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AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*

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The genetic structure within and between USA and European populations of the emerging phytopathogen *Phytophthora ramorum* was examined. Four primer combinations were used for amplified fragment length polymorphism (AFLP) fingerprinting of 67 USA isolates from California and Oregon, and 18 European isolates from Belgium, Germany, The Netherlands, Spain and the UK. In addition, three DNA regions (ITS, *cox* II, and *nad* 5) of additional *Phytophthora* species were amplified by polymerase chain reaction, sequenced, and analysed to provide better phylogenetic understanding of *P. ramorum* within the genus *Phytophthora*. AFLP banding patterns indicate that the 85 isolates form two distinct lineages within a monophyletic group, distinct from the closely related outgroup species *P. lateralis*. With the exception of two isolates from an Oregon nursery, European and USA isolates clustered separately within individual clades. The AFLP profiles also indicate that a single clonal lineage dominates the North American population, while the European population consists of an array of mainly unique, closely related AFLP types. Sequences from the three DNA regions were identical among all *P. ramorum* isolates, and phylogenetic analysis indicates that *P. ramorum* is closely related to *P. lateralis* and *P. hibernalis*.

INTRODUCTION

Phytophthora ramorum is considered an emerging pathogen that has received worldwide attention as the causal agent of sudden oak death (SOD; Rizzo & Garbelotto 2003). In California and Oregon, *P. ramorum* causes a deadly canker disease of tanoak (*Lithocarpus densiflora*), coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*) and Shreve's oak (*Q. parvula* var. *shrevei*), and non-lethal foliar and twig infections of numerous native hardwood and coniferous forest trees, understory shrubs and herbaceous plants (Davidson *et al.* 2003). Symptoms of SOD were first observed during 1994–95 in the San Francisco bay area in California; subsequently *P. ramorum* has reached epidemic proportions in oak forests along a 650 km stretch of the Pacific coastline from central California to southern Oregon. Recently the pathogen has been found in nurseries in California, Oregon, Washington and British Columbia that are located outside the natural range of *P. ramorum* (Davidson *et al.* 2003). The pathogen was first isolated in 1993 in Germany and The Netherlands from rhododendrons in nurseries and formally described in 2001 (Werres *et al.* 2001). Since then, *P. ramorum* has been detected in at least nine European countries, where it is reported to cause twig blight of *Rhododendron*, *Viburnum*, *Camellia*, *Kalmia*, *Pieris*, *Vaccinium* and other important nursery plant species (http://www.eppo.org; Davidson *et al.* 2003). Recently the pathogen was also isolated from multiple tree species in the vicinity of infected rhododendrons in the UK and The Netherlands (http://www.defra.gov.uk). A comparison of morphology and ITS DNA sequences of isolates from both geographic locations confirmed that western USA and European isolates were the same species (Rizzo *et al.* 2002).

The global genetic structure and geographic origin of *P. ramorum* remain unknown. There are no reports of this species in either the USA or Europe before the mid 1990s. Due to its limited geographic range in relation to

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Table 1. Isolates of *Phytophthora* species used in this study.

Isolate number[a]	Host	Origin	Experiments	GenBank
***P. ramorum* (USA)**				
Pr-01^{DR}, CBS110534	*Quercus agrifolia*	Marin Co., CA	AFLP, ITS	AF521564
Pr-02^{DR}	*Lithocarpus densiflora*	Marin Co., CA	AFLP	
Pr-03^{DR}, CBS110535	*Lithocarpus densiflora*	Marin Co., CA	AFLP	
Pr-04^{DR}, CBS110536	*Q. kelloggii*	Marin Co., CA	AFLP	
Pr-05^{DR}, ATCC MYA-2434	*L. densiflora*	Marin Co., CA	AFLP	
Pr-06^{DR}, ATCC MYA-2435	*Q. agrifolia*	Marin Co., CA	AFLP, ITS	AY423276
Pr-08^{DR}	*Q. agrifolia*	Napa Co., CA	AFLP	
Pr-10^{DR}	*L. densiflora*	Monterey Co., CA	AFLP	
Pr-11^{DR}	*Q. agrifolia*	Monterey Co., CA	AFLP	
Pr-13^{DR}	*Q. agrifolia*	Santa Cruz Co., CA	AFLP, ITS	AY423277
Pr-16^{DR}	*Q. agrifolia*^{N}	Santa Cruz Co., CA	AFLP	
Pr-19^{DR}	*Q. agrifolia*	Napa Co., CA	AFLP	
Pr-20^{DR}	*Q. agrifolia*	Sonoma, CA	AFLP	
Pr-22^{DR}	*Q. agrifolia*	Sonoma Co., CA	AFLP	
Pr-24^{DR}	*Q. agrifolia*	Sonoma Co., CA	AFLP	
Pr-27^{DR}	*Q. agrifolia*	Marin Co., CA	AFLP	
Pr-28^{DR}	*L. densiflora*	Sonoma Co., CA	AFLP	
Pr-35^{DR}	*Q. agrifolia*	Sonoma Co., CA	AFLP	
Pr-36^{DR}, CBS110953	*Q. agrifolia*	Sonoma Co., CA	AFLP, ITS	AY423278
Pr-47^{DR}	*Q. agrifolia*	Sonoma Co., CA	AFLP	
Pr-50^{DR}	*Q. agrifolia*	Sonoma Co., CA	AFLP	
Pr-52^{DR}, CBS110537, ATCC MYA-2436	*Rhododendron* sp.^{N}	Santa Cruz Co., CA	AFLP, ITS	AF521567
Pr-57^{DR}	*L. densiflora*	Santa Clara Co., CA	AFLP	
Pr-58^{DR}	*Vaccinium ovatum*	Marin Co., CA	AFLP	
Pr-65^{DR}, CBS110538, ATCC MYA-2437	*Q. parvula*^{N}	Santa Cruz Co., CA	AFLP	
Pr-70^{DR}, CBS110539	*V. ovatum*	Marin Co., CA	AFLP	
Pr-71^{DR}, CBS110540	*Q. agrifolia*	Sonoma Co., CA	AFLP	
Pr-72^{DR}, CBS110954	*Rhododendron* sp.^{N}	Santa Cruz Co., CA	AFLP, ITS	AY423279
Pr-73^{DR}	*Rhododendron* sp.^{N}	Alameda Co., CA	AFLP	
Pr-75^{DR}	*Q. agrifolia*	Monterey Co., CA	AFLP	
Pr-80^{DR}	*V. ovatum*	Marin Co., CA	AFLP	
Pr-82^{DR}	*V. ovatum*	Marin Co., CA	AFLP	
Pr-84^{DR}	Soil	Marin Co., CA	AFLP	
Pr-86^{DR}, CBS110541, ATCC MYA-2440	*Arbutus menziessi*	Marin Co., CA	AFLP	
Pr-87^{DR}	*A. menziessi*	Marin Co., CA	AFLP	
Pr-88^{DR}	*Umbellularia californica*	Sonoma Co., CA	AFLP	
Pr-89^{DR}	*U. californica*	Sonoma Co., CA	AFLP	
Pr-90^{DR}	*Q. agrifolia*	Marin Co., CA	AFLP	
Pr-91^{DR}	*U. californica*^{N}	Santa Cruz Co., CA	AFLP	
Pr-97^{DR}, CBS110955	*Q. agrifolia*	Napa Co., CA	AFLP	
Pr-102^{DR}, ATCC MYA-2949	*Q. agrifolia*	Marin Co., CA	AFLP, ITS	AY423280
			coxII	AY423303
			nad5	AY423320
Pr-103^{DR}	*L. densiflora*	Marin Co., CA	AFLP, ITS	AY423281
Pr-104^{DR}	*L. densiflora*	Marin Co., CA	AFLP	
Pr-105^{DR}	*L. densiflora*	Marin Co., CA	AFLP, ITS	AY423282
Pr-106^{DR}, CBS110956	*U. californica*	Sonoma Co., CA	AFLP, ITS	AY423283
Pr-107^{DR}	*U. californica*	Sonoma Co., CA	AFLP	
Pr-108^{DR}	*Umbellularia californica*	Sonoma Co., CA	AFLP, ITS	AY423284
Pr-110^{DR}, CBS110542	*U. californica*	Marin Co., CA	AFLP	
Pr-112^{DR}	*U. californica*	Marin Co., CA	AFLP	
Pr-113^{DR}	*U. californica*	Marin Co., CA	AFLP	
Pr-114^{DR}	*U. californica*	Marin Co., CA	AFLP, ITS	AY423285
Pr-116^{DR}	*U. californica*	Napa Co., CA	AFLP	
Pr-120^{DR}	*L. densiflora*	Mendocino Co., CA	AFLP, ITS	AY423286
Pr-136^{DR}, ATCC MYA-2441	*U. californica*	Mendocino Co., CA	AFLP	
Pr-146^{DR}	*L. densiflora*	San Mateo Co., CA	AFLP	
Pr-153^{DR}	*U. californica*	Solono Co., CA	AFLP	
Pr-156^{DR}	*L. densiflora*	Curry Co., OR	AFLP	
Pr-157^{DR}	*L. densiflora*	Curry Co., OR	AFLP	
Pr-158^{DR}, CBS110957	*L. densiflora*	Curry Co., OR	AFLP	
Pr-159^{DR}, CBS110543	*L. densiflora*	Curry Co., OR	AFLP, ITS	AF521574
Pr-201c^{DR}	*Rhododendron* sp.^{N}	Santa Cruz Co., CA	AFLP	
Pr-343^{DR}, PrJL3.1^{DR}	*Sequoia sempervirens*	Sonoma Co., CA	AFLP	
Pr-345^{DR}, PrJL3.5.3^{DR}, CBS110544	*S. sempervirens*	Sonoma Co., CA	AFLP, ITS	AF521576

Table 1. (*Cont.*)

Isolate number[a]	Host	Origin	Experiments	GenBank
Pr-SDC21.6^{DR}	*S. sempervirens*	Sonoma Co., CA	AFLP, ITS	AF521577
			coxII	AY423304
			nad5	AY423321
Pr-3-74-1^{EH}	*Pieris* sp.^{N}	Clackamas Co., CR	AFLP, ITS	AY423287
Pr-3-74-2^{EH}	*Viburnum bodnantense* 'Dawn'^{N}	Clackamas Co., OR	AFLP	
PDR1282257^{CB}	*Camellia sasanqua* 'Bonanza'^{N}	Stanislaus Co., CA	AFLP	
***P. ramorum* (Europe)**[b]				
PD 94/844^{PB}, CBS101332	*Rhododendron* sp.	The Netherlands	AFLP	
PD 98/8/2627^{PB}, CBS101331	*Rhododendron* sp.	The Netherlands	AFLP	
PD 98/8/5233^{PB}, CBS101330	*Viburnum* sp.	The Netherlands	AFLP	
PD 98/8/6285^{PB}, CBS101329	*Rhododendron* sp.	The Netherlands	AFLP	
PD 98/8/6743^{PB}, CBS101328	*Rhododendron* sp.	The Netherlands	AFLP	
PD 98/8/6933^{PB}, CBS101326	*Rhododendron* sp.	The Netherlands	AFLP	
BBA 69082^{SW}, CBS101548	*Rhododendron* sp. 'Schneewolke'	Germany	AFLP	
BBA 104/5^{SW}, CBS101549	Recycling water in nursery	Germany	AFLP	
BBA 9/95^{SW}, CBS101553	*R. catawbiense*	Germany	AFLP	
BBA 12/98^{SW}, CBS101551	*R. catawbiense* 'Grandiflorum'	Germany	AFLP, ITS	AY423288
			coxII	AY423305
			nad5	AY423322
BBA 13/99-1^{SW}, CBS109279	*Rhododendron* sp.	Germany	AFLP	
BBA 16/99^{SW}, CBS109278	*V. bodnantense*	Germany	AFLP	
Phyram1^{EM}	*R. catawbiense* 'Grandiflorum'	Mallorca, Spain	AFLP, ITS	AY423289
			coxII	AY423306
			nad5	AY423323
Phyram5^{EM}	*Rhododendron* sp.	Mallorca, Spain	AFLP	
CSL 1571^{AI}	*Viburnum tinus*	England	AFLP	
CSL 1599^{AI}	*R. ericaceae* cv Cheer	Yorkshire, England	AFLP	
CSL 1652^{AI}	*Rhododendron* sp.	West Sussex, England	AFLP	
PRI 508^{PB}	*V. bodnantense*	Belgium	AFLP	
P. lateralis				
PL16^{MG}	Soil	Josephine Co., CA	AFLP, ITS	AF521579
			coxII	AY423312
			nad5	AY423335
PL27^{MG}	*Taxus brevifolia*	Del Norte Co., CA	AFLP, ITS	AF521580
			coxII	AY423313
			nad5	AY423336
PL33^{MG}	*Chamaecyparis lawsoniana*	Del Norte Co., CA	AFLP, ITS	AF521581
			coxII	AY423314
			nad5	AY423337
P. cactorum				
P25^{DR}, 6B 2759^{GB}	*Prunus* sp.	California	ITS	AY423290
			nad5	AY423324
P. cambivora				
PDR198513^{CB}	*Q. agrifolia*	California	ITS	AY423291
			coxII	AY423307
			nad5	AY423325
P. capsici				
P141^{DR}, 3300^{GB}	*Lycopersicon esculentum*	N/A	ITS	AY423292
			coxII	AY423308
			nad5	AY423326
P. cinnamomi				
P2444^{MC} (A2)	*Persea americana*	California	nad5	AY423327
P6379^{MC} (A1)	*Ananas comosus*	Taiwan	coxII	AY423309
			nad5	AY423328
P. cryptogea				
IMI 045168	*Lycopersicon esculentum*	New Zealand	nad5	AY423329
P. erythroseptica				
355^{PT}, LaQ^{DL}	*Solanum tuberosum*	Maine	ITS	AY423293
			coxII	AY423310
			nad5	AY423330
P. gonapodyides				
393^{PT}, NY353^{WW}	*Malus sylvestris*	New York	ITS	AY423294
			nad5	AY423331

Table 1. (*Cont.*)

Isolate number[a]	Host	Origin	Experiments	GenBank
P. hibernalis				
379[PT], 1895[DR], ATCC 64708	*Aquilegia vulgaris*	New Zealand	ITS	AY423295
			nad5	AY423332
380[PT], 1896[DR], ATCC 60352	*Citrus sinensus*	Portugal	ITS	AY423296
			nad5	AY423333
P. ilicis				
4175a[EH]	*Ilex aquifolium*	Oregon	ITS	AY423297
			coxII	AY423311
			nad5	AY423334
P. megasperma				
309[PT], 336[PH]	*Pseudotsuga menziesii*	Washington	ITS	AY423298
			nad5	AY423338
P. nemorosa				
P13[DR]	*L. densiflora*	California	coxII	AY423315
			nad5	AY423339
P. nicotianae				
331[PT], P1352[MC]	*Nicotiana tabacum*	North Carolina	ITS	AY423299
			coxII	AY423316
			nad5	AY423340
P. palmivora				
P1-10[DJM]	*Theobroma cacao*	Costa Rica	ITS	AY423300
			nad5	AY423341
P. pseudosyringae				
IFB PSEU16[TJ]	*Fagus sylvatica*	Germany	coxII	AY423317
			nad5	AY423342
P40[DR]	*Q. agrifolia*	California	coxII	AY423318
			nad5	AY423343
P. sojae				
312[PT], ATCC 48068	*Glycine max*	Wisconsin	ITS	AY423301
			nad5	AY423344
P. syringae				
PDR115773A[CB]	*Rhododendron* sp.	California	ITS	AY423302
			coxII	AY423319
			nad5	AY423345

[a] CB, Cheryl Blomquist; PB, Peter Bonants; PH, Phil Hamm; MC, Mike Coffey; MG, Matteo Garbelotto; EH, Everett Hansen; AI, Alan Inman; DJM, Dave Mitchell; EM, Eduardo Moralejo; DR, Dave Rizzo; PT, Paul Tooley; TJ, Thomas Jung; WW, Wayne Wilcox.

[b] Due to quarantine regulations, lyophilized tissue (rather than cultures) of European isolates was obtained from sources listed in Acknowledgements.

[N] USA *P. ramorum* isolate from nursery.

the distribution of host species and very high mortality levels associated with infection of some hosts (e.g. tanoak), it is widely assumed that *P. ramorum* is exotic to North America. In an earlier study of 14 isolates from Germany and The Netherlands, AFLP analysis indicated these isolates formed a distinct cluster, representing a different lineage from those of the other eight *Phytophthora* species analysed, with small amounts of genetic variability within *P. ramorum*. These 14 isolates also shared identical ITS sequences and isozyme patterns of malate dehydrogenase and malic enzyme (Werres *et al*. 2001).

P. ramorum is heterothallic, requiring interaction of opposite mating types (A1 and A2) for sexual recombination. Until recently, all European isolates tested were shown to belong to the A1 mating type, and all American isolates tested were shown to belong to A2 (Werres *et al*. 2001, Moralejo & Werres 2002, Brasier 2003, Werres & Zielke 2003). These data, taken as a whole, suggest that *P. ramorum* may have been introduced separately into the USA and Europe. In 2002, the first European A2 isolate was collected from infested plant material in Belgium (Werres & de Merlier 2003). In spring 2003, the first USA A1 isolates were identified during a national nursery survey in northern Oregon (Hansen *et al*. 2003). Subsequently more A1 isolates have been recovered from nurseries in Washington and British Columbia. *In vitro* (Clive M. Brasier, pers. comm.) and *in vivo* (Werres & Zielke 2003) matings have been successful between European A1 and USA A2 isolates. As well, USA A2 isolates from the wild and USA A1 isolates from nurseries have been mated successfully (Daniel Hüberli, pers. comm.).

An obvious consequence of sexual recombination in heterothallic *Phytophthora* species is the creation of new genotypes, although mixed mating type populations of some *Phytophthora* species (e.g. *P. cinnamomi*, *P. infestans*) have coexisted in the same location with

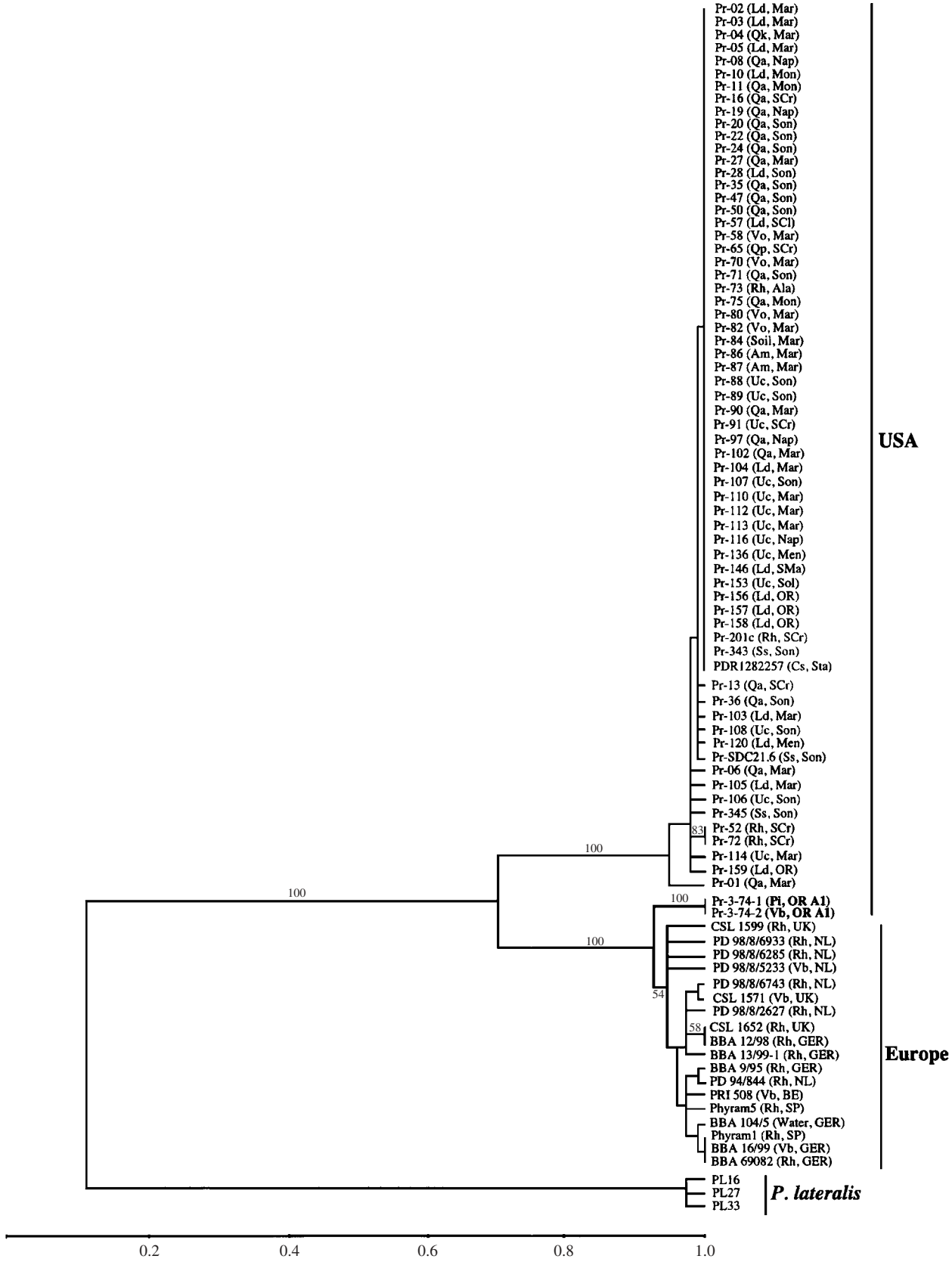

Fig. 1. Neighbour-joining tree obtained from S_J similarity coefficients of 85 *Phytophthora ramorum* and three *P. lateralis* isolates generated from AFLP fingerprint data. The scale is proportion similarity, and numbers near branches indicate bootstrap values obtained from 1000 replicate analyses. Detailed isolate information (host and location) is located next to isolate ID on tree. Hosts: Am, *Arbutus menziesii*; Cs, *Camellia sasanqua*; Ld, *Lithocarpus densiflora*; Pi, *Pieris* spp.; Qa, *Quercus agrifolia*; Qk, *Quercus kelloggii*; Qp, *Quercus parvula*; Rh, *Rhododendron* spp.; Ss, *Sequoia sempervirens*; Uc, *Umbellularia californica*; Vo, *Vaccinium ovatum*; and Vb, *Viburnum* spp. USA locations: Ala, Alameda Co.; Mar, Marin Co.;

extremely low levels, if any, of sexual reproduction (Goodwin *et al*. 1992, Linde *et al*. 1997, Dobrowolski *et al*. 2003). However, these recent discoveries of both mating types of *P. ramorum* in the USA and Europe bring up important questions regarding dispersal and genetic recombination mechanisms of this plant pathogen. The risk of introducing *P. ramorum* outside of its present range is of great concern. A better understanding of its origin, evolution and diversity is important for developing regulatory and disease management strategies. Information on the origin of this pathogen could also provide an effective basis for the search and application of disease resistance in economically and ecologically important hosts species.

The work reported here has a number of objectives related to the overall aim of understanding the origin and genetic variability of *P. ramorum*, and its genetic placement within the genus *Phytophthora*. Our first objective was to characterize the genetic structure of *P. ramorum* populations present in the USA and Europe. This was done by examining a broad range of isolates with AFLP fingerprinting to determine if recombination is taking place within and between populations, and if population subdivisions exist based on geography or host species. Our second objective was to provide a better phylogenetic understanding of this species within the genus *Phytophthora* by inferring gene phylogenies for numerous *Phytophthora* species from three different loci: portions of the nuclear rDNA ITS region, and portions of the mitochondrially-encoded NADH dehydrogenase subunit 5 (*nad* 5) and cytochrome oxidase subunit II (*cox* II) genes.

MATERIALS AND METHODS

Isolates

Isolates of *Phytophthora ramorum* were chosen to represent a broad range of geographic locations and hosts (Table 1). 67 *P. ramorum* isolates from the USA and 18 from Europe, along with three *P. lateralis* isolates used as an outgroup species, were analysed with AFLP. Other *Phytophthora* species were used to generate sequence data for phylogenetic analysis (Table 1). Isolates were maintained on V8 agar at 15–20 °C, depending on species. Cultures used in this study are currently stored under liquid nitrogen in the Department of Plant Pathology, University of California, Davis. Representative voucher cultures have also been deposited with ATCC and CBS (Table 1).

DNA isolation

Isolates were grown in potato dextrose broth on a rotary shaker at room temperature for approx. 10 d. Genomic DNA was isolated from 75 mg of lyophilized mycelium using the following modified CTAB extraction procedure. Lyophilized tissue was pulverized with glass beads in a FastPrep® instrument (Bio101, Carlsbad, CA) for 5 s at 4000 rpm. Pulverized tissue was incubated in 500 ml CTAB on dry ice for 2 min, and then thawed at 75 ° for 2 min. DNA was purified in phenol:chloroform:isoamyl alcohol (25:24:1), further cleaned by using the Geneclean® Turbo Nucleic Acid Purification kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, and eluted in 30 µl ultra-pure water. DNA extracts were stored at −20 °.

DNA amplification

Templates of the *nad* 5 gene were amplified for sequencing by PCR using primers P1bfwd (5′-ATGCTATGGAAGGTCCTACA-3′) and P1brev (5′-AGGTAGTATACGTCTTAAAC-3′) as previously described (Jung *et al*. 2003), and resulted in a 388 bp fragment. Templates of the *cox* II gene were amplified using primers FM 35 (5′-CAGAACCTTGGCAATTAGG-3′) and FM 58 (5′-CCACAAATTTCACTACATTGA-3′) as previously described (Martin 2000), resulting in a 540 bp fragment. ITS amplification was performed with primers ITS1 and ITS4 (White *et al*. 1990) as previously described (Bonants *et al*. 1997), resulting in a *ca* 910–940 bp fragment depending on the species sequenced. All PCR products were sequenced with initial amplification primers on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA).

AFLP analysis

AFLP reactions were performed as described by Vos *et al*. (1995) with slight modifications, using the AFLP core reagent kit (Invitrogen, Carlsbad, CA). AFLP® technology is covered by patents and patent applications owned by Keygene. Genomic DNA (250 ng) was digested with 1.25 U each of the restriction enzymes *Eco*RI and *Mse*I for 2 h at 37 ° in reaction buffer supplied with the kit. Adapters were ligated to restriction fragments in a total volume of 25 µl for 2 h at 20 ° using 0.5 U of T4 DNA ligase. Ligation products were diluted 1:9 with ultra-pure water. Following ligation, DNA was first amplified by PCR using nonselective *Eco*RI (E00) (5′-GACTGCGTACCAATTC-3′) and *Mse*I (M00) (5′-GATGAGTCCTGAGTAA-3′) primers. Each reaction mixture contained buffer (10 mM Tris/HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 50 µM dNTP, 5 ng μl^{-1} of each primer, and 1 U of Platinum *Taq* DNA polymerase (Invitrogen). PCR was performed in an iCycler thermocycler (Bio-Rad

Men, Mendocino Co., Mon, Monterey Co.; Nap, Napa Co.; Sol, Solono Co.; Son, Sonoma Co.; SMa, San Mateo Co.; SCr, Santa Cruz Co.; SCl, Santa Clara Co.; Sta, Stanislaus Co.; OR, Oregon. European locations: BE, Belgium; GER, Germany; NL, The Netherlands; SP, Spain; and UK, United Kingdom. See Table 1 for additional isolate details.

Laboratories, Hercules, CA) under the following conditions: 20 cycles of 30 s at 94 °, 1 min at 56 °, and 1 min at 72 °. The amplification products were diluted 1:30, and 5 µl was used as a template for selective PCR. Based on the results from an initial screening of multiple primer combinations, four informative pairs of selective-base primers were used in the analysis: (E00-AC) + (M00-AC), (E00-TC) + (M00-AG), (E00-GC) + (M00-AC), and (E00-GG) + (M00-CC). Selective PCR conditions were described as above, except using 5 ng of the *Eco*RI primer (labelled with FAM at the 5′ end), 15 ng of the *Mse*I primer, and 5 mM $MgCl_2$. The cycling profile was as follows: 13 cycles of 30 s at 94 °, 30 s at 65 ° (with annealing temperature lowered 0.7 ° each cycle), and 1 min at 72 °, followed by 23 cycles of 30 s at 94 °, 30 s at 56 °, and 1 min at 72 °. Selective amplification products were diluted 1:10 with deionized formamide, denatured for 5 min at 95 °, and placed on ice before processing. AFLP fragments were sized by capillary electrophoresis on an automated ABI 3100 genetic analyzer using the molecular standard GeneScan-500 ROX and GeneScan 3.1.2 software (Applied Biosystems). Electropherograms were scored manually and side by side for the presence (1) or absence (0) of bands of the same apparent size, and only fragments (ranging in size from 70–600 bp) that could be scored unambiguously were analysed. AFLPs of most *P. ramorum* isolates were repeated starting at the ligation step to ensure reproducibility.

Data from all primer combinations were combined and the resulting binary matrix was transformed into a distance matrix (1 − similarity coefficient) using the Jaccard coefficient of similarity (Jaccard 1908) with the program package LE PROGICEL R (version 4; Casgrain & Legendre 1999), which measures the proportion of shared bands. This transformation was utilized to reduce error caused by the dominant nature of the AFLP markers. The matrix was translated into a distance cladogram with the neighbour-joining algorithm in MEGA, version 2.1 (Kumar *et al.* 2001). The resulting tree was rooted using AFLP profiles of three isolates of *P. lateralis*, the closest known relative of *P. ramorum* based on ITS sequences (Rizzo *et al.* 2002). Bootstrap values of branch points were generated in PAUP, version 4.0b10 (Swofford 2002) using 1000 replicates.

MultiLocus analysis

In order to test for reproductive mode (i.e. clonal or recombining), a clone-corrected AFLP binary dataset of the two separate geographic populations, as well as the entire selection of isolates, was analysed for linkage disequilibrium using a modified index of association (I_A) equation in the software program MultiLocus V.1.2.2. (Agapow & Burt 2001). Significance of I_A was determined by randomization (1000 times) procedures by comparing the observed value of I_A to that expected under the null hypothesis of complete panmixis.

Phylogenetic analysis

Published sequences and alignments of other *Phytophthora* species were downloaded from GenBank and TreeBASE, aligned with generated sequences using the multialignment program Sequencher 4.1.2, and manually optimized using the program Se-Al version 2 (Rambaut 1996). Phylogenetic analyses were performed using PAUP* version 4.0b10 (Swofford 2002). For the ITS dataset, nucleotide sites were equally weighted and gaps were treated as missing data. Bases 94 to 661 of the *cox* II and 731 to 1119 of the *nad* 5 genes were used for phylogenetic analysis. For all three datasets, phylogenetic relationships among *Phytophthora* spp. were inferred with maximum-parsimony (MP) analysis by performing heuristic searches with MULPARS on, steepest-descent off, random addition of sequences, and tree bisection reconnection (TBR) branch swapping. Support for internal branches was obtained by bootstrap analysis using 1000 replications (Felsenstein 1985). All sequences and alignments generated in this study have been deposited in GenBank (Table 1) and TreeBASE (S1036), respectively.

RESULTS

AFLP analysis

67 isolates from the USA were analysed with AFLPs, originating from 11 California (Alameda, Marin, Mendocino, Monterey, Napa, San Mateo, Santa Clara, Santa Cruz, Solono, Sonoma, and Stanislaus) and two Oregon (Curry and Clackamas) counties. Stanislaus and Clackamas County isolates were from nurseries outside the natural range of *Phytophthora ramorum*. The 18 European isolates originated from five countries: Belgium, Germany, The Netherlands, the UK, and Spain. The AFLP fingerprints from all four primer combinations resulted in 269 bands that could be unambiguously scored, of which 74 (27.5%) were polymorphic among *P. ramorum* isolates. There were differences in the numbers of AFLP loci produced by each of the different primer pairs, which presumably reflect differences in sequence composition in the genome. All isolates that did not differ by at least one fragment were considered the same AFLP genotype. Dendrograms constructed from each individual AFLP primer combination were concordant, and only differed in degree of resolution (data not shown). Fingerprints from all four primer combinations were combined and used to generate a dendrogram (Fig. 1). A high degree of AFLP similarity among all *P. ramorum* isolates was observed, with S_J values ranging from 0.70 to 1.0 (mean 0.90). In comparison, a low degree of AFLP similarity was observed between *P. ramorum* and *P. lateralis* isolates, with S_J values ranging from 0.10 to 0.12 (mean 0.11). In the USA and European populations, S_J varied from 0.94 to 1.0 (mean 0.99) and 0.91 to 1.0 (mean 0.96), respectively.

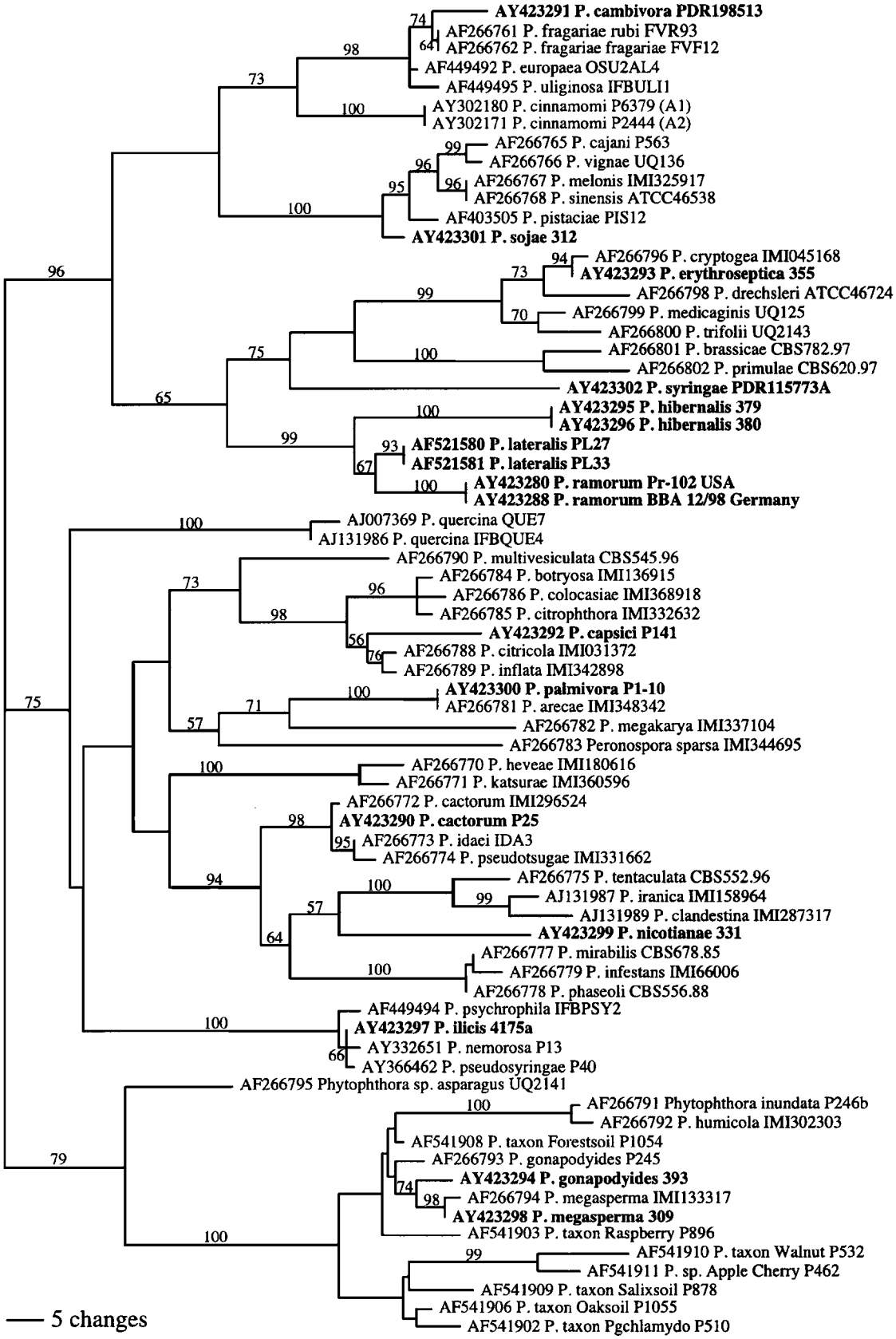

Fig. 2. Phylogenetic relationships among *Phytophthora* species using ITS rDNA sequence data, based on maximum parsimony inferred by a heuristic tree search. Numbers near branches are the percentage of the trees from bootstrap analysis that support the observed topography (values above 50% indicated). This is one of 1891 trees with the shortest length. Of the 878 total characters, 503 were constant, 63 were variable and parsimony-uninformative, and 312 were parsimony-informative. Tree length = 1075, consistency index (CI) = 0.487, homoplasy index (HI) = 0.513, retention index = 0.832. The sequences of taxa in bold were generated for this study.

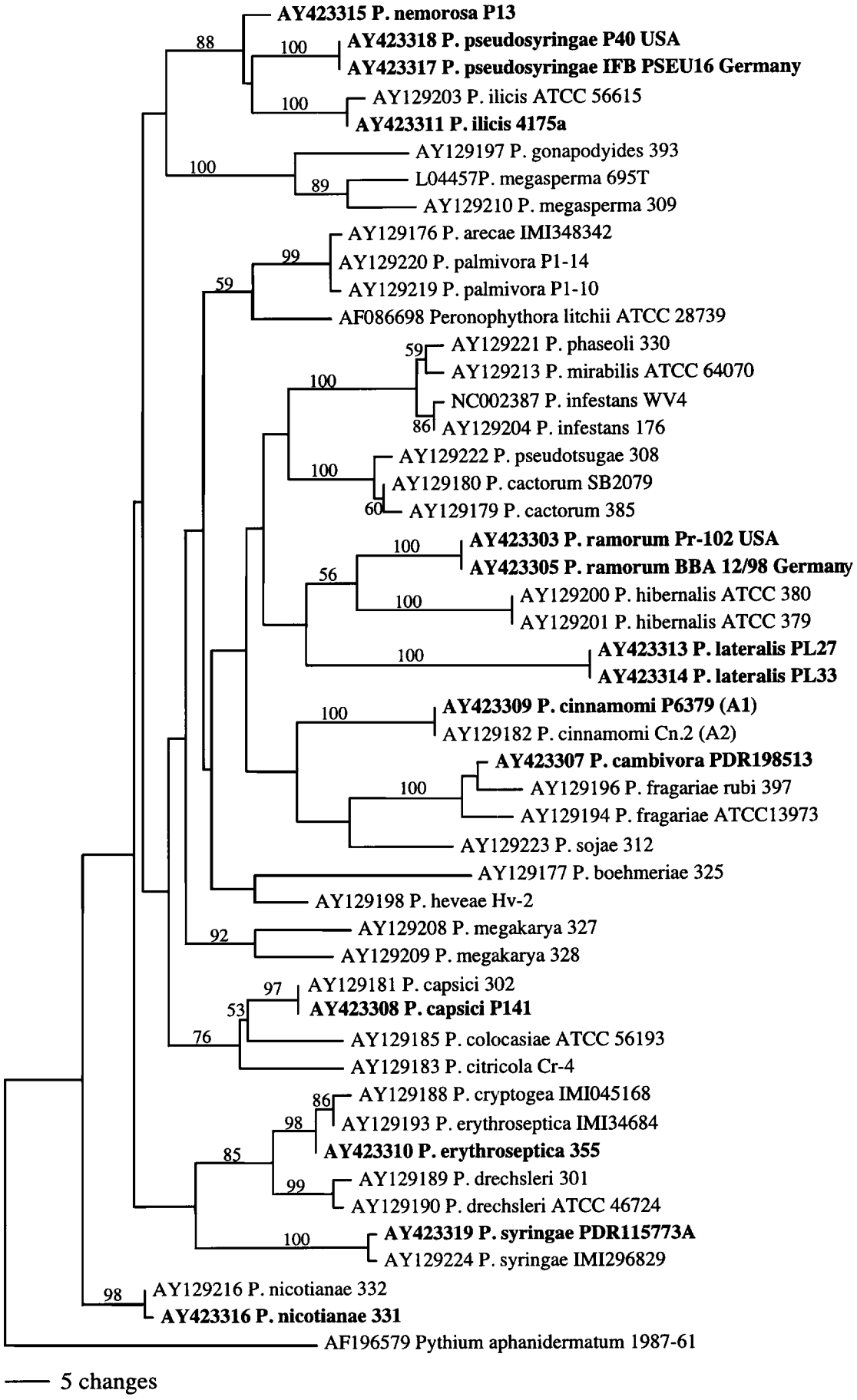

Fig. 3. For legend see opposite page.

With the exception of the two A1 Oregon nursery isolates Pr-3-74-1 and Pr-3-74-2, AFLP analysis showed a clear distinction between USA and European isolates (100% bootstrap support). The two Oregon nursery isolates clustered within the European population, as reported by Hansen *et al.* (2003). In all, 31 distinct AFLP genotypes were identified among all 85 *P. ramorum* isolates. 16 AFLP fingerprints were observed among USA isolates, with a single clone genotype dominating the population and representing over 75% of all USA isolates. This genotype was widely distributed and represented isolates from all 11 California counties, as well as Curry County, Oregon. Unique USA genotypes were only observed once, except with isolates Pr-52 and Pr-72 (from the same nursery in Santa Cruz County, CA) sharing the same AFLP fingerprint. Among the 18 European isolates analysed, 15 AFLP fingerprints were observed. Most European genotypes were unique, however two AFLP types recurred in different countries. Isolates Phyram1 (Spain), BBA 16/99 (Germany) and BBA 69082 (Germany), and isolates CSL 1652 (UK) and BBA 12/98 (Germany) shared the same AFLP fingerprints. Isolates BBA 16/99 and BBA 69082 had identical AFLP profiles, whereas in a previous AFLP study using different enzyme and primer combinations they were shown to be distinct (Werres *et al.* 2001).

MultiLocus analysis

The distribution of AFLP bands among all isolates, as well as among the two separate *Phytophthora ramorum* geographic populations, was analysed to determine whether there was any evidence that recombination may have affected the population structure over the time and area represented by the samples. The index of association, I_A, has an expected value of zero if there is no association of alleles at unlinked loci as assumed in a randomly mating population, therefore high within-population levels of linkage disequilibria are expected in largely clonal species. At the species level, the observed I_A value ($I_A = 19.297$) for all *P. ramorum* isolates was significantly higher ($P = < 0.001$) than the I_A calculated from 1000 artificially recombined datasets. Therefore these results do not confirm recombination events among the entire selection of isolates; i.e. results suggest the two geographic populations are not recombining. However, when populations were considered separately, the observed I_A across loci in the USA population ($I_A = -0.126903$) fell within the distribution for randomized datasets, indicating no significant correlation ($P = 0.607$) of alleles across loci. Likewise, the observed I_A across loci in the European population ($I_A = 0.420827$) fell within the distribution for randomized datasets, also indicating no significant correlation ($P = 0.117$) of alleles across loci. Hence, the null hypothesis of recombination within each individual geographic population could not be rejected. Since the recent Oregon nursery A1 isolates were determined to be of separate and distinct origins than other USA isolates (Hansen *et al.* 2003), they were not considered part of the USA or European population and were excluded from the individual population MultiLocus analyses.

Sequence analysis

The ITS, *cox* II and *nad* 5 regions of *Phytophthora ramorum* isolates representing the 31 different AFLP genotypes were sequenced to determine if they had any detectable sequence polymorphisms. However, all sequenced USA and European isolates had identical ITS, *cox* II and *nad* 5 sequences and showed no nucleotide variation. ITS rDNA trees, constructed by a heuristic search based on maximum parsimony, placed *P. ramorum* within ITS subclade 8a (Cooke *et al.* 2000) along with *P. lateralis* and *P. hibernalis* with strong bootstrap support; *P. ramorum* was more distantly related to species such as *P. syringae*, *P. drechsleri*, and *P. cryptogea* (Fig. 2). The topology of the 1891 most parsimonious trees generated by the heuristic search were almost identical except for branch lengths, and were similar to a majority-rule consensus tree. Of the 878 nucleotides analysed, ITS sequences differed by 11 nucleotides between *P. ramorum* and *P. lateralis*, and by 45 nucleotides between *P. ramorum* and *P. hibernalis*.

No gaps were observed in either mtDNA-encoded *cox* II and *nad* 5 sequence alignment, and all information for parsimony analysis was inferred from base pair substitutions. Most of the major nodes were unresolved for the *cox* II and *nad* 5 gene trees, although branches for some clades and species groupings had moderate to strong bootstrap support (Figs 3–4). Phylogenetic relationships within the clades described by ITS were mostly lost within trees produced by mtDNA sequences; these trees were not largely discordant with ITS, but less resolved. The three species *P. ramorum*, *P. lateralis* and *P. hibernalis* appeared as a cluster of related species; however, there was low bootstrap support for such relationships due to this loss of resolution. No intraspecific variation was observed in these gene regions for the two related species

Fig. 3. Phylogenetic relationships among *Phytophthora* species using *cox* II DNA sequence data, based on maximum parsimony inferred by a heuristic tree search. Numbers near branches are the percentage of the trees from bootstrap analysis that support the observed topography (values above 50% indicated). This is one of 24 trees with the shortest length. Of the 568 total characters, 394 were constant, 39 were variable and parsimony-uninformative, and 135 were parsimony-informative. Tree length = 531, consistency index (CI) = 0.482, homoplasy index (HI) = 0.518, retention index = 0.720. The sequences of taxa in bold were generated for this study.

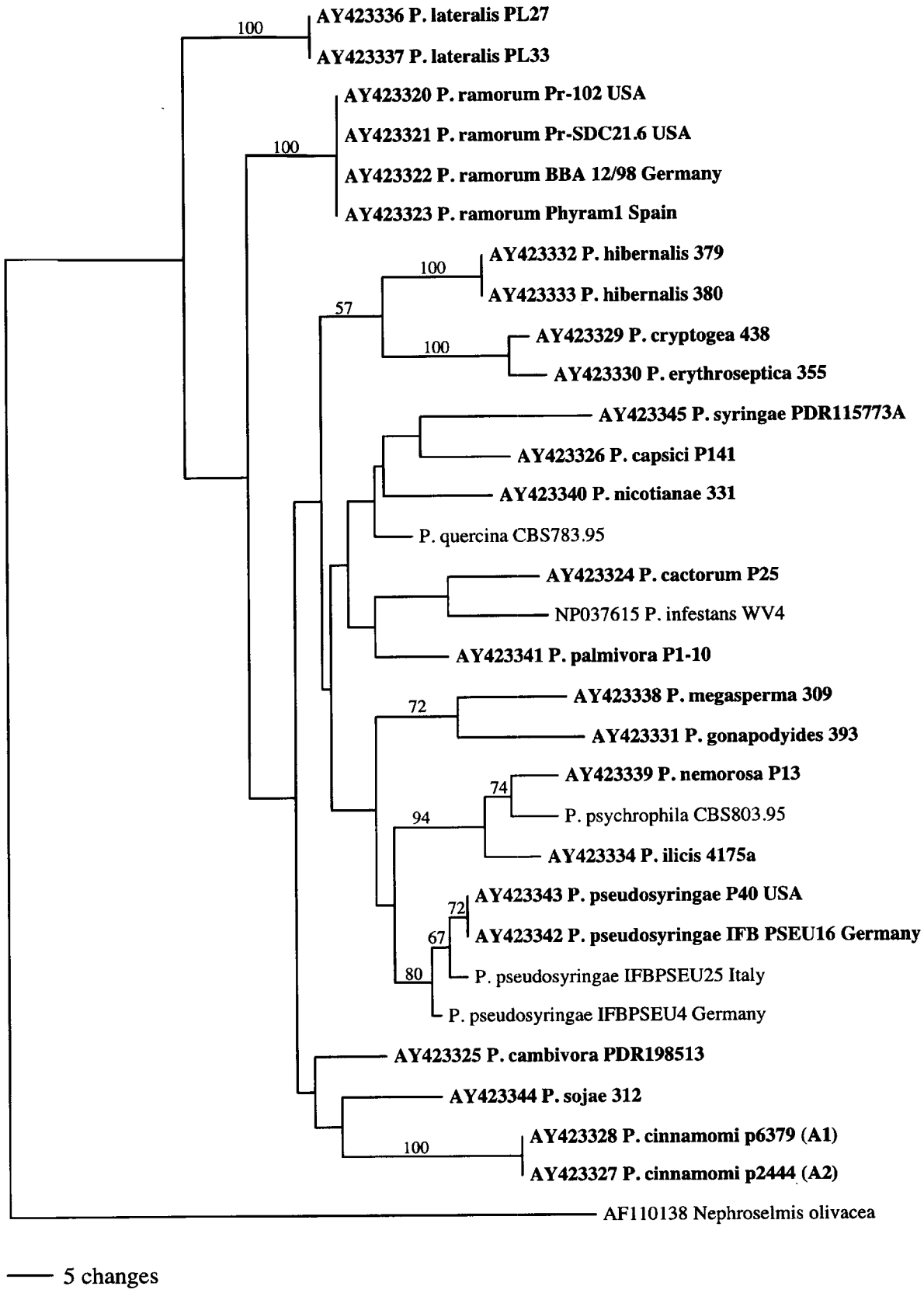

Fig. 4. Phylogenetic relationships among *Phytophthora* species using *nad* 5 DNA sequence data, based on maximum parsimony inferred by a heuristic tree search. Numbers near branches are the percentage of the trees from bootstrap analysis that support the observed topography (values above 50% indicated). This is the single most parsimonious tree. Of the 388 total characters, 243 were constant, 65 were variable and parsimony-uninformative, and 80 were parsimony-informative. Tree length = 383, consistency index (CI) = 0.525, homoplasy index (HI) = 0.475, retention index = 0.631. The sequences of taxa in bold were generated for this study.

P. hibernalis and *P. lateralis*. Phylogenetic analysis of all three datasets combined was not performed, as the results of a partition homogeneity test in a previous study suggested that combining datasets from ITS and *cox* II sequences may not be justifiable (Martin & Tooley 2003).

DISCUSSION

Our AFLP profiles demonstrate a low level of genetic variation in both USA and European *Phytophthora ramorum* populations. Most of the variation among isolates within each population was due to a very limited number of differences (absence or presence) in fragment profiles. The greatest differences were between the USA and European populations, which clustered into two distinct phylogenetic lineages. When AFLP similarity within individual populations from each continent was calculated, values were much higher than those reported for sexually reproducing populations (Péros, Berger & Lahogue 1997, Lamour & Hausbeck 2001), suggesting that most of the AFLP variability within the species is due to continental provenance rather than to true population subdivision. Overall similarity of banding patterns between the two populations confirms they belong to the same species and are distinct from the closely related species *P. lateralis*. In addition, all sequenced USA and European isolates of *P. ramorum* had identical ITS, *cox* II, and *nad* 5 sequences. This indicates a high degree of relatedness and common ancestry within this species. It may mean that *P. ramorum* has more recently evolved or that the amplified regions are highly conserved within its genome. Some variability within *Phytophthora* species was observed in the mtDNA gene regions; hence such loci could be useful for studies investigating intraspecific variation (Jung *et al*. 2003, Martin & Tooley 2003). The multiple gene phylogenies generated in our study are limiting because they do not provide high resolution of genetic relationships among the close relatives of *P. ramorum*. However, these phylogenies support the view that *P. ramorum* shares recent common ancestors with, but is distinct from *P. lateralis* and *P. hibernalis*.

In the USA, a single AFLP genotype comprised 75% of the isolates and was recovered throughout the known natural geographic range of the pathogen. This suggests a primarily clonal USA population structure of *P. ramorum*. No correlation was demonstrated between host and AFLP genotype, supporting the idea of host non-specificity, which has also been suggested by previous inoculation studies (Garbelotto *et al*. 2003). In comparison to the USA, European isolates clustered as a number of unique, but closely related, lineages. The higher amounts of intraspecific variation in the European population suggest the introduction of multiple strains into Europe, a longer residence time in Europe, or different selection pressures (e.g. different conditions in European nurseries compared to wild land settings in USA). There were no correlations between geography and AFLP genotype within the European population. This lack of geographic structuring may be due to the relatively recent introduction of this pathogen into Europe and(or) to the human-related gene flow from frequent movement of *P. ramorum* on infected plant material. The probable role of plant trade pathways in the creation of an 'artificial' panmictic population at the continental level is highlighted by the observation that two AFLP types were found more than once within Europe, and in different countries.

In contrast with our AFLP results, recent phenotypic studies of *P. ramorum* indicate that USA isolates are more variable in morphology, growth rate and virulence than European isolates (Brasier *et al*. 2002, Pogoda & Werres 2002, Brasier 2003). In addition, a recent study detected differences in sporangial morphology between isolates from the USA and Europe (D. Hüberli & M.G., unpubl.). The lack of correlation among phenotypic and genotypic variation has been demonstrated in other *Phytophthora* species (Dudzinski, Old & Gibbs 1993, Abu-El Samen, Secor & Gudmestad 2003). Phenotypic variation within a molecular clone was also reported for *P. cinnamomi* in Western Australia (Hüberli *et al*. 2001). The continuous range of variability in phenotypic characters rather than discrete groupings suggests such traits may be multigenic; consequently, an AFLP or microsatellite clone should not be considered a genetic clone (Brasier 2003). It is possible that the adaptive genes conferring such fitness variables and those associated with our AFLP fingerprints may be evolving at different rates (Brasier 2003).

One potential hypothesis to explain the source of the limited AFLP variation in *P. ramorum* populations is that the level of genetic variation seen within each geographic population has arisen via asexual mechanisms. Linkage disequilibrium was detected by computation of the I_A when all isolates were treated as a single population. Within the limits of the isolates available for this study, this result strongly suggests that recombination has not been occurring between the two continents, and hence between the two mating types. Alternatively, the genetic variation seen within each geographic population may have arisen *via* asexual mechanisms, such as mutation and(or) mitotic recombination; we were unable to exclude this possibility. Mitotic crossing-over produces much lower levels of genetic variation in comparison to sexual recombination. When pairwise Jaccard similarity values for the AFLP data were extracted in all combinations and plotted by geographic population, frequency distribution was not informative (data not shown). Due to the high similarity among isolates, the distribution of pairwise data appeared as a tall, narrow peak rather than a unimodal or bimodal Gaussian curve (Redecker *et al*. 2001), suggesting that genotypes in each population were not generated by true recombination, but by other means as suggested above.

Evidence of mitotic recombination in *Phytophthora* species has been previously reported (Sansome 1980, Goodwin, Cohen & Fry 1994, Abu-El Samen, Secor & Gudmestad 2003, Dobrowolski *et al*. 2003) and could explain the slight variations observed in our AFLP profiles within each population. *P. ramorum* is known to rapidly reproduce asexually through the formation of large numbers of sporangia, which either germinate directly or differentiate into motile zoospores. A recent study reported considerable levels of inherent genetic variability among single-zoospore isolates of *P. infestans* asexually derived from the same parent culture (Abu-El Samen, Secor & Gudmestad 2003) and implicated mitotic recombination as a possible cause.

Another hypothesis is that sexual recombination has generated the observed AFLP variation. In the populations surveyed, the present data cannot rule out the possibility of sexual recombination in *P. ramorum* populations, but indicate that sexual recombination is restricted, and highlighted by mating type segregation within each population. In a population where sexual reproduction is frequent, the two mating types are expected to occur in similar numbers (Anagnostakis 1988), unless one mating type demonstrates reduced fitness. The underlying equilibrium among loci within each population could be indicative of earlier recombination events. It is also possible that *P. ramorum* exhibits an 'epidemic' population structure (Maynard Smith *et al*. 1993) within the USA, meaning that a highly successful individual from an originally heterogeneous sexual population increases rapidly, subsequently producing an epidemic clone, possibly due to clonal or episodic selection (Brasier 1995). It should be noted, however, that in populations with low genetic diversity it is not possible to estimate the importance of sexual and asexual reproduction with DNA fingerprints alone (Hoegger *et al*. 2000). Codominant markers, including microsatellite loci, should provide a better understanding of the contribution of mitotic recombination to overall population structure of *P. ramorum* populations.

The predominance of a single mating type and low levels of genotypic diversity within each geographic population support the hypothesis that *P. ramorum* was recently introduced into both North America and Europe. Lower geographical population structure and overall genetic diversity is expected for a disease that has emerged due to a recent introduction or translocation (Morehouse *et al*. 2003). However, the observed phenotypic differences in isolates, in conjunction with mating type segregation, support the hypothesis of separate introductions onto both continents from a third, as yet unknown, location. The existence of *P. ramorum* elsewhere is not documented, although a thorough search of hosts on other continents has not been conducted. A direct European-North American route, followed by differential selection of phenotypes on the two individual continents is also possible. The establishment of *P. ramorum* on both continents is most likely due to increased movement of plants from the wild into cultivation, and from one part of the world to another through the international nursery trade.

Opportunities for sexual recombination and gene flow between the A1 and A2 types have probably been limited in North America and Europe, as it appears that these mating types have been geographically separated until recently, with no current evidence of gene flow between the two populations. The recent discovery that A1 isolates in an Oregon nursery located outside the known natural range of *P. ramorum*, represented by isolates Pr-3-74-1 and Pr-3-74-2 in our analyses, share very similar AFLP profiles with European isolates, as supported by Hansen *et al*. (2003), indicates similar origins. Although introduction from a third location cannot be excluded, a European–North American pathway for movement of these genotypes is also possible. This possibility, combined with the evidence of genetically and phenotypically distinct European and North American lineages supported by our results and other studies, highlights the need for regulatory control of plant trade between different continents.

A centre of origin has been unequivocally demonstrated for only a few *Phytophthora* species (Tooley, Fry & Villareal Gonzalez 1985, Zentmyer 1988). The origin of many presumed exotic species of *Phytophthora* is unknown. For example, *P. lateralis* is assumed to be an introduced forest pathogen in the USA but more than 80 yr after it was first identified, the origin of this pathogen is still undetermined (Hansen *et al*. 2000). Although represented by only one species in North America, 100–200 species of the genus *Lithocarpus* are distributed throughout eastern and southern Asia and Indomalaysia (Little 1979). Several other reported hosts of *P. ramorum* have their origins in Asia, including *Rhododendron* and *Viburnum*, thus an Asiatic origin for this plant pathogen seems plausible. More survey and population studies are required before the origin of *P. ramorum* can be determined. With a better understanding of the epidemiology, evolution and population biology of *P. ramorum*, regulatory solutions can be developed for the continued spread and management of the disease in sensitive uninfected forests and commercial nurseries.

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REFERENCES

Abu-El Samen, F. M., Secor, G. A. & Gudmestad, N. C. (2003) Genetic variation among asexual progeny of *Phytophthora infestans* detected with RAPD and AFLP markers. *Plant Pathology* **52**: 314–325.

Agapow, P. M. & Burt, A. (2001) Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* **1**: 101–102.

Anagnostakis, S. L. (1988) *Cryphonectria parasitica* cause of chestnut blight. *Advances in Plant Pathology* **6**: 123–136.

Bonants, P. J. M., Hagenaar-de Weerdt, M., van Gent-Pelzer, M. P. E., Lacourt, I., Cooke, D. E. L. & Duncan, J. M. (1997) Detection and identification of *Phytophthora fragariae* Hickman by the polymerase chain reaction. *European Journal of Plant Pathology* **103**: 345–355.

Brasier, C. M. (1995) Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. *Canadian Journal of Botany* **73** (Suppl. 1): S1213–S1221.

Brasier, C. M., Rose, J., Kirk, S. A. & Webber, J. F. (2002) *Pathogenicity of Phytophthora ramorum isolates from North America and Europe to bark of European Fagaceae, American Quercus rubra and other forest trees. Paper abstract. Sudden Oak Death Science Symposium, 15–18 December 2002 in Monterey, CA*. http://danr.ucop.edu/ihrmp/sodsymp/paper/paper09.html.

Brasier, C. M. (2003) Sudden oak death exhibits transatlantic differences. *Mycological Research* **107**: 258–259.

Casgrain, P. & Legendre, P. (1999) *The R package for multivariate and spatial analysis*. University of Montreal, Montreal.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. & Brasier, C. M. (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30**: 17–32.

Davidson, J. M., Werres, S., Garbelotto, M., Hansen, E. M. & Rizzo, D. M. (2003) Sudden oak death and associated diseases caused by *Phytophthora ramorum*. Online. *Plant Health Progress* doi: 10.1094/PHP-2003-0707-01-DG.

Dobrowolski, M. P., Tommerup, I. C., Chearer, B. L. & O'Brien, P. A. (2003) Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. *Phytopathology* **93**: 695–704.

Dudzinski, M. J., Old, K. M. & Gibbs, R. J. (1993) Pathogenic variability in Australian isolates of *Phytophthora cinnamomi*. *Australian Journal of Botany* **41**: 721–732.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Garbelotto, M., Davidson, J. M., Ivors, K., Maloney, P. E., Hüberli, D., Koike, S. T. & Rizzo, D. M. (2003) Non-oak native plants are main hosts for sudden oak death pathogen in California. *California Agriculture* **57**: 18–23.

Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N. & Fry, W. E. (1992) Clonal diversity and genetic recombination of *Phytophthora infestans* populations in northern and central Mexico. *Phytopathology* **82**: 955–961.

Goodwin, S. B., Cohen, B. A. & Fry, W. E. (1994) Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences, USA* **91**: 11591–11595.

Hansen, E. M., Goheen, D. J., Jules, E. S. & Ullian, B. (2000) Managing Port-Orford-Cedar and the introduced pathogen *Phytophthora lateralis*. *Plant Disease* **84**: 4–10.

Hansen, E. M., Reeser, P. W., Sutton, W., Winton, L. & Osterbauer, N. (2003) First report of A1 mating type of *Phytophthora ramorum* in North America. *Plant Disease* **87**: 1267.

Hoegger, P. J., Rigling, D., Holdenrieder, O. & Heiniger, U. (2000) Genetic structure of newly established populations of *Cryphonectria parasitica*. *Mycological Research* **104**: 1108–1116.

Jaccard, P. (1908) Nouvelles recherches sur la distribution florale. *Bulletin de la Société Vaudoise des Sciences Naturelles* **44**: 223–270.

Jung, T., Nechwatal, J., Cooke, D. E. L., Hartmann, G., Blaschke, M., Oswald, W. F., Duncan, J. M. & Delatour, C. (2003) *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. *Mycological Research* **107**: 772–789.

Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001) Mega2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**: 1244–1245.

Lamour, K. H. & Hausbeck, M. K. (2001) Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. *Phytopathology* **91**: 973–980.

Linde, C., Drenth, A., Kemp, G. H. J., Wingfield, M. J. & von Broembsen, S. L. (1997) Population structure of *Phytophthora cinnamomi* in South Africa. *Phytopathology* **87**: 822–827.

Little, E. L. jr. (1979) *Checklist of United States Trees* (*Native and Naturalized*). [Agriculture Handbook no. 541.] USDA, Washington, DC.

Martin, F. N. (2000) Phylogenetic relationships among some *Pythium* species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. *Mycologia* **92**: 711–727.

Martin, F. N. & Tooley, P. W. (2003) Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. *Mycologia* **95**: 269–284.

Maynard Smith, J., Smith, N. H., O'Rourke, M. & Spratt, B. G. (1993) How clonal are bacteria? *Proceedings of the National Academy of Sciences, USA* **90**: 4384–4388.

Moralejo, E. & Werres, S. (2002) First report of *Phytophthora ramorum* on *Rhododendron* sp. in Spain. *Plant Disease* **86**: 1052.

Morehouse, E. A., James, T. Y., Ganley, A. R. D., Vilgalys, R., Berger, L., Murphy, P. J. & Longcore, J. E. (2003) Multilocus sequence typing suggests the chytrid pathogen of amphibian is a recently emerged clone. *Molecular Ecology* **12**: 395–403.

Péros, J.-P., Berger, G. & Lahogue, F. (1997) Variation in pathogenicity and genetic structure in the *Eutypa lata* population of a single vineyard. *Phytopathology* **87**: 799–806.

Pogoda, F. & Werres, S. (2002) *Pathogenicity of European and American Phytophthora ramorum isolates to Rhododendron. Poster abstract. Sudden Oak Death Science Symposium, 15–18 December 2002 in Monterey, CA*. http://danr.ucop.edu/ihrmp/sodsymp/poster/poster26.html.

Rambaut, A. (1996) *Se-Al: Sequence Alignment Editor*. http://evolve.zoo.ox.ac.uk/software.html.

Redecker, D., Szaro, T. M., Bowman, R. J. & Bruns, T. D. (2001) Small genets of *Lactarius xanthogalactus*, *Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. *Molecular Ecology* **10**: 1025–1034.

Rizzo, D. M., Garbelotto, M., Davidson, J. M., Slaughter, G. W. & Koike, S. (2002) *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Disease* **86**: 205–214.

Rizzo, D. M. & Garbelotto, M. (2003) Sudden oak death: endangering California and Oregon forest ecosystems. *Frontiers in Ecology and the Environment* **1**: 197–204.

Sansome, E. (1980) Reciprocal translocation heterozygosity in heterothallic species of *Phytophthora* and its significance. *Transactions of the British Mycological Society* **74**: 175–185.

Swofford, D. L. (2002) *PAUP*: phylogenetic analysis using parsimony* (* *and other methods*). Version 4. Sinauer Associates, Sunderland, MA.

Tooley, P. W., Fry, W. E. & Villareal Gonzalez, M. J. (1985) Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. *Journal of Heredity* **76**: 431–435.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M.

(1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407–4414.

Werres, S., Marwitz, R., Man in't Veld, W. A., De Cock, A. W. A. M., Bonants, P. J. M., de Weerdt, M., Themann, K., Ilieva, E. & Baayen, R. P. (2001) *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycological Research* **105**: 1155–1165.

Werres, S. & de Merlier, D. (2003) First detection of *Phytophthora ramorum* mating type A2 in Europe. *Plant Disease* **87**: 1266.

Werres, S. & Zielke, B. (2003) First studies on the pairing of *Phytophthora ramorum*. *Journal of Plant Disease and Protection* **110**: 129–130.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a guide to methods and applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. Academic Press, San Diego.

Zentmyer, G. A. (1988) Origin and distribution of four species of *Phytophthora*. *Transactions of the British Mycological Society* **91**: 367–378.

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