Host-Induced Phenotypic Diversification in *Phytophthora ramorum*[1]

Takao Kasuga,[2] Mai Bui,[2] Christine Shoemaker,[2] Elizabeth Bernhardt,[3] Tedmund Swiecki,[3] Kamyar Aram,[4] David Rizzo,[4] Melina Kozanitas,[5] and Matteo Garbelotto[5]

Abstract

Forestry, agriculture, and native ecosystems face ever-increasing threats by invasive species. Not all introduced species are, however, invasive. In order to establish and persist in a non-native land, introduced species have to adapt to different environments, unfamiliar food, and predators. There are a number of examples where invasive species evolved quickly in non-native regions (Brasier 1995, Davis 2009), such as leg size of cane toad (Phillips et al. 2006) and leaf size of St John's wart (Maron et al. 2004). Large phenotypic variation has been observed in clonal lineages of the generalist plant pathogen *Phytophthora cinnamomi* (Hüberli et al. 2001). Rapid phenotypic changes in invasive animals and plants are believed to result from selection of existing genetic variation; in a non-native location, under different selective pressure, different traits from their native range may be selected. Contribution of *de-novo* mutations for adaptation is probably not sufficient to explain rapid phenotypic changes observed in invasive species. *De-novo* DNA mutations are rare: spontaneous mutation rate estimated for a plant is only one base substitution per genome per generation (Becker et al. 2011) and non-neutral mutation rate in eukaryotes is approximately 1/300 per genome per generation (Drake 1999). Hence, it is unclear how an invasive clonal pathogen, which is devoid of genetic variation, will adapt to new hosts and a new environment. It may be that, in response to environmental stress, invasive clones may generate variation on which selection acts.

There are several mechanisms proposed for the generation of *de novo* variation in response to stress. For instance, adaptive mutation proposes that DNA mutation rates may increase as an immediate and direct response to selective pressures (Cairns et al. 1988). Environmental stress may also induce epigenetic variation (Eichten et al. 2011), which is a heritable variation in gene regulation that cannot be attributed to DNA sequence variation. Transposable elements (TEs), which are mobile genetic elements and are highly mutagenic, may also be involved in the generation of *de novo* variation. A typical eukaryotic genome harbors thousands of TEs; however, their activities are epigenetically suppressed. Stress is known to activate TEs, which may contribute to genome diversification (Madlung and Comai 2004).

Isolates of *Phytophthora ramorum* belonging to the NA1clonal lineage display a large variation in colony morphology and growth rate. In culture, *P. ramorum* grows in a circular mycelial mat, which is defined as wild type (*wt*) (Brasier et al. 2006). However, some NA1 isolates show irregular and fluffy colony types, which are referred to non-wild type (*nwt*). Some isolates stop growing upon subculturing, which is referred to early senescence. Isolates showing *nwt* morphology are less aggressive on *Rhododendron* leaves (Elliott et al. 2011). We have recently shown that dramatic phenotypic variation among *P. ramorum* isolates is associated with the host species from which the microbe was originally cultured, but not with multilocus genotypes (Kasuga et al. 2012). Isolates originating from oaks (*Quercus* spp.) are less virulent on oak seedlings than those originating from California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.). Furthermore, oak isolates are more likely to show *nwt* phenotype and early senescent phenotype than bay laurel isolates (Kasuga et al. 2012). Microarray mRNA profiling was conducted to detect gene regulation differences between oak and California bay laurel isolates. As a result, derepression of hundreds of transposable elements (TEs) and down-regulation of Crinkler effector homologs were observed in the majority of oak isolates, but this expression pattern was rare in

isolates from California bay laurel. In some instances, oak and California bay laurel isolates originating from the same geographic location had identical multilocus genotypes, but had different phenotypes. Because (1) no genetic subdivision has been detected between California bay laurel and oak isolates, and (2) oak is a dead-end host and infection is initiated from asexual propagules produced on nearby foliage host such as California bay laurel, we hypothesized that *P. ramorum* derived from a foliar host undergoes host-dependent genetic/epigenetic alterations inside an oak host. The observed phenomenon may be described as host-induced phenotypic diversification (HIPD); heritable phenotypic changes of the pathogen can occur inside host plants and the rate of change is host species dependent.

Direct evidence of HIPD and associated genetic/epigenetic alteration need to be established. We examined phenotypes of re-isolates from artificially inoculated canyon live oak (*Quercus chrysolepis* Liebm.) with an isolate displaying *wt* colony type typical of California bay laurel isolates. Nine months post inoculation, 20 percent of re-isolates (n=67) showed *nwt* colony phenotype. Both *wt* and *nwt* colony types were frequently recovered from the periphery of a single lesion in the cambium, which indicates a stochastic nature of the phenotypic conversion. In contrast, the vast majority of isolates obtained from California bay laurel consistently showed wild type (*wt*) colony morphology. Global mRNA profiling confirmed that some of the re-isolates growing in culture showed expression patterns typical for those isolated from oak trunks (i.e., derepression of TEs and repression of genes belonging to Crinkler effector family).

We have demonstrated that *P. ramorum* growing in oak undergoes phenotypic conversion; colony phenotypes and gene expression patterns of California bay laurel isolates were drastically changed after 9 months under the bark of canyon live oak. Although actual TE transposition and genome diversification are yet to be experimentally validated, the observed TE activity in re-isolates strongly indicates the occurrence of TE-mediated genome diversification in *P. ramorum* growing in oak hosts. According to an epi-transposon hypothesis, which is a theory of genome evolution to explain punctuated equilibria observed in the fossil records of animals, physiological stress, associated with major climatic change or invasion of new habitats, disrupts epigenetic silencing of TEs, which results in TE reactivation and genome diversification (Zeh et al. 2009). Brasier (1986, 1995) proposed a theory of episodic selection to explain rapid evolution observed in invasive plant pathogens: species will undergo episodic selection under sudden environmental disturbances such as geographical transposition, alterations in resource availability, exposure to a new host, and sudden climatic changes, which may result in the emergence of a highly fitted pathogenic clone or allopatric hybrid. Abiotic stress as well as interspecific hybridization proposed in the theory of episodic selection are known to cause transcriptional activation of TEs that may subsequently lead to restructuring of the genome (Madlung and Comai 2004). The generated genome diversity may then be served as raw material on which episodic selection can act. The epi-transposon hypothesis provides a molecular mechanism for accelerated evolution and hence complements the theory of episodic selection. Activation of TEs in suboptimal hosts may thus be a widespread strategy among invasive pathogens for adaptation and host shifts.

Acknowledgments

Literature Cited

Becker, C.; Hagmann, J.; Muller, J.; Koenig, D.; Stegle, O.; Borgwardt, K.; Weigel, D. 2011. Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. Nature. 480: 245–U127.

Brasier, C.M. 1986. The population biology of Dutch elm disease: its principal features and some implications for other host-pathogen systems. In: Ingram, D.S.; Williams, P.H., eds. Advances in plant pathology. London: Academic Press: 53–118.

Brasier, C.M. 1995. Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. Canadian Journal of Botany-Revue Canadienne De Botanique. 73: S1213–S1221.

Brasier, C.M.; Kirk, S.A.; Rose, J. 2006. Differences in phenotypic stability and adaptive variation between the main European and American lineages of *Phytopthora ramorum*. In: Brasier, C.M.; Jung, T.; Oßwald, W., eds. Progress in research on Phytophthora diseases of forest trees. Proceedings of the Third International IUFRO Working Party S07.02.09 meeting at Freising, Germany. Farnham, Surrey, UK: Forestry Commission, Forest Research: 166–173.

Cairns, J.; Overbaugh, J.; Miller, S. 1988. The origin of mutants. Nature. 335: 142–145.

Davis, M. 2009. Invasion biology. New York: Oxford University Press.

Drake, J.W. 1999. The distribution of rates of spontaneous mutation over viruses, prokaryotes, and eukaryotes. Annals of the New York Academy of Sciences. 870: 100–107.

Eichten, S.R.; Swanson-Wagner, R.A.; Schnable, J.C.; Waters, A.J.; Hermanson, P.J.; Liu, S.Z.; Yeh, C.T.; Jia, Y.; Gendler, K.; Freeling, M.; Schnable, P.S.; Vaughn, M.W.; Springer, N.M. 2011. Heritable epigenetic variation among maize inbreds. PLoS Genet. 7(11): e1002372.

Elliott, M.; Sumampong, G.; Varga, A.; Shamoun, S.F.; James, D.; Masri, S.; Grünwald, N.J. 2011. Phenotypic differences among three clonal lineages of *Phytophthora ramorum*. Forest Pathology. 41: 7–14.

Hüberli, D.; Tommerup, I.C.; Dobrowolski, M.P.; Calver, M.C.; Hardy, G.E.St.J. 2001. Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia. Mycological Research. 105: 1053–1064.

Kasuga, T.; Kozanitas, M.; Bui, M.; Hüberli, D.; Rizzo, D.M.; Garbelotto, M. 2012. Phenotypic diversification is associated with host-induced transposon derepression in the sudden oak death pathogen *Phytophthora ramorum*. PLoS ONE 7:e34728.

Madlung, A.; Comai, L. 2004. The effect of stress on genome regulation and structure. Annals of Botany. 94: 481–495.

Maron, J.L.; Vila, M.; Bommarco, R.; Elmendorf, S.; Beardsley, P. 2004. Rapid evolution of an invasive plant Ecological Monographs. 74: 261–280.

Phillips, B.L.; Brown, G.P.; Webb, J.K.; Shine, R. 2006. Invasion and the evolution of speed in toads. Nature. 439: 803.

Zeh, D.W.; Zeh, J.A.; Ishida, Y. 2009. Transposable elements and an epigenetic basis for punctuated equilibria. BioEssays. 31: 715–726.