pathogen *Phytophthora ramorum*. BMC Genomics. 17:385.