

# Emergence of the sudden oak death pathogen *Phytophthora ramorum*

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**The recently emerged plant pathogen *Phytophthora ramorum* is responsible for causing the sudden oak death epidemic. This review documents the emergence of *P. ramorum* based on evolutionary and population genetic analyses. Currently infection by *P. ramorum* occurs only in Europe and North America and three clonal lineages are distinguished: EU1, NA1 and NA2. Ancient divergence of these lineages supports a scenario in which *P. ramorum* originated from reproductively isolated populations and underwent at least four global migration events. This recent work sheds new light on mechanisms of emergence of exotic pathogens and provides crucial insights into migration pathways.**

## ***Phytophthora ramorum* is a recently emerged, exotic plant pathogen**

The pathogen *Phytophthora ramorum* is responsible for causing sudden oak death in the Western US and ‘sudden larch death’ on Japanese larch in the UK. It has emerged rapidly and repeatedly, with devastating impact upon forest ecosystems and causing severe economic hardship to some nursery operations (Figure 1). *P. ramorum* emerged in the mid-1990s nearly simultaneously on woody ornamental plants in Germany and causing sudden oak death on oaks and tanoaks in the US [1–3] (Figure 2). Since the emergence of this pathogen several research groups have applied a range of population genetic and evolutionary tools to understand the pattern and process of its emergence [4]. This review tracks the emergence of *P. ramorum* based on these recent evolutionary and population genetic studies.

## **Biology and impact of *P. ramorum***

*Phytophthora* species are members of the Oomycetes, which are fungus-like diploid eukaryotes commonly referred to as water molds. They belong to the Stramenopiles and are most closely related to the golden-brown algae and diatoms [5]. The typical *Phytophthora* life cycle includes both an asexual and a sexual phase. Two asexual spores are recognized: chlamydospores and sporangia (Glossary). Sexual reproduction results in the formation of oospores. In heterothallic *Phytophthora* species, sexual structures

can only be formed when the two mating types encounter and mate. *P. ramorum* is heterothallic and produces sporangia and chlamydospores. Oospores have not been observed in the known geographic distribution of *P. ramorum*.

*Phytophthora ramorum* is adapted to cool temperatures with optimal growth at 20 °C [3]. The life cycle of *P. ramorum* resembles that of other splash-dispersed *Phytophthora* species [1]. Deciduous sporangia are formed on the surface of infected leaves or twigs and, depending upon environmental conditions, are locally splash-dispersed or spread over long distances by wind and wind-driven rain [6] (Box 1). Motile zoospores are released from sporangia, and upon contact with susceptible host tissue then encyst, germinate, and penetrate host tissue. Rapid repetition of this asexual cycle results in epidemics when environmental conditions are favorable. Chlamydospores are abundantly produced within infected plant tissue and allow *P. ramorum* to survive adverse conditions in infected stems and leaves on the plant, in plant debris on the soil surface, or in the soil [7]. Within the genus-wide phylogeny, *P. ramorum* is classified in *Phytophthora* clade 8c and its closest relatives include *Phytophthora lateralis*, *Phytophthora foliorum* and *Phytophthora hibernalis* [8].

Currently, the known host-range of *P. ramorum* encompasses more than 100 plant species in over 40 genera

## **Glossary**

**Chlamydospore:** an asexual reproductive structure providing a resting spore that can survive adverse conditions better than sporangia.

**Clonal lineage:** a population of asexually reproducing individuals descended from the same ancestor.

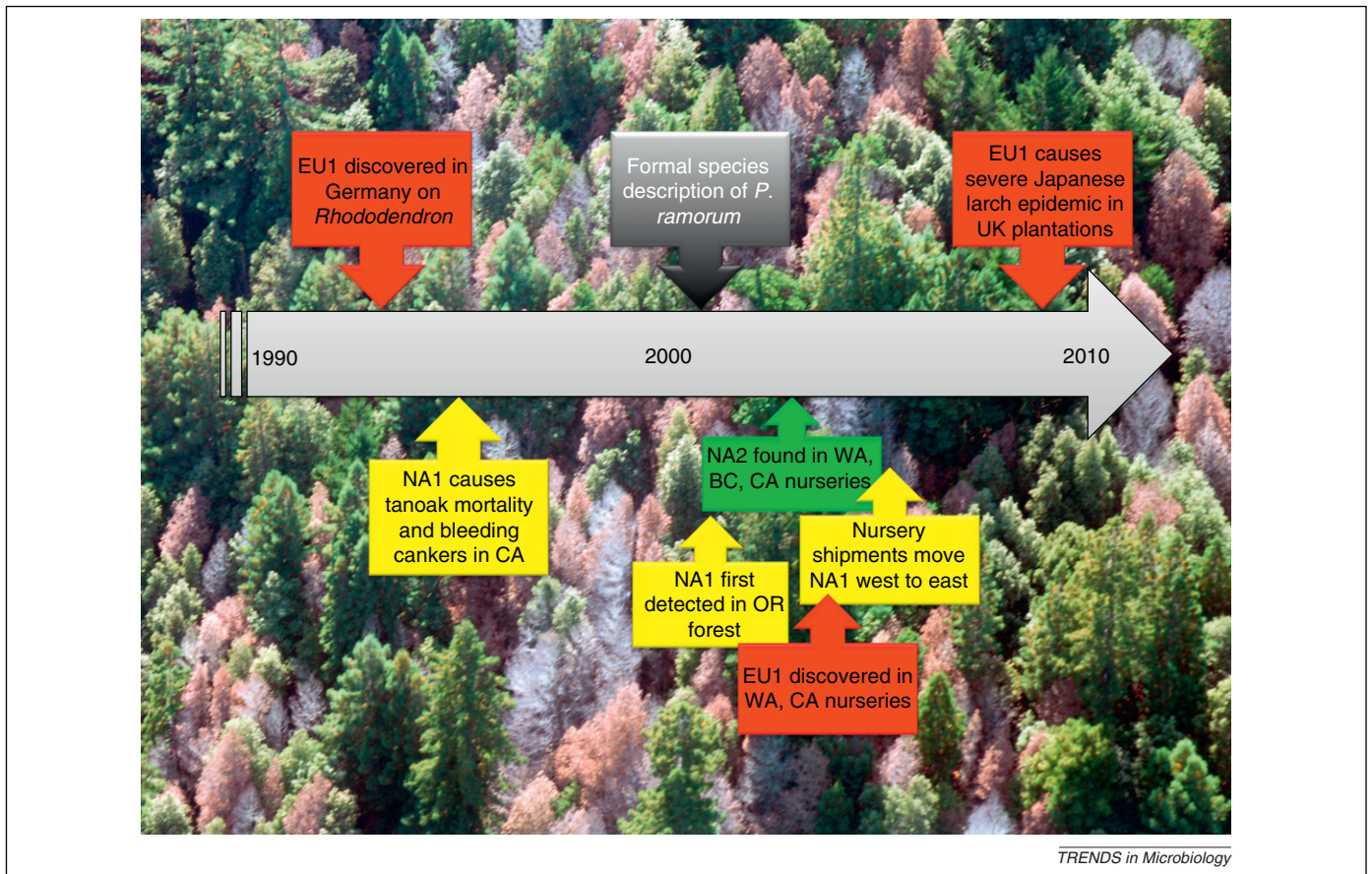
**Heterothallic:** requiring two opposite mating types for sexual reproduction via oospores as opposed to homothallic species. Homothallism is thought to result in inbreeding or selfing with low rates of outcrossing.

**Mating type:** heterothallic *Phytophthora* species require mating between two sexes for sexual reproduction. These sexes are termed mating types, designated A1 and A2. Homothallic species do not require mating types.

**Oospore:** sexual reproductive structure giving Oomycetes their name. Oospores are formed by fusion of antheridial (male) and oogonial (female) reproductive organs. Oospores are also biologically important as resting spores given their strong, double walls that provide protection against desiccation and microbial decay.

**Sporangium:** asexual reproductive spores that can differentiate into motile zoospores or germinate directly to produce hyphae. Sporangia are the spores that provide a means of rapid reproduction and are thought to contribute to rapid disease progress in an epidemic.

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**Figure 1.** Approximate chronology of the emergence of *Phytophthora ramorum*, the causal agent of sudden oak death. Clonal lineages are distinguished in colors: red, EU1; yellow, NA1; green, NA2.

observed to be infected in forest, landscape or nursery environments [1]. The pathogen causes two main categories of symptoms, depending on the host plant [9,10]. On hosts where foliar infection occurs (e.g. California bay laurel, *Rhododendron* species, and tanoak), *P. ramorum* infection results in necrotic lesions on the leaves and in shoot dieback. Such hosts play a key role as abundant sporulators in the sudden oak death epidemic (e.g. California bay laurel in California or tanoak in southwestern Oregon). On canker hosts (e.g. oaks, tanoak, and European beech), *P. ramorum* typically causes bleeding cankers on the stem. These cankers predominantly appear as discolored areas in the phloem (inner bark), often extending into the outer portion of the xylem. A decline in sap flow and stem hydraulic conductivity results in rapid crown decline and tree death (sudden oak death) [11,12]. Tanoak is an intriguing host that shows both bleeding cankers and shoot dieback symptoms.

The impact of *P. ramorum* differs depending on the ecosystem and host. In natural (forest) and semi-natural (plantation) ecosystems, extensive mortality of trees and shrubs can have severe short- and long-term ecological consequences, including significant changes in the structure and composition of the plant and animal communities, and increased water run-off and associated soil erosion. *P. ramorum* has significant economic impact upon forests owing to reductions in recreational, cultural, or commodity value and the costs of monitoring and eradication

measures. In North America, the major environmental impact of *P. ramorum* has been in the coastal woodlands of California and southwestern Oregon [10,13]. In Europe, widespread tree dieback and mortality caused by *P. ramorum* have only been recently reported in Japanese larch plantations in England [14] and, as of 2011, in Wales, Scotland, Northern Ireland and the Republic of Ireland. In nurseries, *P. ramorum* has had a significant financial impact ranging from bankruptcy at one extreme to losses due to quarantine restrictions, losses due to mandatory destruction of infested ornamentals, and trade losses. Generally, the detection of *P. ramorum* in a nursery forces the adoption of a series of quarantine and regulatory measures ranging from the immediate destruction of the infected nursery stock to regular monitoring of host plants [European Union regulatory measures 2002/757/EC (<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:252:0037:0039:EN:PDF>), further amended by 2004/426/EC ([http://www.furs.si/law/EU/zvr/zakonodaja\\_CIRCA/EU\\_circa/nadzor\\_les\\_phytophthora/32004\\_426\\_EC.pdf](http://www.furs.si/law/EU/zvr/zakonodaja_CIRCA/EU_circa/nadzor_les_phytophthora/32004_426_EC.pdf)) and 2007/201/EC (<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:090:0083:0085:EN:PDF>); United States federal domestic quarantine 301.92 ([http://www.oregon.gov/ODA/PLANT/NURSERIES/docs/pdf/federal\\_order.pdf?ga=t](http://www.oregon.gov/ODA/PLANT/NURSERIES/docs/pdf/federal_order.pdf?ga=t)) and federal order DA-2011-04 ([http://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/pram/downloads/pdf\\_files/SPROWithPramPrenotificationFO.pdf](http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/SPROWithPramPrenotificationFO.pdf)).



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**Figure 2.** Disease and symptoms of sudden oak death and ramorum blight. (a) Sudden oak death symptoms include bleeding cankers often observed on affected trees, such as oaks and beech, that eventually die. (b) Tanoak death in Big Sur, California. Ramorum foliar or shoot blight is observed on woody ornamentals such as (c) *Rhododendron* (shown), *Viburnum*, *Pieris*, and *Kalmia* or native species such as (d) bay laurel (shown) and tanoak.

### Repeated emergence of *P. ramorum*

#### Population structure

*P. ramorum* is currently only recognized in Europe and North America (Figure 3). In Europe, the pathogen exists as the EU1 clonal lineage [15,16]. In North America three lineages are currently recognized: NA1, NA2, and EU1 [16,17] (Figure 4). The clonal lineages were named after the continent (North America or Europe) where they were first detected, and lineages are readily distinguished by all molecular marker systems employed to date, including amplified fragment length polymorphism analysis, simple sequence repeat genotypes, and DNA sequences for mitochondrial or nuclear loci [18].

#### Emergence of NA1 in the US

The emergence of the NA1 clonal lineage has distinct dynamics in the forest and the nursery environments. By the time *P. ramorum* was first isolated from a bleeding canker on coast live oak, the epidemic in California was well established [2]. Detailed population genetic analysis determined that the NA1 clone was most probably introduced by importation of infected nursery host plants to a local nursery in Scotts Valley, CA in the late 20<sup>th</sup> century from an unknown source population [19,20] (Figure 3; yellow). The presence of genetically indistinguishable forest populations at distances of over 50 km [19] is incompatible with the short-distance dispersal of the large airborne sporangia [6,20]. However, this population structure can be explained by human-assisted movement of the pathogen on plants or soil (including soil on shoes or vehicle tires) [19]. From the initial focus of introduction, *P. ramorum* rapidly spread to native tanoak (*Notholithocarpus densiflorus*) and

oaks (*Quercus* spp), presumably through foliar infection of California bay laurel, and thus started the sudden oak death epidemic in forest ecosystems. In the decade since its discovery this clonal lineage has spread to the current limits of the sudden oak death epidemic up and down the California coast [21] (Figure 3; yellow). Interestingly, the Curry County infestation in Oregon was probably the result of a different introduction from infected nursery stock because this population cannot be genetically linked to a specific California forest infestation that could have served as a source population [20,22].

By 2004 the NA1 lineage was widespread in the nursery trade and its long-distance dispersal can be largely traced to trade in ornamental plants [16,17,19,20,22–24]. Throughout North America, several distinct pathways of migration are apparent among nurseries (Figure 3; yellow). First, NA1 has regularly been found in nurseries in California, Oregon, Washington and British Columbia spreading bi-directionally on the west coast [22–24]. Second, *P. ramorum* is spreading west to east via nursery shipments, with two apparent migration pathways from southern California and the Pacific Northwest [17] (Figure 3; yellow). The pathogen is now found intermittently in nursery environments in several states including Alabama, Missouri, Florida, Georgia, South and North Carolina, and potentially also in others.

#### Emergence of EU1 in Europe and North America

The EU1 clonal lineage was first detected in nurseries and established gardens in Germany and the Netherlands on ornamentals including *Rhododendron* and *Viburnum* [3]. A few years later *P. ramorum* was discovered in other areas

### Box 1. Dispersal mechanisms of *P. ramorum*

**Rain and wind:** rain splash and wind-driven rain are thought to explain the rapid spread of *P. ramorum* within a forest. In mixed-evergreen forests in California, rain-splash dispersal of *P. ramorum* 10–15 m has been detected [6]. In southwestern Oregon, the pattern of new *P. ramorum* infections across the landscape is consistent with local rain splash and long-distance (up to 4 km) inoculum dispersal in turbulent air currents [20,54].

**River and streams:** as with other *Phytophthora* species (e.g. *P. lateralis* [55]), *P. ramorum* can be dispersed over long distances in moving water bodies such as rivers and streams. Both in California and Oregon, stream monitoring using leaf baits could detect *P. ramorum* up to 1–20 km downstream from the probable inoculum source [6,56]. However, considering that infection patterns in forests do not seem to follow stream courses, secondary infection through sporangia and zoospores present in waterways is probably a rare event.

**Human activities:** human-mediated movement of infected plant material or soil has resulted in the dispersal of *P. ramorum* both in natural and nursery environments [57]. The national and international trade of infected nursery stock contributed significantly to the spread of the pathogen among nurseries in North America and Europe [17,23] and started the sudden oak death epidemic in California [20,21]. *P. ramorum* has been detected in the sapwood of several tree species [11,58]. However, because sporulation on the bark surface is rare, the role of infected wood (i.e. firewood and timber) in *P. ramorum* dispersal remains to be established. However, the movement of green waste containing infected leaves might contribute to the dispersal of the pathogen. Soil samples collected from vehicle or bike tires and the shoes of hikers were found to contain *P. ramorum* spores, indicating that recreational activities in infested areas could promote the spread of *P. ramorum* [6]. Similarly, infested potting media in nurseries can act as an inoculum reservoir for new root and shoot infections [59].

**Animals:** vertebrates (e.g. deer, squirrels, and birds) and invertebrates (e.g. snails) might act as vectors of *P. ramorum* by dispersing infected plant material or soil. One study showed that sporangia and chlamydospores that were ingested, digested and excreted by snails could infect detached leaves [60].

of Europe. Currently, *P. ramorum* is present in many European countries where it is mainly found in ornamental nurseries or gardens with the exception of the UK where it is now causing landscape-scale epidemics in some larch plantations (<http://rapra.csl.gov.uk/pr/index.cfm>) [14]. However, neither the site nor the origin of the first introduction of *P. ramorum* into Europe is clear.

The EU1 clonal lineage is also found in North America and appears to have been introduced into the Pacific Northwest [23]. A coalescent analysis with migration [4,25] provided support for a unidirectional migration of the EU1 clonal lineages from Europe to North America [23] (Figure 3; red). However, this effort could not establish whether the pathogen was introduced into British Columbia, Canada, or Washington, USA. After its first introduction into the Pacific Northwest, EU1 isolates have since migrated to California and Oregon.

#### Emergence of NA2 in the US

The NA2 clonal lineage was first observed in 2004 in California and Washington [16]. However, it appears that this lineage was introduced into either British Columbia or Washington from an unknown source [23] (Figure 3; green). It is currently only rarely found in California and is more commonly found in Washington and British Columbia.

#### Clonal divergence and microevolution

In both Europe and the US the clonal lineages are accumulating mutations and gradually diversifying genetically from the ancestral, invasive NA1, NA2, or EU1 clones in a process of clonal divergence [15,17,23] (Figure 4). This pattern is most prominent for the NA1 clonal lineage owing to the use of hypervariable loci for genotyping and its possibly larger population sizes, but is apparent in all lineages [15,17,19,20,22–24].

Despite the emergence of new genotypes in forest ecosystems, there is no clear evidence that selection has favored any specific genotypes in established infestations where the dominant genotypes have remained unchanged over the past ten years. Changes in the dominance of genotypes in new forest and nursery infestations have been documented and have been ascribed to stochastic effects including founder effects during establishment followed by genetic drift [17,19,22].

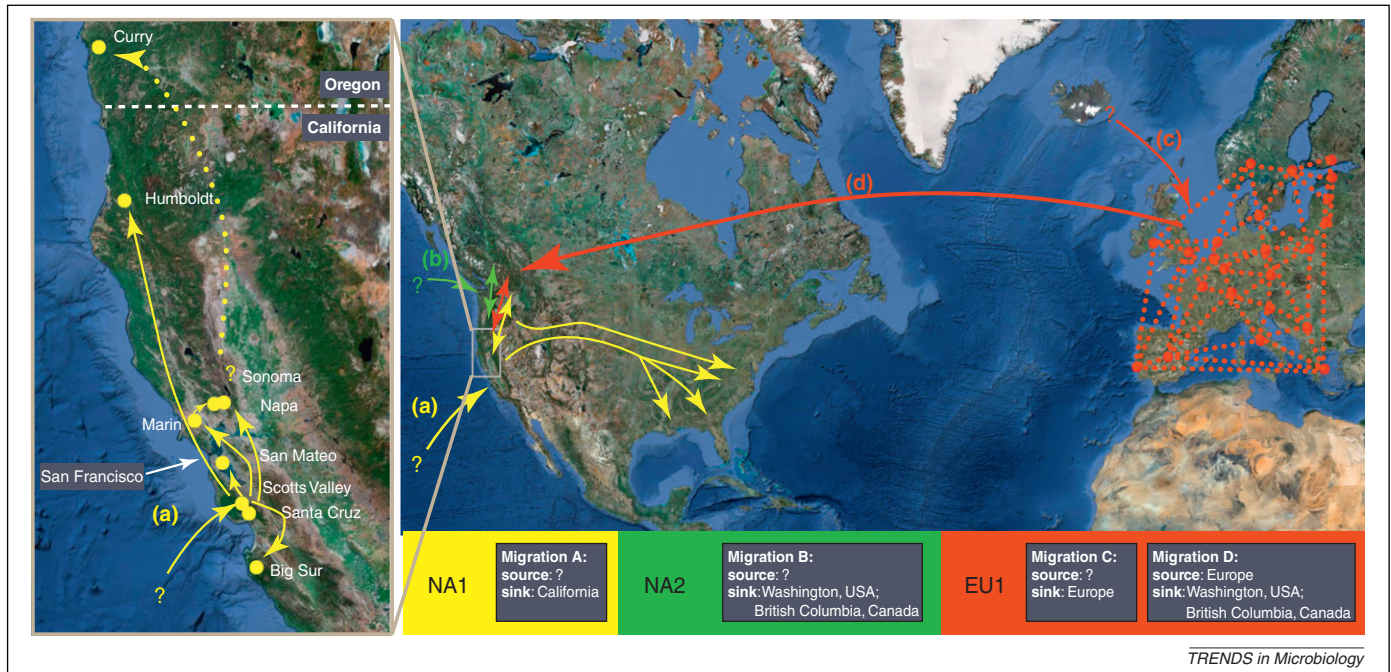
#### What factors are involved in the repeated emergence of *P. ramorum*?

Given the fact that *P. ramorum* has migrated globally at least four times, further introduction of novel genotypes and migration into new areas is very likely. Environmental conditions typical for cultivation of ornamental plants – moderate to low temperatures and seasonally high moisture – are ideal for the establishment and maintenance of *P. ramorum* in nurseries [26,27]. It is thus unlikely that *P. ramorum* can be eradicated given its current extensive distribution, although eradication is currently a standard practice in nurseries.

Several epidemiological factors provide perspective into why this pathogen had the opportunity to emerge repeatedly. One important factor is the large host-range that includes widely grown woody ornamentals, most notably *Rhododendron*, *Camellia*, *Viburnum*, *Pieris* and *Kalmia*. The use of fungicides can mask the presence of *P. ramorum* on many of these ornamentals grown in nursery environments and provide a reservoir for cryptic infections. Although *P. ramorum* is a foliar and stem pathogen, latent infection of roots and foliage has been observed [27–30]. Furthermore, all *Phytophthora* species known to infect the foliage of nursery crops produce similar symptoms. Nursery crops are shipped intra- and inter-continently [31] and are only inspected for visual symptoms when imported to the US or Europe. Between 2004 and 2010 the USDA Animal and Plant Health Inspection Service reported imports ranging between 5000 and 320 000 plants per year of *Rhododendron*, *Camellia*, *Viburnum* and *Pieris*, which are all considered to be high-risk hosts for movement of *P. ramorum* ([http://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/pram/downloads/pdf\\_files/NPB-RWGR.pdf](http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/NPB-RWGR.pdf)).

#### Is there a center of origin?

In North America and Europe *P. ramorum* has a typical clonal population structure. However, the genome sequence of an NA1 isolate and resequencing of nuclear genes in the introduced lineages has revealed substantial heterozygosity within each clonal lineage [32,33]. This heterozygosity and evidence of recombination in the history of nuclear genes suggest that these clonal introductions



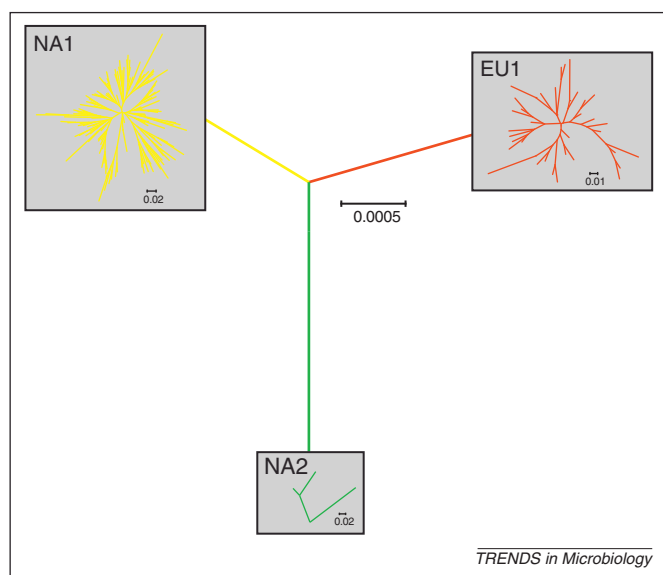
**Figure 3.** Scenarios depicting repeated emergence and migration of the sudden oak death pathogen *Phytophthora ramorum*. (Left) Scenario for the most likely introduction and spread of the NA1 clone of *P. ramorum* in California. (Right) The currently recognized, four intercontinental migrations of *P. ramorum* supported by population genetic and evolutionary studies. Shown are the most likely scenarios for repeated introduction of the three known color-coded clonal lineages NA1 (yellow), NA2 (green), and EU1 (red) into North America and EU1 (red) into Europe. The network presented for Europe reflects considerable uncertainty about the primary introduction and subsequent migration pathways within Europe. Red dots mark the country in which infection was observed and do not indicate the actual focus of infection.

are descended from a sexual population [32]. At the center of origin of *P. ramorum* one would expect a diverse and sexual population and probably the presence of close relatives, as is the case for *Phytophthora infestans* [34–36]. The expectation is that *P. ramorum* coevolved with its host or hosts over evolutionary time at this center of origin. To date, a sexual population has not been found and the

source or sources of the introduced lineages are unknown. Evolutionary analysis of genes in the *P. ramorum* lineages and the closest known relatives of *P. ramorum* also revealed a substantial number of lineage-specific mutations [32]. These mutations indicate that the lineages have evolved independently for an extended period of time. In fact, crude estimates of their age suggest that they may be more than 100 000 years old. These data are supported by the poor genetic compatibility between the A1 and A2 mating types when *in vitro* mating is attempted between lineages [37–39] (discussed below). Importantly, these lineage-specific mutations indicate that the observed divergence among the lineages was not a recent event, and instead the lineages were probably members of three diverged populations before their introduction. Thus, the three introductions of *P. ramorum* were probably the result of multiple movements of *P. ramorum* inoculum from several source populations.

#### Genetics of *P. ramorum*

*P. ramorum* is a heterothallic eukaryote and thus has two mating types. All isolates of the NA1 and NA2 lineages are of A2 mating type [40]. EU1 lineage isolates are almost exclusively of A1 mating type [40,41]. Three EU1 isolates of A2 mating type were detected in Europe in 2002–2003, but after eradication measures were taken no such isolates have since been identified. The current European *P. ramorum* population is assumed to be exclusively of the A1 mating type [41] and the only known risk for sexual recombination in nature is at North American sites where A1 and A2 strains coexist. So far, no natural recombinants have been detected [17,24], which has raised questions about the functionality of the sexual reproductive system



**Figure 4.** Lineage divergence and clonal expansion of *P. ramorum*. A maximum-likelihood tree showing the extent of divergence between the three clonal lineages as calculated using mitochondrial DNA (mtDNA) sequences [52] and the Tamura three-parameter model. Distances between microsatellite genotypes illustrating clonal divergence within lineages are shown at the tips of the mtDNA tree. Distances among genotypes were calculated using Bruvo's distance and visualized with unrooted neighbor-joining trees using published data [15,17,19,23,24,53].

in *P. ramorum*. Oospore production rates are generally low [37]. Boutet *et al.* [39] adapted a mating assay from Brasier and Kirk [37] and used maturation periods of up to 500 days to generate 38 progeny of EU1 × EU1 lineage isolates and 13 progeny of EU1 × NA1 lineage isolates. Oospore germination rates were 0.03% to 0.62% and all progeny proved to be recombinants based on their simple sequence repeat (SSR) profiles. None of the progeny had increased pathogenicity as compared to the parental strains; several were significantly less pathogenic [39]. Non-Mendelian inheritance profiles of SSR loci and a significant increase in genome content in 35% of these progeny suggest a high incidence of aneuploid chromosomes [38]. Several progeny were also genotypically and morphologically unstable, indicating post-meiotic chromosome rearrangements. After one year, the growth rate of several progeny had significantly increased whereas no changes were observed in the parental isolates. Although these data document the presence of a functional meiotic system for *P. ramorum* in F1 progeny, inheritance is often non-Mendelian, the rate of reproduction is very low and progeny are often not viable or have reduced fitness, at least in their early stages. The production of viable F2 progeny and the possibility of post-zygotic barriers remain to be explored. The evidence for genetic divergence of lineages NA1, NA2, and EU1 [32] (above) and the apparent dysfunction of the sexual system argues that these lineages have been reproductively isolated for a long time. This clonal divergence and reproductive isolation is reminiscent of the divergence observed for A1 and A2 clonal lineages in *Phytophthora cinnamomi* where sex remains to be documented in nature [42,43].

### Emergence of other *Phytophthora* species

*P. ramorum* has received close attention due to its devastating impacts, but the global movement of other *Phytophthora* species is also well known. The potato late blight pathogen *P. infestans* has experienced several major global migrations in its nearly 200 year history as a major pathogen [44]. *P. cinnamomi*, *P. lateralis*, and *Phytophthora pinifolia* represent a fraction of the other damaging *Phytophthora* forest pathogens that are thought to be exotic in their known range but whose origins are unknown [43,45,46]. The attention on *P. ramorum* in particular has spurred renewed interest in the diversity and distribution of other *Phytophthora* species in ornamental nurseries and forests. As a result, the number of described *Phytophthora* species has more than doubled in recent years and continues to grow at an exponential rate. Among problematic *Phytophthora* species, pathogens that exhibit limited genetic diversity and/or clonality are the rule rather than the exception, suggesting that the introduction of *Phytophthora* to new hosts and environments is a common mechanism of pathogen emergence (Box 2).

A disturbing new trend in *Phytophthora* research is the discovery of species hybrids. Hybrid species can have expanded host-ranges as compared to the parental species. *Phytophthora alni* is a species complex causing die-back of alder in Europe and is the result of two or more hybridization events between species not known to cause disease on alder [47,48]. *P. infestans* has hybridized with another closely related *Phytophthora* species in South

### Box 2. Major mechanisms for the emergence of fungal and oomycete plant pathogens

**Pathogen migration:** *P. ramorum* provides a clear example of the emergence of a pathogen via human-assisted global migration. Many costly and destructive plant pathogens have emerged via movement from their native geographic range to a new environment [35,44,61]. Successful colonization and establishment of the pathogen in the new location often involves an expansion or shift of host-range [43,62,63]. These new hosts have not coevolved with the pathogen and may have little resistance or tolerance to infection.

**Host introduction:** another mechanism of pathogen emergence occurs when a new host, usually a cultivated crop, is introduced into the native geographic range of the pathogen, which then 'jumps' to the crop plant. Although initially a host-range expansion, subsequent adaptation to the new host and movement of the pathogen in association with the crop can in time lead to divergence and speciation. Several pathogens of high-value crops are suspected to have emerged in this manner, but there are few cases with strong supporting data (e.g. [64]).

**Hybridization and introgression:** gene and genome sequencing of plant pathogens have revealed a surprising level of horizontal gene transfer [48,49,65–71]. The amount of genetic material involved ranges from single toxin genes to multiple chromosomes and even whole genomes in hybrid species. Examples of hybridization and introgression between species can be found across different pathogen groups and may be a common mechanism in the evolution and adaptation of emerging plant pathogens [51].

America to form a pathogen with a novel host-range [49]. *Phytophthora* hybrids have also been found on ornamental plants [50]. Hybridization may be a direct result of the global movement of *Phytophthora* [51]. An increased awareness of hybridization as an evolutionary process should be built into phytosanitary risk protocols.

### Concluding remarks

It is now obvious that *P. ramorum* has emerged repeatedly, at least three times in North America and once in Europe. Continued migration of this pathogen is thus a realistic scenario. What can be done to avoid further introductions? Migration of this pathogen is most probably dependent on the movement of infested host plants. Therefore, one possible scenario would be initial quarantine and propagation of imported plant material at a certified plant introduction station to assure that plants are pathogen free. Many questions about the historic emergence, the potential for

### Box 3. Outstanding questions

- Where is the center of origin of the sudden oak death pathogen?
- Will *P. ramorum* eventually reproduce sexually?
- Can *P. ramorum* hybridize with other *Phytophthora* species, as has been observed for *P. alni* and *P. andina*?
- How was *P. ramorum* introduced into Europe and what was the pattern of spread?
- Will there be further new epidemics, as observed for the recent Japanese larch epidemic in the UK, and can we predict them?
- What would be the consequences of introduction of the EU1 or NA lineages into US forests, were this to occur? – or of the introduction of NA1 or NA2 lineages into Europe?
- How do we improve the current biosecurity protocols to reduce further spread of *P. ramorum* and how can we avoid further pathogen introductions?
- Can breeding for resistance help the management of sudden oak death on oaks and tanoak and related epidemics on larch?
- How will climate change affect the sudden oak death epidemic?

continued adaptation through sexual reproduction, and source populations of *P. ramorum*, among others, beg to be answered (Box 3). The search for the center of origin should be continued because this could clarify our understanding of this pathogen's biology. Furthermore, it is very likely that the center of origin of *P. ramorum* will also be a source of other exotic *Phytophthora* species because the source area is poorly explored for *Phytophthora* diversity. Thus, a concerted search for *P. ramorum* will help to identify other potential biosecurity threats posed by the genus *Phytophthora*.

### Acknowledgments

We thank Jennifer Parke and Chris Mundt for reviewing an earlier draft of this manuscript. Financial support to N.J.G. was provided by the USDA Agricultural Research Service (USDA-ARS) – Current Research Information System (CRIS) 5358-22000-034-00, the USDA-ARS Floriculture Nursery Research Initiative, the US Forest Service, and the USDA Animal and Plant Health Inspection Service (USA APHIS).

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