Migration Patterns of the Emerging Plant Pathogen *Phytophthora ramorum* on the West Coast of the United States of America

S. Prospero, N. J. Grünwald, L. M. Winton, and E. M. Hansen

First author: Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331-2902, and WSL Swiss Federal Research Institute, 8903 Birmensdorf, Switzerland; second author: Horticultural Crops Research Laboratory, U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS), Corvallis OR 97330; third author: Subarctic Agricultural Research Unit, USDA-ARS, Fairbanks, AK 99775; and fourth author: Department of Botany and Plant Pathology, Oregon State University, Corvallis.
Accepted for publication 19 February 2009.

ABSTRACT

Prospero, S., Grünwald, N. J., Winton, L. M., and Hansen, E. M. 2009. Migration patterns of the emerging plant pathogen *Phytophthora ramorum* on the West Coast of the United States of America. Phytopathology 99:739-749.

Phytophthora ramorum (oomycetes) is the causal agent of sudden oak death and ramorum blight on trees, shrubs, and woody ornamentals in the forests of coastal California and southwestern Oregon and in nurseries of California, Oregon, and Washington. In this study, we investigated the genetic structure of *P. ramorum* on the West Coast of the United States, focusing particularly on population differentiation potentially indicative of gene flow. In total, 576 isolates recovered from 2001 to 2005 were genotyped at 10 microsatellite loci. Our analyses of genetic diversity and inferences of reproductive mode confirm previous results for the Oregon and California populations, with the strong majority of the genotypes belonging to the NA1 clonal lineage and showing no evidence for sexual reproduction. The high incidence of genotypes shared among populations and the lack of genetic structure among populations show that important large-scale, interpopulation genetic exchanges have occurred. This emphasizes the importance of human activity in shaping the current structure of the *P. ramorum* population on the West Coast of the United States.

Additional keywords: simple sequence repeats.

In the last two decades, rapid advances in molecular techniques have provided novel tools for developing a better understanding of the population biology and genetics of microorganisms. For example, neutral genetic markers such as amplified fragment length polymorphism (AFLP), single nucleotide polymorphism (SNP), and simple sequence repeats (SSR or microsatellites) allow characterization of the genetic diversity of plant pathogen populations (12,48). Based on population genetic data, inferences can then be made about the origin of a pathogen (native versus introduced), its reproductive mode (clonal versus recombinant), and its evolutionary history and potential. Knowing the population genetics and dynamics of a pathogenic microorganism may provide information crucial to the development of sustainable control strategies (47).

The Oomycete genus *Phytophthora* currently contains more than 90 recognized species, most of which are destructive plant pathogens (16,54). Since the mid 1990s, *Phytophthora ramorum* Werres, De Cock & Man in't Veld has been causing extensive mortality (sudden oak death [SOD]) of oak species (*Quercus* spp.) and tanoak (*Lithocarpus densiflorus*) in native forest ecosystems of coastal California and southwestern Oregon (32,34,60). Given the ecological importance of the main host species of *P. ramorum*, SOD can be considered to be a "key threat" to North American native forests (3,40,59,60). Moreover, in the last several years, *P. ramorum* has also been repeatedly isolated from diseased plants in horticultural nurseries in California (since 2001), Oregon (since 2003), and Washington (since 2003) (22,53,55,64). Trace-forward surveys have shown that, from 2003 to 2007 through nursery trade, *P. ramorum*-infected plants have been shipped from the Pacific coast to 32 states across the continental United States, including states on the East Coast (2,68) (available online by the California Oak Mortality Task Force [COMTF]). Unfortunately, it appears that this pathogen has recently been able to establish in the Eastern United States, given its repeated recovery from nurseries or adjacent landscape or riparian environments in two states (39,68). In Europe, where the heterothallic *P. ramorum* was first isolated in 1993, it has mainly been found in nurseries and woodland gardens and only sporadically on trees (9,71). In both North America and Europe, *P. ramorum* is considered to be a quarantine organism and severe regulations have been adopted in order to prevent its further spread (32).

Since recognition of the high destructive potential of *P. ramorum* on a landscape scale, intense research efforts have been undertaken to understand its biology and ecology. In 2004, the draft genome sequence of *P. ramorum* was completed (available online by the Department of Energy [DOE] Joint Genome Institute), providing important new opportunities for genetic studies (27,28, 32,67). Recent molecular analyses based on microsatellites have revealed that North American and European isolates cluster into three separate clades (32,37,44). The first clade (EU1 lineage) is the only clonal lineage found in Europe and is sporadically present in North American nurseries, the second clade (NA1 lineage) is dominant in North American forests and nurseries, and the third clade (NA2 lineage) has only been observed in a few nurseries in Washington State and California (37). Clonal lineages differ not only in mating type (NA1 = A2, NA2 = A2, and EU1 = A1, with a few exceptions probably representing mating type switch within the lineage) (69) (A. Chandelier, *personal communication*) but also in culture morphology (70), aggressiveness (7), nucleotide sequences in conserved genes (e.g., mitochondrial cytochrome *c* oxidase subunit 1) (41), and genetic diversity (37,38). Despite the sporadic presence of the opposite mating types on both continents (31,69), the production of sexual spores (oospores), to date, has

Corresponding author: S. Prospero; E-mail address: simone.prospero@wsl.ch

doi:10.1094/PHYTO-99-6-0739

only been observed under laboratory conditions (8,71). Further support of the idea of a reproductive barrier comes from an analysis of nuclear sequence data, showing that *P. ramorum* clonal lineages diverged a long time ago, possibly on the order of 165,000 to 500,000 years (25).

So far, all main studies on the genetic structure of *P. ramorum* in North America have focused on two specific geographic populations: California and Oregon. Ivors et al. (37,38) demonstrated a higher genetic diversity in California nurseries than in forests and Mascheretti et al. (45) identified three widespread genotypes as the probable founders of the California population. Recently, we showed that the *P. ramorum* population in Oregon is characterized by low genetic diversity, with the forest population (Curry County) being distinctly differentiated from the nursery population (57). Only 9 of the 24 genotypes found in the infested forest in Oregon were also identified in California (45).

The purpose of the present study was to assess the genetic structure of the overall *P. ramorum* population on the West Coast of the United States across California, Oregon, and Washington with the same microsatellite markers that we previously used to analyze the Oregon population. The specific objectives we addressed were to (i) verify if the results obtained for the California and Oregon populations (e.g., genetic diversity and reproductive mode) are also valid for the overall *P. ramorum* population, (ii) determine if the overall *P. ramorum* population is geographically differentiated, (iii) establish if migration between populations in different states can be detected, and (iv) analyze the impact of human activity on the overall population structure of *P. ramorum*.

MATERIALS AND METHODS

Origin of the *P. ramorum* isolates. In total, 576 isolates recovered between 2001 and 2005 were analyzed in this study, representing the overall *P. ramorum* population on the West Coast of the United States (i.e., Oregon, California, and Washington) (Table 1). Isolates from Oregon were divided into two different populations according to their origin (i.e., forest [OR-fo] or nurseries [OR-nu]). Forest isolates were collected in southwestern Oregon (Curry County) in an area of ≈50 km^2 where an intense eradication effort has been going on since 2001 (34). Most of the Oregon isolates (272 from the forest and 51 from nurseries) were previously analyzed by Prospero et al. (57). The large majority of the California (CA) isolates were obtained from a broad range of infected plants (e.g., tanoak; coast live oak, *Quercus agrifolia*; California bay laurel, *Umbellularia californica*; and evergreen huckleberry, *Vaccinium ovatum*) in forests and parks located in seven different counties (from north to south: Humboldt, Mendocino, Sonoma, Marin, Contra Costa, Alameda, and Santa Cruz). These isolates were chosen to cover most of the current geographic range of *P. ramorum* in California, which encompasses >10,000 km^2 (≈500 by 30 km). Three California isolates were recovered from infected rhododendron plants in a nursery in Sacramento County. In both the OR-fo and CA populations, some isolates originated from soil samples collected within infested sites and from streams draining *P. ramorum*-infested sites. All isolates belonging to the Washington (WA) population were collected from symptomatic plants (i.e., *Camellia*, *Rhododendron*, *Viburnum*, and *Pieris* spp.) grown in horticultural nurseries located in seven different counties. Thirty-three CA isolates and five WA isolates were previously analyzed in the study of Ivors et al. (37).

DNA isolation. Genomic DNA was extracted from *P. ramorum* cultures using a standard cetyltrimethylammonium bromide–chloroform–isopropanol protocol (72).

AFLP analysis. In all, 45 North American isolates (43 from the NA1 lineage, 1 from the NA2 lineage, and 1 from the EU1 lineage), each representing a different microsatellite genotype, and three additional European isolates not analyzed with microsatellites in this study were characterized using AFLP analysis to link our work to the study of Ivors et al. (38). AFLP analysis was performed on genomic DNA using the AFLP Microbial Fingerprinting protocol (Applied Biosystems, Foster City, CA) with slight modifications as described previously (33). Selective polymerase chain reaction (PCR) was performed on 1.5 µl of diluted, preamplified product in a 10-µl reaction volume with 0.5 µl of *Mse*I-XX primer at 5 µM, 0.5 µl of dye-labeled *Eco*RI-XX primer at 1 µM, and 3.75 µl of AFLP core amplification mix (Applied Biosystems), where *Mse*I-XX/*Eco*RI-XX corresponds to one of three primer combinations: AC/AC, GC/AC, and GG/CC as described by Ivors et al. (38). PCR products (1.0 µl) were run with 10 µl of loading buffer (9.8 µl of deionized formamide and 0.2 µl of GeneScan-500 size standard; Applied Biosystems) on a capillary sequencer (3100 Avant Genetic Analyzer; Applied Biosystems). Electropherograms were analyzed using GeneMapper software (version 3.7; Applied Biosystems) to extract a matrix of presence and absence of alleles of 100 to 500 bp after visual inspection for further downstream analysis. All AFLP analyses were replicated on independent DNA extractions and PCR reactions until all polymorphic alleles could be unambiguously binned for a final total of eight alleles.

Microsatellite analysis. All isolates were genotyped at 10 microsatellite loci developed previously for *P. ramorum* (56,57). The loci were amplified in fluorescent, multiplex PCR following the protocol of Prospero et al. (57). Loci PrMS43a and PrMS43b were amplified by the same primer set. Alleles were sized on an ABI Prism 3100 sequencer (Applied Biosystems) and results were analyzed using GeneScan and Genotyper software (Applied Biosystems). Locus PrMS42 was excluded from the population analyses because of unclear allele pattern (57).

Data analyses. For most genetic analyses, geographical populations (i.e. OR-fo, OR-nu, CA, and WA) were considered separately. Multilocus genotypes PrNA2-1 (CA and WA, NA2 lineage) and PrEU1-1 (OR-nu and WA, EU1 lineage) were

TABLE 1. Collection data of the four populations of *Phytophthora ramorum* analyzed in this study

Population code	Geographic location	Isolates[y]		
		Origin	Year of isolation	*N*
OR-fo (*N* = 302)	Oregon, forest (Curry County)	Infected plants	2001–2005	235
		Streams	2002–2004	33
		Soil	2002–2005	34
OR-nu (*N* = 66)	Oregon, nurseries	Infected plants	2003–2005	66
CA (*N* = 117)	California, forest[z]	Infected plants	2001–2005	109
		Streams	2004–2005	6
		Soil	2004–2005	2
WA (*N* = 91)	Washington, nurseries	Infected plants	2003–2004	91

[y] Nursery isolates from Oregon were kindly provided by N. Osterbauer (Oregon Department of Agriculture), the California isolates by M. Garbelotto (University of California, Berkeley) and D. Rizzo (University of California, Davis), and the isolates from Washington State by A. Wagner and J. Falacy (Washington State Department of Agriculture).

[z] Three isolates from California (NA2 clonal lineage) were recovered from infected rhododendron plants in a nursery in Sacramento County.

excluded from those analyses in which genetic characteristics of the NA1 lineage were investigated. Given that clonal reproduction of genotypes may influence data interpretation, most genetic analyses were conducted with both all isolates of a population and clone-corrected data sets (i.e., only one representative isolate for each multilocus genotype).

In each population, genotypic diversity was quantified by the number of genotypes observed (MG_{Obs}); the number of genotypes expected in a sample of $N = 54$ (MG_{Exp}) (i.e., smallest population being analyzed); the Stoddart and Taylor's index $G = 1/\Sigma_i p_i^2$, where p_i = observed frequency of ith genotype (63); and the evenness index $E_5 = (1/\lambda - 1)/(e^{H'} - 1)$, where λ corresponds to the Simpson's index (62) and H' to the Shannon-Wiener's index (61). The indices G and E_5 (mean values and standard confidence intervals with bias correction) were calculated using the SAS macro <jack-boot.sas> whereas MG_{Exp} was calculated using the macro <Rarefac.C> (30). Differences in the G and E_5 values among populations were tested for significance with bootstrapping (2,000 resamples, confidence level of 90%). The significance of the correlation between number of genotypes shared and minimal geographic distance between populations was tested by conducting a Spearman's rank correlation test. Cluster analysis was based on allele frequencies observed for either AFLP or microsatellite loci. The tree based on AFLP data was constructed using the unweighted pair-group method of averages (UPGMA) algorithm from Nei's (51) genetic distance matrix. Statistical support for tree branches was obtained using 1,000 bootstrapped samples with Tools for Population Genetic Analyses (TFPGA) version 1.3 (50). To analyze relationships among microsatellite genotypes, a matrix of pairwise Cavalli-Sforza and Edwards (10) distances among all multilocus genotypes was generated with Populations 1.2.28 (O. Langella, available online by Bioinformatics Organization) and subsequently used to construct a split network in SplitsTree4 V4.10 (36).

Gene diversity was quantified by the allelic richness averaged over loci (A_R) and by the expected heterozygosity (H_E) using Fstat 2.9.3.2 (26). Excesses or deficits in genetic diversity were measured for each population by Wright's (73) fixation index F_{IS} (i.e., inbreeding coefficient). Significant deviations from Hardy-Weinberg equilibrium expectations were evaluated by Fisher's exact tests, with unbiased P values estimated through a Markov chain method (10,000 dememorizations, 100 batches, 5,000 iterations per batch) as implemented in Genepop version 3.1c (58). The hypothesis of asexual (i.e., clonal) reproduction within the *P. ramorum* populations was tested as follows. First, allele patterns of all multilocus genotypes were checked to detect eventual recombinant genotypes (i.e., sharing alleles of both the North American and the European lineages of *P. ramorum*). Second, the index of association statistics implemented in Multilocus version 1.3 (1) (http://www.agapow.net/software/multilocus) was used to test for linkage disequilibrium among loci. To remove the dependency of the statistics on the number of loci analyzed, instead of I_A we used the slightly modified measure r_d. In total, 1,000 randomizations were performed to compare the observed r_d values with that expected under completely random mating (46). Given the difficulty to detect significant linkage disequilibrium when all isolates analyzed belong to the same clonal lineage (15,57), for r_d calculation, representatives of genotypes PrNA2-1 (NA2 lineage) and PrEU1-1 (EU1 lineage) were added to the data sets of those populations in which these genotypes were not found.

Population differentiation was assessed using three different approaches. First, Cavalli-Sforza and Edwards (10) genetic distances between each multilocus genotype (considered as a sample of two genes) and the four populations were calculated and each genotype was then assigned to the genetically closest population (i.e., the population where a genotype should be based on allele frequencies: expected population) as implemented in Geneclass 2.0 (14). To avoid the bias introduced by counting a current genotype in its population (i.e., the population where it has been found: source population) when estimating allelic frequencies, assignment of genotypes was performed using the "leave one out" option (i.e., genotype to be assigned to a population not considered by the calculation of allele frequencies). Analyses were conducted only with clone-corrected data sets and excluding genotypes PrNA2-1 and PrEU1-1. Second, an UPGMA tree based on Nei's (51) genetic distance among populations was constructed using TFPGA. Support for internal branches was obtained using 1,000 bootstrapped samples of the data set. Multilocus genotypes PrNA2-1 and PrEU1-1 were not included in the original populations but were used as representative of the NA2 and EU1 lineages, respectively. Third, the presence of genetic structure was evaluated by performing an analysis of molecular variance (AMOVA) (18) with Arlequin version 3.1 (17). For this, two models were considered in which populations were divided into two groups. In the first model, populations were grouped according to their environmental origin (i.e., forest [OR-fo and CA] and nurseries [OR-nu and WA]). In the second model, the OR-fo population was assigned to a group and the OR-nu, CA, and WA populations were attributed to another group. In both models, genetic variation was partitioned among groups, among populations within groups, and within populations. Calculations were performed using clone-corrected data sets and multilocus genotypes PrNA2-1 and PrEU1-1 were excluded from the analyses. The significance of covariance components and fixation indices was tested with 1,000 permutations.

Gene flow among populations was estimated by performing a maximum-likelihood-based analysis. Migrate-N 2.4.2 was used to estimate Θ, M, and the amount and direction of gene flow between populations based on microsatellite data (4,5). Theta is defined as $4N_e\mu$ for a diploid system with nuclear microsatellite loci, where N_e is the effective population size and μ is the mutation rate per generation and site, whereas M is defined as m/μ, where m is the immigration rate. The number of migrants per generation was calculated as $N_e m = \Theta M/4$. The continuous Brownian motion model was used instead of the discrete stepwise mutation model to allow for faster parameter estimation for large samples as specified in the Migrate-N user manual. Because lineages NA1, NA2, and EU1 are reproductively isolated and effectively evolving as separate populations (37,57), Migrate-N analysis was restricted to only individuals belonging to the NA1 clonal lineage to satisfy the assumptions of a stepwise mutation or Brownian mutation model. Resulting populations included CA-fo, OR-fo, OR-nu, and WA isolates. Data was analyzed with Migrate-N by using 10 short chains with 5,000 sampled genealogies and 3 long chains with 50,000 sampled genealogies. Heating was active with four temperatures (1.0, 1.2, 1.5, and 3.0). The process was repeated five times. Maximum-likelihood estimates for Θ and M for four independent runs were conducted and are presented as mean and range of four runs.

RESULTS

AFLP analyses. AFLP analysis was conducted to assess whether clonal lineages established here correspond to those characterized previously by Ivors et al. (37,38). Of 108 loci scored, 45 were polymorphic and selected for analysis. AFLP analysis resulted in the same three clades characterized previously by Ivors et al. (37,38) and showed similar placements as microsatellite data (Fig. 1). All clades had strong statistical bootstrap support (>78%). All further analysis shown below is based solely on microsatellite data.

Genotypic diversity. In total, 72 multilocus microsatellite genotypes were identified in the *P. ramorum* population (Appendix 1). The most frequent genotype (PrNA1-56) included 34.5% of the isolates screened and was only found in the OR-fo population (66.2% of the isolates). Thirty-five genotypes (48.6%) were represented by a single isolate (i.e., frequency of 0.2%). The

number of genotypes detected in the individual populations ranged from 13 (OR-nu) to 30 (OR-fo and WA) (Fig. 2). Twenty genotypes (27.8%) were present in more than one population. The WA and OR-nu populations showed a higher incidence of genotypes shared among populations (19 of 30 and 9 of 13, respectively) than the CA (13 of 29) and OR-fo (7 of 30) populations (Fig. 2). The highest number of shared genotypes was observed between the two furthest populations (CA and WA: minimum 590 km, 13 genotypes shared) (Table 2). In both populations, PrNA1-16 was the most frequent genotype (32.5 and 19.8% of the isolates, respectively). In contrast, the two Oregon populations had only two genotypes in common (PrNA1-27 and PrNA1-40). Based on Spearman's correlation test, the number of genotypes shared was not correlated with the minimal distance between pairs of populations ($t = 0.8$, $P = 0.468$). The number of genotypes expected in a sample of $N = 54$ ranged from 5.4 (OR-fo) to 19.6 (WA) (Table 3). Genotypic diversity quantified with the Stoddart and Taylor's index G varied strongly among populations. Based on 2,000 bootstrapped resamples, differences in G values were significant between the OR-fo and the other three populations, as well as between the WA and the other three populations. The values of the evenness index E_5 ranged from 0.4 (OR-fo and CA) to 0.7 (OR-nu) and differences were only significant between the OR-nu and the other three populations (Table 3).

Gene diversity. Only loci PrMS39b, PrMS43a, PrMS43b, and PrMS45 were variable among the multilocus genotypes PrNA1-1 to PrNA1-70 (Appendix 1), with a maximal number of mutational steps ranging from 10 (PrMS45) to 22 (PrMS39b). The split network based on the Cavalli-Sforza and Edwards (10) distance showed that multilocus genotypes PrNA1-1 to PrNA1-70 grouped together and were clearly separated from genotypes PrNA2-1 and PrEU1-1 (Fig. 3). Within the PrNA1-1 to PrNA1-70 clade, multilocus genotypes unique to the OR-fo population were not intermingled with genotypes unique to the CA or WA populations. The four genotypes unique to the OR-nu population were dispersed in both groups (data not shown). Multilocus genotype PrNA2-1 (lineage NA2), which was recovered from infected plants in nurseries in CA and WA at four loci (PrMS6, PrMS9, PrMS13, and Pr9C3) shared alleles with genotypes PrNA1-1 to PrNA1-70. However, this genotype at three loci had different alleles (PrMS27,

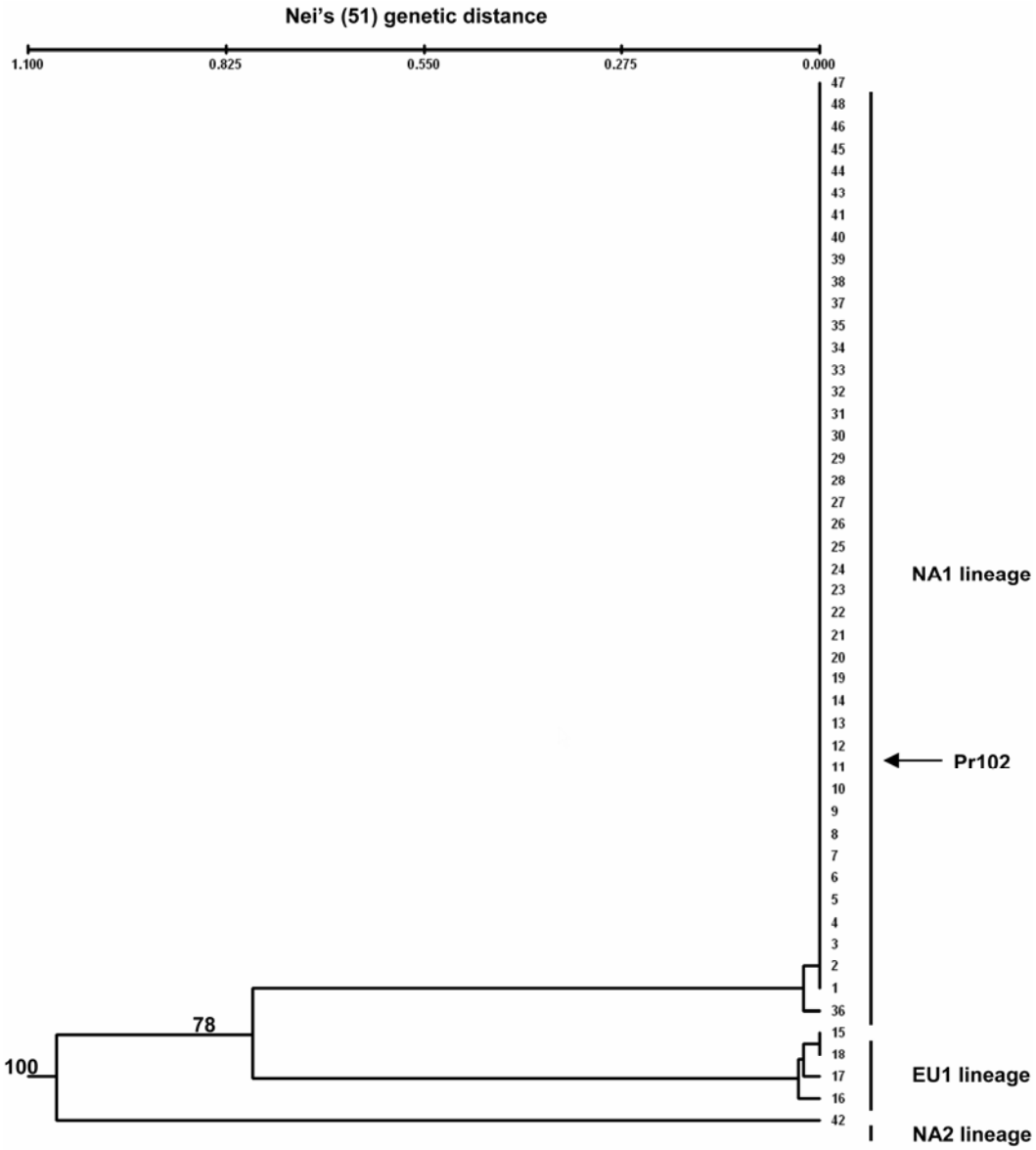

Fig. 1. Unweighted pair-group method of averages cluster of the 45 North American isolates (each representing a different microsatellite multilocus genotype) and three additional European isolates of *Phytophthora ramorum* based on Nei's (51) genetic distance calculated for amplified fragment length polymorphism allele frequencies. Statistical support for branches (indicated at nodes with support >70%) was obtained using 1,000 bootstrapped samples of the data set. The isolate Pr102 was used for sequencing the genome of *P. ramorum* (67).

PrMS39b, and PrMS43a) and failed to amplify at the loci PrMS21, PrMS43b, and PrMS45 (Appendix 1). By contrast, multilocus genotype PrEU1-1 had characteristic allele patterns at all loci except at locus PrMS43b, where no amplicon was produced (Appendix 1). This genotype was found in the OR-nu and WA populations and belonged to the EU1 lineage of *P. ramorum* (57).

Excluding multilocus genotypes PrNA2-1 and PrEU1-1 from the calculations, only small variations in allelic richness were observed among populations (range: 2.3 to 2.6 non-clone-corrected, 2.3 to 2.5 clone-corrected) (Table 3). In all four populations as well as in the overall population, the observed heterozygosity was significantly higher than the expected heterozygosity, which indicates significant deviations from Hardy-Weinberg expectations. The OR-fo population was characterized by the highest heterozygosity excess (F_{IS} = –0.57) and the OR-nu population showed the smallest difference between observed and expected heterozygosity (F_{IS} = –0.18).

Reproductive mode. None of the multilocus genotypes detected shared alleles of both the North American (A2 mating type) and the European (A1 mating type) lineage of *P. ramorum* (Table 3). High levels of linkage disequilibrium among loci were indicated with both non-clone-corrected and clone-corrected data sets. In all individual *P. ramorum* populations, as well as in the overall population, the observed r_d value was significantly higher than the r_d value calculated from 1,000 artificially recombined data sets.

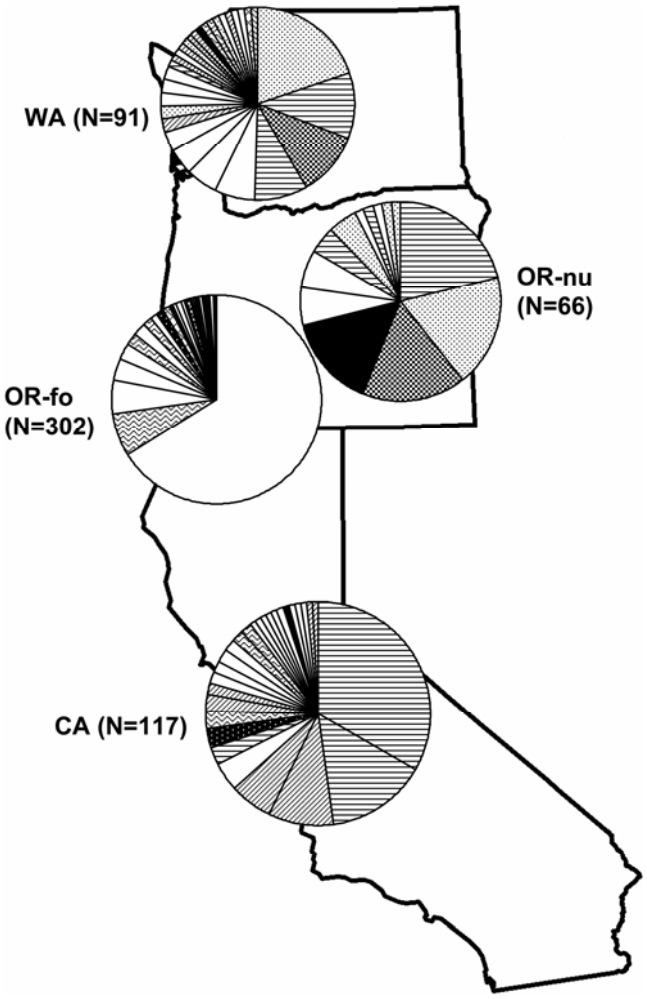

Fig. 2. Incidence of *Phytophthora ramorum* multilocus genotypes in the Oregon forest (OR-fo), Oregon nursery (OR-nu), California (CA), and Washington (WA) populations. White sectors represent genotypes unique to the specific population. Sectors with the same filling pattern indicate genotypes shared among specific populations.

Population differentiation. In the assignment tests, only 39 multilocus genotypes (40.2%) were assigned to the source population (Table 4). Assignments to a nonsource population were particularly frequent in the WA and OR-nu populations (27 of 28 genotypes and all 12 genotypes, respectively). Only in the OR-fo population for the majority of the genotypes (86.7%) did the expected population correspond to the source population. Accordingly, the UPGMA tree based on Nei's (51) genetic distance showed that the CA, OR-nu, and WA populations grouped in the same cluster and were genetically very close (0.03 Nei's genetic distance) (Fig. 4). The OR-fo population was clearly differentiated from these three populations with a node appearing in 99% of the bootstrapped samples. Multilocus genotype PrNA2-1 clustered with the four North American populations analyzed but the genetic distance from them was large (0.82 Nei's [51] genetic distance). By contrast, multilocus genotype PrEU1-1 was completely separated from all North American genotypes. A comparative tree derived from clone-corrected data sets showed qualitatively similar results (tree not shown).

In both models of AMOVA structure considered (forest versus nursery and OR-fo versus OR-nu, CA, and WA), most (>90%) of the total genetic variance resided within populations (Table 5). When comparing forest and nursery populations, a significant proportion of the variance (5.8%) was also detected among populations within groups, whereas the genetic variation residing between groups was not significantly different from zero.

Gene flow. Maximum-likelihood-based analysis using Migrate-N revealed that all populations are well connected in both directions, with the exception of the OR-nu and OR-fo populations, where drift is more significant than migration in some Migrate-N runs although population sizes are roughly equivalent (Table 6). Given that most values of M (= m/μ) were >1, overall, the effect of migration (m) is slightly larger than the effect of mutation (μ). In all populations, the effective number of migrants per generation $N_e m$ (= $\Theta M/4$) was <0.61. This suggests that, even though migration brings additional genetic variability into populations, local populations will continue diverging due to the combined effects of mutation and genetic drift.

DISCUSSION

The levels of genotypic and gene diversity of the overall *P. ramorum* population on the West Coast of the United States roughly correspond to those previously reported for California and Oregon (45,57). Comparable studies on other forest species of *Phytophthora* are rare, because most genetic investigations have dealt with species causing significant economic losses in agricultural crops, such as *P. infestans* (13,29), *P. sojae* (19), and *P. capsici* (6,42). Using restriction fragment length polymorphism markers, Linde et al. (43) showed that, in Australia and South Africa, the population of *P. cinnamomi*, an exotic pathogen of *Eucalyptus* spp., is characterized by low genetic diversity. In Europe, a low level of AFLP diversity was observed in the population of the oak-specific fine root pathogen *P. quercina* (11). The results of the present study support the conclusion of Ivors et al. (38) that the current genetic structure of *P. ramorum* probably reflects a genetic bottle-

TABLE 2. Minimal distances (km, above the diagonal) and number of shared multilocus genotypes (below the diagonal) between pairs of *Phytophthora ramorum* populations[z]

Population	OR-fo	OR-nu	CA	WA
OR-fo	⋯	90	230	440
OR-nu	2	…	250	20
CA	5	4	…	590
WA	6	9	13	…

[z] OR-fo, OR-nu, CA, and WA = populations from Oregon forest, Oregon nurseries, California, and Washington nurseries, respectively.

neck which occurred when the pathogen was introduced as clones belonging to lineages NA1, NA2, and EU1 into North America. Despite the relatively high number of genotypes detected in lineage NA1, all were genetically very close; only four microsatellite loci showed differences among them and a split network analysis indicated that they all cluster together. These NA1 genotypes are likely the descendants of a single *P. ramorum* individual (25) or of few very closely related individuals. The other two genotypes identified (PrNA2-1 and PrEU1-1) had different allele patterns. Genotype PrEU1-1, which was recovered in North America for the first time in 2003 in a nursery in Oregon, belongs to the European clonal lineage and is of A1 mating type (35). In contrast, genotype PrNA2-1 is similarly of A2 mating type yet is genetically differentiated from all other North American genotypes. This genotype belongs to the NA2 lineage described by Ivors et al. (37) as clade 3 and suggests that total genetic diversity within the A2 mating type of *P. ramorum* is higher than the diversity so far observed within the West Coast population of North America.

Several lines of evidence suggest that sexual recombination is currently not occurring in the *P. ramorum* population on the West Coast of the United States, as previously observed in the California and Oregon populations (37,38,57). First, despite the presence of isolates of both mating types, no recombinant genotypes were observed. Recently, we documented the coexistence of A1 and A2 mating type individuals in the same retail nursery in Northern California and established that sexual recombination had not occurred (31). Second, all populations analyzed deviated significantly from Hardy-Weinberg equilibrium in form of excess heterozygosity. Negative fixation indices were previously reported for clonal populations of *P. infestans* (66), *P. cinnamomi* (43), and *P. ramorum* in Oregon (57). Third, the modified index of association value (r_d) observed in the four geographic populations and in the overall population was significantly higher than the value calculated for artificially recombined data sets, indicating high within-population levels of linkage disequilibrium. Fourth, nuclear sequence analysis provided further evidence for ancient divergence of the three clonal lineages, indicating reproductive isolation since before the emergence of modern agriculture (25). Yet, the same work also established that the ancestors of the extant *P. ramorum* lineages were members of a sexually reproducing population. If sexual reproduction is still possible, the absence of sexual reproduction in contemporary populations could be due to the strong disproportion in frequencies of occurrence of the two mating types. In addition, in nursery environments where both mating types have been occasionally observed, infected plant material is rapidly destroyed, which further reduces opportunities for sexual reproduction. The coexistence of both mating types in the same population without sexual reproduction was previously reported for other *Phytophthora* spp.; for example, for *P. cinnamomi* in Australia (15) and for *P. infestans* in Ecuador (52) and the United States (24,49). According to Goodwin (23), this situation may be observed when the two mating types are genetically very different, when they have different host preferences, or when the migration bringing them together has occurred too recently for detecting sexual recombinants. In *P. ramorum*, the difficulty of producing viable oospores by crossing tester strains under laboratory conditions (8) may suggest that the mating system is not functional, possibly because of long isolation of the two mating types (20,25).

The genetic differentiation of the overall *P. ramorum* population on the West Coast of the United States is low, with most genetic variation residing within geographic populations. The UPGMA phylogram showed that genetic distances among OR-nu, CA, and WA populations are very small. The recent study of Mascheretti et al. (45) on the genetic structure of *P. ramorum* in California evidenced extensive genetic variability not only within but also among populations, suggestive of a limited natural long-distance spread of the pathogen. Our findings are not necessarily in conflict with this study. Because we undertook a large-scale, regional analysis, our samples were chosen to represent the entire *P. ramorum* population on the West Coast of the United States (comprising different sampling years and samples from numerous locations) and not for a detailed within-state analysis. In California, a lack of structure was only observed among some particular populations (nurseries and forests) and was explained by a combination of natural spread (escapes from nurseries) and human-induced transport of *P. ramorum*, which ensured gene flow among some sites (45). Widespread gene flow among geographic populations may also account for the lack of differentiation observed in our study. Given fixation of alleles within lineages, it might be more appropriate to refer to genotype flow rather than gene flow (47). An indirect evidence of frequent exchanges of genotypes among geographic populations is the fact that most populations were composed of genotypes which, based on allele frequencies, should rather belong to another population. Several factors suggest that human activity and not the spontaneous spread of *P. ramorum* is responsible for the significant genotype flow observed. First, the large geographic distances (minimum of 20 to 590 km) among populations indicate that there is a mechanism of long-distance dispersal. In the forest, most new infections arise within 10 to 500 m of previously infected trees and long-distance

TABLE 3. Indices of genetic diversity and reproduction mode in the four populations (i.e., OR-fo, OR-nu, CA, and WA) and in the overall population of *Phytophthora ramorum*

		Genotypic diversity[p]				Gene diversity[p,q]			Reproduction mode[q]	
Population[n]	N[o]	MG_{Obs}[r]	MG_{Exp}[s]	G[t]	E_5[u]	A_R[v]	H_E[w]	F_{IS}[x]	Rec. MG[y]	r_d[z]
OR-fo	302	30	5.4	2.2 b (1.4–3.0)	0.4 a (0.2–0.5)	2.4 (2.5)	0.32 (0.42)	–0.57* (–0.20)	No	0.10* (0.09*)
OR-nu	54 (+12)	12 (+1)	11.8	6.7 a (5.3–8.1)	0.7 b (0.6–0.8)	2.3 (2.3)	0.42 (0.46)	–0.18* (–0.09)	No	0.44* (0.28*)
CA	114 (+3)	27 (+1)	18.2	6.6 a (4.3–9.0)	0.4 a (0.2–0.5)	2.6 (2.5)	0.38 (0.45)	–0.32* (–0.15)	No	0.13* (0.16*)
WA	87 (+4)	28 (+2)	19.6	12.4 c (9.6–15.3)	0.5 a (0.4–0.6)	2.5 (2.4)	0.42 (0.45)	–0.19* (–0.14)	No	0.15* (0.13*)
Total	557 (+19)	70 (+2)	20.2	6.7 (3.9–9.6)	0.1 (0.03–0.3)	2.7 (2.6)	0.41 (0.46)	–0.23* (–0.11)	No	0.03* (0.07*)

[n] OR-fo, OR-nu, CA, and WA = populations from Oregon forest, Oregon nurseries, California, and Washington nurseries, respectively.
[o] N = sample size. Values in parentheses consist of isolates belonging to the multilocus genotypes PrNA2-1 and PrEU1-1.
[p] Multilocus genotypes PrNA2-1 (CA and WA) and PrEU1-1 (OR-nu and WA) were excluded from the calculation of the indices of genetic diversity.
[q] Values of A_R, H_E, F_{IS}, and r_d in parentheses were calculated using clone-corrected data sets.
[r] Number of multilocus genotypes (MGs) observed. Additional genotypes found which belong to the EU1 and NA2 lineages: OR-nu (+1) = PrEU1-1; CA (+1) = PrNA2-1; WA (+2) = PrNA2-1 and PrEU1-1.
[s] Number of MGs expected in a population of $N = 54$.
[t] Stoddart and Taylor's index (90% confidence interval).
[u] Genotypic evenness index (90% confidence interval). Values followed by different letters differ significantly based on 2,000 bootstrapped resamples.
[v] Allelic richness averaged over loci, based on a sample size of $N = 49$ individuals (non-clone-corrected data sets) and $N = 10$ individuals (clone-corrected data sets).
[w] Expected heterozygosity.
[x] Fixation index (i.e., inbreeding coefficient). Statistically significant deviations from Hardy-Weinberg equilibrium are indicated by * ($P < 0.001$).
[y] Recombinant genotypes (based on allele pattern).
[z] Index of linkage disequilibrium among loci. Statistically significant linkage disequilibrium among loci is indicated by * ($P < 0.001$).

spread (5 to 10 km) is rarely observed (34,45,60) whereas, in nurseries, new infections generally occur <1 m from previously infected plants (65). Second, the absence of a significant correlation between the number of shared genotypes and geographic distance between populations also indicates the presence of a long-distance spread mechanism. The highest number of shared genotypes was observed between the two furthest populations (i.e., California and Washington). Third, the numerous trace-back and -forward surveys reveal the movement of infected plants among nursery settings (55,64). The first trace-backs in 2003 indicated that infected plant material was shipped from California nurseries to Oregon and Washington. Therefore, the nursery population of *P. ramorum* in California may be the original source from which originated most genotypes found in Oregon and Washington nurseries. This hypothesis is supported by the fact that the two most common genotypes in California (PrNA1-16 and PrNA1-19) are also frequent in the two nursery populations. Later, movement of *P. ramorum*-infected plants most likely occurred also among Oregon and Washington nurseries because these two populations (i) are genetically very similar and, (ii) according to a maximum-likelihood-based gene flow analysis, they are bidirectionally well connected, which suggests important genetic exchanges in both directions. Finally, new genotypes probably also evolved locally from the founder genotype or genotypes because unique genotypes were identified in both nursery populations. The results concerning the California population presented in this study were obtained with isolates coming from infested forests and not from nurseries. Consequently, because most isolates analyzed were recovered before 2005, in California until 2004 the same genotypes may have been present both in the infested forest and in the nurseries, as previously reported by Garbelotto et al. (21). To estimate the genotypic diversity of *P. ramorum* in California more accurately, a larger number of isolates should be considered, given the huge distributional range of the pathogen in this state compared with the infested forest area in southwestern Oregon.

Based on our findings, the forest population in Oregon did not contribute to the other *P. ramorum* epidemics on the West Coast of the United States. This population was dominated by a genotype (PrNA1-56) not found elsewhere and presented the highest incidence of unique genotypes (i.e., nonshared with other populations). The strong predominance of a single genotype may partially be due to the sampling design and to the strategy applied to control the disease. In contrast to California, in the infested

TABLE 4. Results of assignment tests for *Phytophthora ramorum* genotypes using a distance-based method (Cavalli-Sforza and Edwards [10] distance, "leave one out" option)[w]

	Source population[y]			
Expected population[x]	OR-fo (N = 30)	OR-nu (N = 12)	CA (N = 27)	WA (N = 28)
OR-fo	**26**	3	5	10
OR-nu	1	**0**	7	6
CA	2	7	**12**	11
WA	1	2	3	**1**
MG (%)[z]	86.7	0	44.4	3.3

[w] Analyses were conducted with clone-corrected data sets and by excluding multilocus genotypes (MGs) PrNA2-1 (California [CA] and Washington [WA]) and PrEU1-1 (Oregon nurseries [OR-nu] and WA).

[x] Geographic population where a genotype should be based on allele frequencies (i.e., genetically closest population).

[y] Geographic population where a specific genotype has been found; N = number of genotypes in each population.

[z] Percentage of MGs for which the expected population corresponds to the source population.

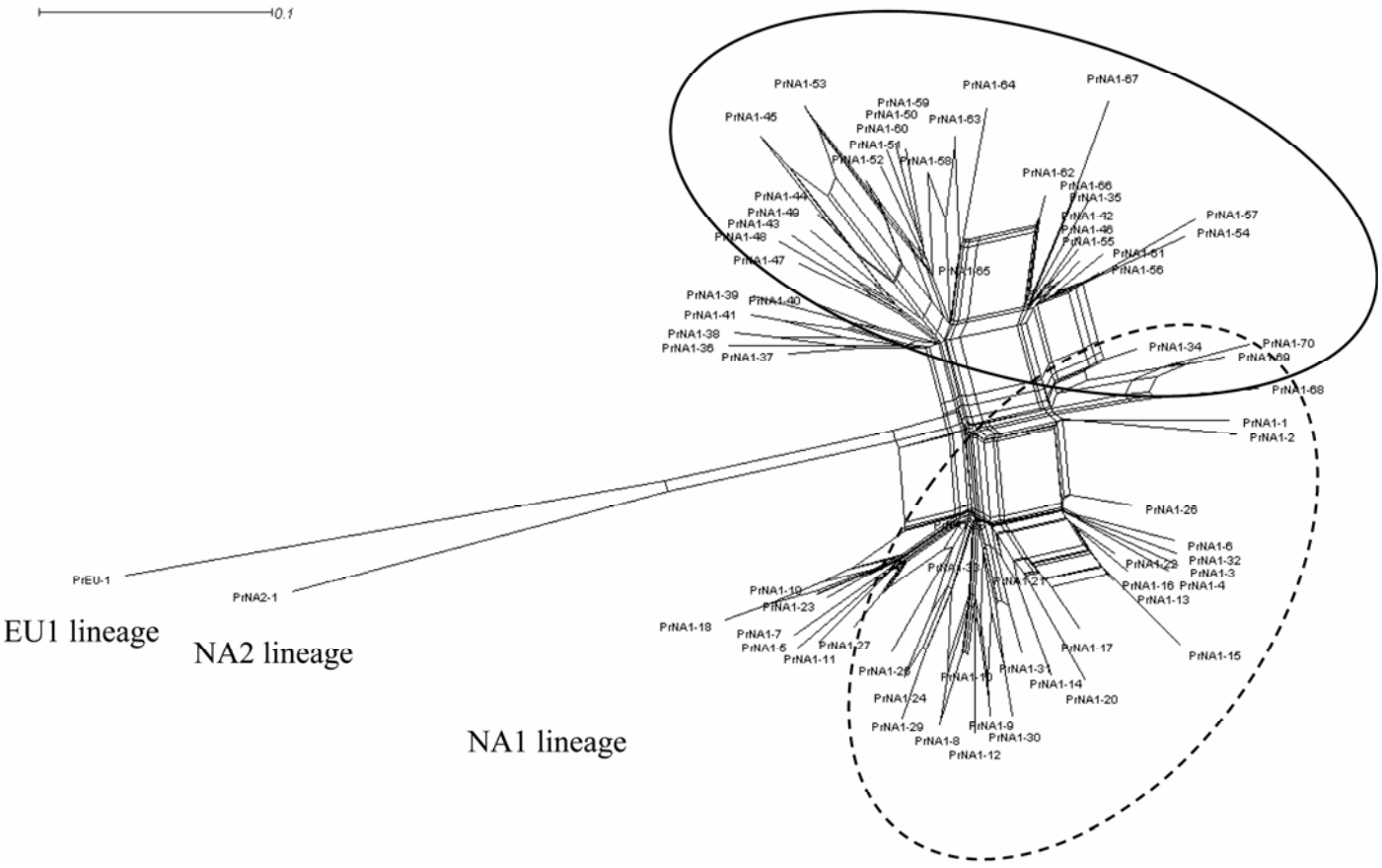

Fig. 3. Split network based on Cavalli-Sforza and Edwards (10) genetic distance of the 72 multilocus genotypes detected in the *Phytophthora ramorum* population on the West Coast of the United States. The solid line encompasses the approximate position of genotypes unique to the Oregon forest population, whereas the broken line encompasses the approximate position of genotypes unique to the California or Washington populations.

forest in southwestern Oregon, a very intensive sampling has been conducted in a relatively small area (≈50 km^2). In addition, the chances of build-up and spread of inoculum of rare genotypes might be severely reduced by the rapid elimination of infected plants. Eradication efforts probably disadvantage more rare genotypes than frequent ones. The dominant genotype, which may be the founder of the forest population in Curry County (57), is present at very low frequency in California (single isolate at China Camp State Park, Marin County) (45), from where it could have been accidentally introduced into southwestern Oregon. The unique genotypes that we found in the infested forest in Curry County may have evolved locally from PrNA1-56 through mutation or mitotic recombination (57). The founder genotype and most unique genotypes at the loci PrMS39b and PrMS43a have alleles which are rare in the other three populations (length of 250 and 377 bp, respectively); therefore, the OR-fo population appears to be slightly different from the other West Coast populations, and most genotypes observed in this population were assigned to this population based on allele frequencies. The 250- and 377-bp alleles, however, are only one mutational step away from the dominant alleles in the other populations (length of 246 and 373 bp). For this reason, analyses using Migrate-N indicate genetic exchanges between the OR-fo population and the other populations, which could seem in conflict with the other findings. The dispersal over long distances from local Curry County genotypes may have been prevented by the ongoing eradication efforts and the absence of commercial shipment, as well as a certain geographic isolation of the infested forest.

In conclusion, our study suggests that human activity can strongly influence the spread and the population structure of an emerging plant pathogen. Accepting the hypothesis of a recent introduction of *P. ramorum* into the United States, genetic analyses indicate that large-scale, regional range expansion within the West Coast resulted mostly from the movement over long distances of infected plants, as previously suggested by trace-back and -forward surveys. Consequently, the current *P. ramorum* population is basically genetically homogeneous from California to Washington over a 600-km range. In the future, regular population analyses will be necessary to assess the efficacy of quarantine regulations at the local (e.g., nurseries) and regional (e.g., between states) scale and to reveal significant changes (e.g., presence of recombinant genotypes, changes in the frequencies of the clonal lineages NA2 and EU1, or appearance of new clonal lineages) in the single *P. ramorum* populations and in the overall population. In addition, such studies would provide basic information on the biology of *P. ramorum*; such as, for example, the rate at which new alleles and consequently new genotypes emerge within a clonal lineage.

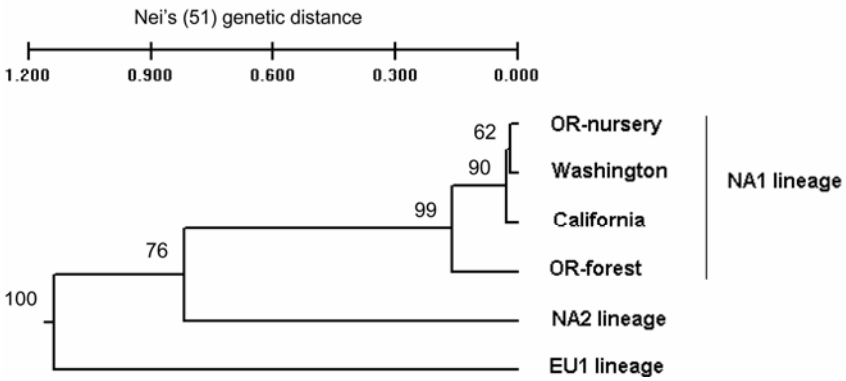

Fig. 4. Unweighted pair-group method of averages tree of the analyzed populations of *Phytophthora ramorum* based on Nei's (51) genetic distance calculated by considering all isolates sampled. Statistical support for branches (indicated at each node) was obtained using 1,000 bootstrapped samples of the data set.

TABLE 5. Results of analyses of molecular variance (AMOVA) with 10 microsatellite loci of *Phytophthora ramorum* from four populations (OR-fo, OR-nu, CA, and WA)[y]

Model of structure[z]	Source of variation	Percentage of variation	Fixation indices	*P* value
Forest (OR-fo, CA) vs. nursery (OR-nu, WA)	Among groups	–1.5	–0.014	ns
	Among populations within groups	5.8	0.06	<0.001
	Within populations	95.7	0.04	<0.001
OR-fo vs. OR-nu, CA, and WA	Among groups	8.7	0.09	ns
	Among populations within groups	–0.5	–0.006	ns
	Within populations	91.8	0.08	<0.001

[y] OR-fo, OR-nu, CA, and WA = populations from Oregon forest, Oregon nurseries, California forest, and Washington, respectively; ns = not significant ($P > 0.05$).
[z] Analyses were conducted by using clone-corrected data sets. Multilocus genotypes PrNA2-1 (CA and WA) and PrEU1-1 (OR-nu and WA) were not included in the calculations.

TABLE 6. Gene flow between four populations of *Phytophthora ramorum* (CA, OR-fo, OR-nu, and WA)[z]

		M estimates for sink populations			
Source population	Θ	CA	OR-fo	OR-nu	WA
CA	0.98 (0.94–1.02)	…	1.19 (0.5–2.0)	1.24 (0.8–1.5)	1.40 (0.8–2.8)
OR-fo	1.03 (0.98–1.07)	2.50 (2.1–3.0)	…	0.84 (0.4–1.2)	1.29 (0.6–1.6)
OR-nu	0.98 (0.90–1.04)	1.523 (1.1–2.3)	0.58 (0.4–0.9)	…	1.13 (0.5–1.5)
WA	0.98 (0.96–1.01)	1.27 (0.5–2.3)	1.20 (0.7–1.4)	1.31 (0.7–1.8)	…

[z] CA, OR-fo, OR-nu, and WA = populations from California forest, Oregon forest, Oregon nurseries, and Washington, respectively. The mean maximum likelihood estimate and corresponding range observed in four independent Migrate-N simulations for $\Theta = 4N_e$, where $N_e\mu$ is the effective population size and μ is the mutation rate per generation and per site, and for *M* (scaled number of migrants m/μ), are given. The effective number of migrants ($N_e m$) can be calculated as $\Theta M/4$. Source (donor) populations are shown in rows and sink (recipient) populations in columns. Only isolates belonging to the NA1 clonal lineage were included in the analysis.

Appendix 1. Allele patterns (sizes in bp) of the 72 multilocus genotypes of *Phytophthora ramorum* identified on the West Coast of the United States (Oregon, California, and Washington)

Genotype	Prospero et al. (57)[x]	MS13	MS9	MS6	Pr9C3	MS21	MS27	MS39b	MS43a	MS43b	MS45	Lineage[y]
PrNA1-1		172/187	149/176	165/168	216/226	247/247	213/213	210/210	377/377	486/486	167/187	NA1
PrNA1-2	PrOR32	172/187	149/176	165/168	216/226	247/247	213/213	242/242	373/373	486/486	167/187	NA1
PrNA1-3		172/187	149/176	165/168	216/226	247/247	213/213	246/246	345/345	486/486	167/187	NA1
PrNA1-4		172/187	149/176	165/168	216/226	247/247	213/213	246/246	357/357	486/486	167/187	NA1
PrNA1-5	PrOR31	172/187	149/176	165/168	216/226	247/247	213/213	246/246	357/357	490/490	167/187	NA1
PrNA1-6		172/187	149/176	165/168	216/226	247/247	213/213	246/246	365/365	486/486	167/187	NA1
PrNA1-7		172/187	149/176	165/168	216/226	247/247	213/213	246/246	365/365	490/490	167/187	NA1
PrNA1-8		172/187	149/176	165/168	216/226	247/247	213/213	246/246	369/369	478/478	167/187	NA1
PrNA1-9		172/187	149/176	165/168	216/226	247/247	213/213	246/246	369/369	482/482	167/187	NA1
PrNA1-10		172/187	149/176	165/168	216/226	247/247	213/213	246/246	369/369	486/486	167/187	NA1
PrNA1-11	PrOR30	172/187	149/176	165/168	216/226	247/247	213/213	246/246	369/369	490/490	167/187	NA1
PrNA1-12		172/187	149/176	165/168	216/226	247/247	213/213	246/246	369/369	494/494	167/187	NA1
PrNA1-13		172/187	149/176	165/168	216/226	247/247	213/213	246/246	369/373	486/486	167/187	NA1
PrNA1-14		172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/373	482/482	167/187	NA1
PrNA1-15		172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/373	486/486	167/167	NA1
PrNA1-16	PrOR20	172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/373	486/486	167/187	NA1
PrNA1-17		172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/373	486/490	167/187	NA1
PrNA1-18		172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/373	490/490	167/167	NA1
PrNA1-19	PrOR29	172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/373	490/490	167/187	NA1
PrNA1-20		172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/373	494/494	167/187	NA1
PrNA1-21	PrOR22	172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/373	–/–[z]	167/187	NA1
PrNA1-22	PrOR24	172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/377	486/486	167/187	NA1
PrNA1-23		172/187	149/176	165/168	216/226	247/247	213/213	246/246	373/377	490/490	167/187	NA1
PrNA1-24		172/187	149/176	165/168	216/226	247/247	213/213	246/246	377/377	478/478	167/187	NA1
PrNA1-25		172/187	149/176	165/168	216/226	247/247	213/213	246/246	377/377	482/482	167/187	NA1
PrNA1-26		172/187	149/176	165/168	216/226	247/247	213/213	246/246	377/377	486/486	167/187	NA1
PrNA1-27	PrOR19	172/187	149/176	165/168	216/226	247/247	213/213	246/246	377/377	490/490	167/187	NA1
PrNA1-28	PrOR21	172/187	149/176	165/168	216/226	247/247	213/213	246/246	377/377	–/–	167/187	NA1
PrNA1-29		172/187	149/176	165/168	216/226	247/247	213/213	246/246	381/381	478/478	167/187	NA1
PrNA1-30		172/187	149/176	165/168	216/226	247/247	213/213	246/246	381/381	482/482	167/187	NA1
PrNA1-31		172/187	149/176	165/168	216/226	247/247	213/213	246/246	381/381	486/486	167/187	NA1
PrNA1-32		172/187	149/176	165/168	216/226	247/247	213/213	246/246	385/385	486/486	167/187	NA1
PrNA1-33		172/187	149/176	165/168	216/226	247/247	213/213	246/246	–/–	478/478	167/187	NA1
PrNA1-34		172/187	149/176	165/168	216/226	247/247	213/213	246/250	377/377	486/486	167/187	NA1
PrNA1-35	PrOR16	172/187	149/176	165/168	216/226	247/247	213/213	250/250	357/357	486/486	167/187	NA1
PrNA1-36		172/187	149/176	165/168	216/226	247/247	213/213	250/250	365/365	482/482	167/187	NA1
PrNA1-37		172/187	149/176	165/168	216/226	247/247	213/213	250/250	365/365	486/486	167/187	NA1
PrNA1-38		172/187	149/176	165/168	216/226	247/247	213/213	250/250	365/365	490/490	167/187	NA1
PrNA1-39		172/187	149/176	165/168	216/226	247/247	213/213	250/250	369/369	482/482	167/187	NA1
PrNA1-40	PrOR25	172/187	149/176	165/168	216/226	247/247	213/213	250/250	369/369	486/486	167/187	NA1
PrNA1-41		172/187	149/176	165/168	216/226	247/247	213/213	250/250	369/369	490/490	167/187	NA1
PrNA1-42		172/187	149/176	165/168	216/226	247/247	213/213	250/250	369/373	486/486	167/187	NA1
PrNA1-43	PrOR26	172/187	149/176	165/168	216/226	247/247	213/213	250/250	373/373	478/478	167/187	NA1
PrNA1-44	PrOR13	172/187	149/176	165/168	216/226	247/247	213/213	250/250	373/373	482/482	167/187	NA1
PrNA1-45	PrOR27	172/187	149/176	165/168	216/226	247/247	213/213	250/250	373/373	482/482	167/167	NA1
PrNA1-46	PrOR4	172/187	149/176	165/168	216/226	247/247	213/213	250/250	373/373	486/486	167/187	NA1
PrNA1-47	PrOR14	172/187	149/176	165/168	216/226	247/247	213/213	250/250	373/373	490/490	167/187	NA1
PrNA1-48		172/187	149/176	165/168	216/226	247/247	213/213	250/250	373/373	494/494	167/187	NA1
PrNA1-49		172/187	149/176	165/168	216/226	247/247	213/213	250/250	373/377	478/482	167/187	NA1
PrNA1-50	PrOR12	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	466/466	167/187	NA1
PrNA1-51	PrOR5	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	478/478	167/187	NA1
PrNA1-52	PrOR6	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	482/482	167/187	NA1
PrNA1-53	PrOR28	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	482/482	167/167	NA1
PrNA1-54	PrOR2	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	486/486	167/167	NA1
PrNA1-55		172/187	149/176	165/168	216/226	247/247	213/213	250/250	373/377	486/486	167/187	NA1
PrNA1-56	PrOR1	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	486/486	167/187	NA1
PrNA1-57		172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	486/486	187/187	NA1
PrNA1-58	PrOR3	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	490/490	167/187	NA1
PrNA1-59	PrOR7	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	494/494	167/187	NA1
PrNA1-60	PrOR11	172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/377	506/506	167/187	NA1
PrNA1-61		172/187	149/176	165/168	216/226	247/247	213/213	250/250	377/381	486/486	167/187	NA1
PrNA1-62	PrOR8	172/187	149/176	165/168	216/226	247/247	213/213	250/250	381/381	486/486	167/187	NA1
PrNA1-63	PrOR9	172/187	149/176	165/168	216/226	247/247	213/213	250/250	381/381	490/490	167/187	NA1
PrNA1-64	PrOR17	172/187	149/176	165/168	216/226	247/247	213/213	250/250	381/381	506/506	167/187	NA1
PrNA1-65	PrOR15	172/187	149/176	165/168	216/226	247/247	213/213	250/250	381/381	–/–	167/187	NA1
PrNA1-66	PrOR18	172/187	149/176	165/168	216/226	247/247	213/213	250/250	385/385	486/486	167/187	NA1
PrNA1-67		172/187	149/176	165/168	216/226	247/247	213/213	250/250	385/385	486/486	167/167	NA1
PrNA1-68		172/187	149/176	165/168	216/226	247/247	213/213	254/254	369/369	486/486	167/187	NA1
PrNA1-69	PrOR10	172/187	149/176	165/168	216/226	247/247	213/213	254/254	377/377	486/486	167/187	NA1
PrNA1-70		172/187	149/176	165/168	216/226	247/247	213/213	254/254	377/377	486/490	167/187	NA1
PrNA2-1		172/187	176/176	165/168	216/216	–/–	210/216	145/151	171/171	–/–	–/–	NA2
PrEU1-1	PrOR33	187/187	149/170	165/165	218/224	247/256	216/216	136/140	146/146	–/–	163/187	EU1

[x] Name adopted by Prospero et al. (57).
[y] *Phytophthora ramorum* lineages according to Grünwald et al. (38).
[z] No PCR amplicons were produced.

ACKNOWLEDGMENTS

This work was in part funded by grants from the United States Forest Service and USDA-ARS. We thank N. Osterbauer, A. Wagner, J. Falacy, D. Rizzo, and M. Garbelotto for providing *P. ramorum* isolates for this study; W. Sutton and M. Larsen for expert technical assistance in the laboratory; A. Krupkin for help with interpretation of microsatellite data; and P. Beerli for useful comments on Migrate-N analysis and interpretation.

LITERATURE CITED

1. Agapow, P.-M., and Burt, A. 2001. Indices of multilocus linkage disequilibrium. Mol. Ecol. Notes 1:101-102.
2. Alexander J. 2006. Review of *Phytophthora ramorum* in European and North American nurseries. Pages 37-39 in: Proc. Sudden Oak Death Second Sci. Symp.: The State of Our Knowledge. S. J. Frankel, P. J. Shea, and M. I. Haverty, tech. coords. Gen. Tech. Rep. GTR-PSW-196, U.S. Dep. Agric. For. Serv. Pac. Southwest Res. Stn. Albany, CA.
3. Barrett, T. M., Gatziolis, D., Fried, J. S., and Waddell, K. L. 2006. Sudden oak death in California: What is the potential? J. For. 104:61-64.
4. Beerli, P. 2007. Estimation of the population scaled mutation rate from microsatellite data. Genetics 177:1967-1968.
5. Beerli, P., and Felsenstein, J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. USA 98:4563-4568.
6. Bowers, J. H., Martin, F. N., Tooley, P. W., and Luz, E. D. M. N. 2007. Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. Phytopathology 97:492-503.
7. Brasier, C. M. 2003. Sudden oak death: *Phytophthora ramorum* exhibits transatlantic differences. Mycol. Res. 107:257-259.
8. Brasier, C. M., and Kirk, S. A. 2004. Production of gametangia by *Phytophthora ramorum* in vitro. Mycol. Res. 108:823-827.
9. Brasier, C., Denman, S., Brown, A., and Webber, J. 2004. Sudden oak death (*Phytophthora ramorum*) discovered on trees in Europe. Mycol. Res. 108:1108-1110.
10. Cavalli-Sforza, L. L., and Edwards, A. W. F. 1967. Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Genet. 19:233-257.
11. Cooke, D. E. L., Jung, T., Williams, N. A., Schubert, R., Osswald, W., and Duncan, J. M. 2005. Genetic diversity of European populations of the oak fine-root pathogen *Phytophthora quercina*. For. Pathol. 35:57-70.
12. Cooke, D. E. L., and Lees, A. K. 2004. Markers, old and new, for examining *Phytophthora infestans* diversity. Plant Pathol. 53:692-704.
13. Cooke, D. E. L., Young, V., Birch, P. R. J., Toth, R., Gourlay, F., Day, J. P., Carnegie, S. F., and Duncan, J. M. 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995–97). Plant Pathol. 52:181-192.
14. Cornuet, J. M., Piry, S., Luikart, G., Estoup, A., and Solignac, M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989-2000.
15. Dobrowolski, M. P., Tommerup, I. C., Shearer, B. L., and O'Brien, P. A. 2003. Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. Phytopathology 93:695-704.
16. Erwin, D. C., and Ribeiro, O. K. 1996. Phytophthora Diseases Worldwide. American Phytopathological Society Press, St. Paul, MN.
17. Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinf. Online 1:47-50.
18. Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes – application to human mitochondrial-DNA restriction data. Genetics 131:479-491.
19. Gally, M., Ramos, A. M, Dokmetzian, D., and Lopez, S. E. 2007. Genetic variability of *Phytophthora sojae* isolates from Argentina. Mycologia 99:877-883.
20. Garbelotto, M., Ivors, K., Hüberli, D., Bonants, P., and Wagner, A. 2006. Potential for sexual reproduction of *Phytophthora ramorum* in Washington State nurseries. Pages 129-130 in: Proc. Sudden Oak Death Second Sci. Symp.: The State of Our Knowledge. S. J. Frankel, P. J. Shea, and M. I. Haverty, tech. coords. Gen. Tech. Rep. GTR-PSW-196, U.S. Dep. Agric. For. Serv. Pac. Southwest Res. Stn. Albany, CA.
21. Garbelotto, M., Vettraino, A. M., and Rosenzweig, N. 2007. Genotyping indicates nursery and early *Phytophthora ramorum* collections are indistinguishable, while showing current geographic diversification of wild populations driven by geography. (Abstr.) Sudden Oak Death Third Sci. Symp. Santa Rosa, CA.
22. Goheen, E. M., Hansen, E., Kanaskie, A., Osterbauer, N., Parke, J., Pscheidt, J., and Chastagner, G. 2006. Sudden Oak Death and *Phytophthora ramorum*. A Guide for Forest Managers, Christmas Tree Growers, and Forest-Tree Nursery Operators in Oregon and Washington. Oregon State University, Corvallis.
23. Goodwin S. B. 1997. The population genetics of *Phytophthora*. Phytopathology 87:462-473.
24. Goodwin, S. B., Cohen, B. A., Deahl, K. L., and Fry, W. E. 1994. Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. Phytopathology 84:553-558.
25. Goss, E. M., Carbone, I., and Grünwald, N. J. Ancient isolation and independent evolution of the three clonal lineages of the exotic sudden oak death pathogen *Phytophthora ramorum*. Mol. Ecol. 18:1161-1174.
26. Goudet, J. 2001. Fstat, a program to estimate and test gene diversities and fixation indices (version 2.9.3). University of Lausanne, Switzerland.
27. Govers, F., and Gijzen, M. 2006. *Phytophthora* genomics: The plant destroyers' genome decoded. Mol. Plant-Microbe Interact. 19:1295-1301.
28. Grünwald, N. J. 2008. What can availability of the *Phytophthora ramorum* genome do for us? Pages 233-238 in: Proc. Sudden Oak Death Third Sci. Symp. S. J. Frankel, J. T. Kliejunas, and K. M. Palmieri, tech. coords. Gen. Tech. Rep. GTR-PSW-214, U. S. Dep. Agric. For. Serv. Pac. Southwest Res. Stn. Albany, CA.
29. Grünwald, N. J., Flier, W. G., Sturbaum, A. K., Garay-Serrano, E., van den Bosch, T. B. M., Smart, C. D., Matuszak, J. M., Lozoya-Saldaña, H., Turkensteen, L. J., and Fry, W. E. 2001. Population structure of *Phytophthora infestans* in the Toluca Valley region of Central Mexico. Phytopathology 91:882-890.
30. Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., and Fry, W. E. 2003. Analysis of genotypic diversity data for populations of microorganisms. Phytopathology 93:738-746.
31. Grünwald, N. J., Goss, E. M., Larsen, M. M., Press, C. M., McDonald, V. T., Blomquist, C. L., and Thomas, S. L. 2008. First report of the European lineage of *Phytophthora ramorum* in a California nursery. Plant Dis. 2:314-314.
32. Grünwald, N. J., Goss, E. M., and Press, C. M. 2008. *Phytophthora ramorum*: a pathogen with a remarkably wide host-range causing sudden oak death on oaks and ramorum blight on woody ornamentals. Mol. Plant Pathol. 9:729-740.
33. Grünwald, N. J., and Hoheisel, G.-A. 2006. Hierarchical analysis of diversity, selfing, and genetic differentiation in populations of the oomycete *Aphanomyces euteiches*. Phytopathology 96:1134-1141.
34. Hansen, E. M., Kanaskie, A., Prospero, S., McWilliams, M., Goheen, E. M., Osterbauer, N., Reeser, P., and Sutton, W. 2008. Epidemiology of *Phytophthora ramorum* in an Oregon tanoak forest. Can. J. For. Res. 38:1133-1143.
35. Hansen, E. M., Reeser, P. W., Sutton, W., Winton, L., and Osterbauer, N. 2003. First report of A1 mating type of *Phytophthora ramorum* in North America. Plant Dis. 87:1267.
36. Huson, D. H., and Bryant, D. 2006. Application of phylogenetics networks in evolutionary studies. Mol. Biol. Evol. 23:254-267.
37. Ivors, K. L., Garbelotto, M., Vries, M., Ruyter-Spira, I. D. E., Hekkert, B. T., Rosenzweig, N., and Bonants, P. 2006. Microsatellite markers identify three lineages of *Phytophthora ramorum* in U.S. nurseries, yet single lineages in US forests and European nursery populations. Mol. Ecol. 15:1493-1505.
38. Ivors, K. L., Hayden, K. J., Bonants, P. J. M., Rizzo, D. M., and Garbelotto, M. 2004. AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*. Mycol. Res. 108:378-392.
39. Jones, J. 2008. Current status of *Phytophthora ramorum* in US nurseries and U.S. regulatory highlights. In: Meet. Calif. Oak Mortality Task Force (COMFT), San Rafael, CA.
40. Kluza, D. A., Vieglais, D. A., Andreasen, J. K., and Peterson, A. T. 2007. Sudden oak death: geographic risk estimates and predictions of origins. Plant Pathol. 56:580-587.
41. Kroon, L. P. N. M., Verstappen, E. C. P., Kox, L. F. F., and Bonants, P. J. M. 2004. A rapid diagnostic test to distinguish between American and European populations of *Phytophthora ramorum*. Phytopathology 94:613-620.
42. Lamour, K. H., and Hausbeck, M. K. 2001. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology 91:973-980.
43. Linde, C., Drenth, A., and Wingfield, M. J. 1999. Gene and genotypic diversity of *Phytophthora cinnamomi* in South Africa and Australia revealed by DNA polymorphism. Eur. J. Plant Pathol. 105:667-680.
44. Martin, F. N. 2008. Mitochondrial haplotype determination in the oomycete plant pathogen *Phytophthora ramorum*. Curr. Genet. 54:23-34.
45. Mascheretti, S., Croucher, P. J. P., Vettraino, A., Prospero, S., and Garbelotto, M. 2008. Reconstruction of the sudden oak death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Mol. Ecol. 17:2755-2768.
46. Maynard Smith, J., Smith, N. H., O'Rourke, M., and Spratt, B. G. 1993. How clonal are bacteria? Proc. Natl. Acad. Sci. USA 90:4384-4388.

47. McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349-379.
48. Milgroom, M. G. 1997. Genetic variation and the application of genetic markers for studying plant pathogen populations. J. Plant. Pathol. 79:1-13.
49. Miller, J. S., Hamm, P. B., and Johnson, D. A. 1997. Characterization of the *Phytophthora infestans* population in the Columbia basin of Oregon and Washington from 1992 to 1995. Phytopathology 87:656-660.
50. Miller, M. P. 1997. Tools for Population Genetic Analyses (TFPGA) 1.3: A Windows Program for the Analysis of Allozyme and Molecular Population Genetic Data. Computer software distributed by the author. Available at http://www.marksgeneticsoftware
51. Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.
52. Oliva, R. F., Erselius, L. J., Adler, N. E., and Forbes, G. A. 2002. Potential of sexual reproduction among host-adapted populations of *Phytophthora infestans* sensu latu in Ecuador. Plant Pathol. 51:710-719.
53. Osterbauer, N. K., Rehms, L., and Hedberg, J. 2003. Official survey of Oregon agricultural commodities for *Phytophthora ramorum*. (Abstr.) Phytopathology 93:S68.
54. Park, J., Park, B., Veeraghavan, N., Jung, K., Lee, Y. H., Blair, J. E., Geiser, D. M., Isard, S., Mansfield, M. A., Nikolaeva, E., Park, S. Y., Russo, J., Kim, S. H., Greene, M., Ivors, K. L., Balci, Y., Peiman, M., Erwin, D. C., Coffey, M. D., Rossman, A., Farr, D., Cline, E., Grunwald, N. J., Luster, D. G., Schrandt, J., Martin, F., Ribeiro, O. K., Makalowska, I., and Kang, S. 2008. *Phytophthora* database: A forensic database supporting the identification and monitoring of *Phytophthora*. Plant Dis. 92:966-972.
55. Parke, J., Pscheidt, J., and Linderman, R. 2003. *Phytophthora ramorum*, a guide for Oregon nurseries. Oreg. State Univ. Ext. Service. EM 8840.
56. Prospero, S., Black, J. A., and Winton, L. M. 2004. Isolation and characterization of microsatellite markers in *Phytophthora ramorum*, the causal agent of sudden oak death. Mol. Ecol. Notes 4:672-674.
57. Prospero, S., Hansen, E. M., Grünwald, N. J., and Winton, L. M. 2007. Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. Mol. Ecol. 16:2958-2973.
58. Raymond, M. L., and Rousset, F. 1995. An exact test for population differentiation. Evolution 49:1280-1283.
59. Rizzo, D. M., and Garbelotto, M. 2003. Sudden oak death: Endangering California and Oregon forest ecosystems. Front. Ecol. Environ. 1:197-204.
60. Rizzo, D. M., Garbelotto, M., and Hansen, E. M. 2005. *Phytophthora ramorum*: Integrative research and management of an emerging pathogen in California and Oregon forests. Annu. Rev. Phytopathol. 43:309-345.
61. Shannon, C. E., and Weaver, W. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana.
62. Simpson, E. H. 1949. Measurement of diversity. Nature 163:688.
63. Stoddart, J. A., and Taylor, J. F. 1988. Genotypic diversity: Estimation and prediction in samples. Genetics 118:705-711.
64. Tjosvold, S. A., Buermeyer, K. R., Blomquist, C., and Frankel, S. 2005. Nursery guide for diseases caused by *Phytophthora ramorum* on ornamentals: diagnosis and management. ANR Univ. Calif. Publ. 8156.
65. Tjosvold, S. A., Chambers, D. L., Koike, S., and Fichtner, E. 2006. Epidemiology of *Phytophthora ramorum* infecting rhododendrons under simulated nursery conditions. Pages 459-461 in: Proc. Sudden Oak Death Second Sci. Symp.: The State of our Knowledge. S. J. Frankel, P. J. Shea, and M. I. Haverty, tech. coords. Gen. Tech. Rep. PSW-GTR-196, U.S. Dep. Agric. For. Serv. Pac. Southwest Res. Stn. Albany, CA.
66. Tooley, P. W., Fry, W. E., and Villareal-Gonzaléz, M. J. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. J. Hered. 76:431-435.
67. Tyler, B. M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R. H. Y., Aerts, A., Arredondo, F. D., Baxter, L., Bensasson, D., Beynon, J. L., Chapman, J., Damasceno, C. M. B., Dorrance, A. E., Dou, D., Dickerman, A. W., Dubchak, I. L., Garbelotto, M., Gijzen, M., Gordon, S. G., Govers, F., Grünwald, N. J., Huang, W., Ivors, K. L., Jones, R. W., Kamoun, S., Krampis, K., Lamour, K. H., Lee, M.-K., McDonald, W. H., Medina, M., Meijer, H. J. G., Nordberg, E. K., Maclean, D. J., Ospina-Giraldo, M. D., Morris, P. F., Phuntumart, V., Putnam, N. H., Rash, S., Rose, J. K. C., Sakihama, Y., Salamov, A. A., Savidor, A., Scheuring, C. F., Smith, B. M., Sobral, B. W. S., Terry, A., Torto-Alalibo, T. A., Win, J., Xu, Z., Zhang, H., Grigoriev, I. V., Rokhsar, D. S., and Boore, J. L. 2006. *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. Science 313:1261-1266.
68. Wamishe, Y. A., Jeffers, S. N., and Hwang, J. 2006. *Phytophthora ramorum* and other species of *Phytophthora* detected in field soil and water at retail nurseries in the southeastern USA. (Abstr.) Phytopathology 96:S120.
69. Werres, D., and De Merlier, D. 2003. First detection of *Phytophthora ramorum* mating type A2 in Europe. Plant Dis. 87:1266.
70. Werres, S., and Kaminski, K. 2005. Characterisation of European and North American *Phytophthora ramorum* isolates due to their morphology and mating behaviour in vitro with heterothallic *Phytophthora* species. Mycol. Res. 109:860-871.
71. Werres, S., Marwitz, R., Veld, W., De Cock, A., Bonants, P. J. M., De Weerdt, M., Themann, K., Ilieva, E., and Baayen, R. P. 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. Mycol. Res. 105:1155-1165.
72. Winton, L. M., and Hansen, E. M. 2001. Molecular diagnosis of *Phytophthora lateralis* in trees, water, and foliage baits using multiplex polymerase chain reaction. For. Pathol. 31:275-283.
73. Wright, S. 1922. Coefficients of inbreeding and relationship. Am. Nat. 56:330-338.