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Low genetic variation detected in New Zealand populations of *Phaeomoniella chlamydospora*

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Summary. *Phaeomoniella chlamydospora* is considered to be the causal agent of Petri disease. This disease causes decline of grapevines in most grape growing regions of the world. Genetic variation within 39 New Zealand isolates of *Phaeomoniella chlamydospora* was compared to six isolates from Italy using randomly amplified polymorphic DNA (RAPD), randomly amplified microsatellites (RAM), amplified fragment length polymorphism (AFLP), and universally primed polymerase chain reaction (UP-PCR). Using each method, genetic variation within New Zealand and Italian isolates of *P. chlamydospora* was shown to be low, with a maximum of seven genetic groups identified by each primer. The greatest amount of genetic variation was shown using AFLP analysis, with 21 different groups identified. RAPD, AFLP and UP-PCR primers detected inter-vineyard, intra-vineyard and intra-vine variation of New Zealand isolates. A subset of five New Zealand and one Italian isolate was further investigated using mycelial compatibility groups (MCGs). One MCG was identified, supporting low genetic variation within *P. chlamydospora* isolates. Low genetic variation within the New Zealand and the Italian populations suggests that asexual reproduction predominates, and the presence of intra-vineyard and intra-vine variation in New Zealand indicates that multiple introductions have occurred.

Key words: Petri disease, DNA polymorphism, genetic variation, mycelial compatibility groups.

Introduction

Esca and esca-like syndromes are devastating diseases of mature grapevines in Europe and North America. Esca is a complex disease involving a succession of different fungi that culminates in white rot of the vine trunk. One of the most common fungi associated with esca-like diseases is *Phaeomoniella chlamydospora* (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Pascoe *et al*., 2000). Traditional methods have yielded some information on the morphology, mode of reproduction, mode of dispersal and possible toxin production of this fungal pathogen (Crous and Gams, 2000; Evidente *et al*., 2000; Tabacchi *et al*., 2000). Since 1998, molecular methods (Dupont *et al*., 1998; Groenewald *et al*., 2000), together with morphological (Crous and Gams, 2000) and pathological criteria (Scheck *et al*., 1998; Ferreira *et al*., 1999), have taxonomically categorised *P. chlamydospora* and identified it as the causal organism of Petri disease (Mugnai *et al*., 1999; Surico *et al*., 2000). Further molecular analysis using RAPD and RAMS provided information about genetic variation in Italian, South African and American isolates (Tegli *et al*., 2000). These studies have indicated low genetic variation within Italian *P. chlamydospora* isolates, support-

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ing the hypothesis that this pathogen predominantly reproduces asexually (Tegli, 2000).

In this study, a cohort of four molecular techniques and one macroscopic technique were used to determine genetic variation in *P. chlamydospora*. RAPD and RAMs analysis can reveal extensive molecular variation in closely related taxonomic groups and have been used informatively in many genera and species of *Penicillium*, *Aspergillus* and *Trichoderma* (Majer *et al*., 1996), *Eutypa lata* (Péros and Larignon, 1998), *Scytalidium thermophilum* (Lyons *et al*., 2000), *Gremmeniella abietina* (Hantula and Müller, 1997), *Phlebiopsis gigantea* (Vainio and Hantula, 2000), and *Venturia* spp. (Kasanen *et al*., 2001). Universally primed polymerase chain reaction (UP-PCR) is similar to RAPD analysis; however, unlike RAPDs, its primers are longer and target intergenic, more variable areas of the genome (Bulat *et al*., 1998). UP-PCR has been used to study isolates of *Trichoderma* and *Gliocladium* (Bulat *et al*., 1998), *Ascochyta pisi* (Lübeck *et al*., 1998) and *Fusarium avenaceum* (Yli-Mattila *et al*., 1997). In contrast to RAPD, RAM and UP-PCR, AFLP involves amplification of subgroups of fragments obtained by restriction digestion, and therefore inspects the entire genome for polymorphism (Blears *et al*., 1998). AFLP has been used in many genetic studies of fungi including the *Melampsora epitea* complex (Samils *et al*., 2001), *Cladosporium fulvum* (syn. *Fulvia fulva* [Cooke] Cif.) and *Pyrenopeziza brassicae* (Majer *et al*., 1996). In addition, MCGs were determined for *P. chlamydospora,* which involved a multi-loci analysis of genetic relatedness (Saupe, 2000). When mycelia of genetically distinct isolates from the same species confront one another, a distinct zone of demarcation can develop between the colonies (Worrall, 1997). Isolates that exhibit a compatible reaction with one another can be placed in the same MCG, the members of which are presumed to be more genetically similar to one another. As such, this test is a macroscopic measure of genetic variability (Leslie, 1993).

This paper describes genetic variation among New Zealand populations. The research used the techniques outlined above to determine variation within a group of isolates representative of New Zealand vineyards and this diversity was compared to that of Italian isolates.

Materials and methods

Fungal isolates and growth conditions

Thirty-nine isolates of *P. chlamydospora* were isolated from grapevine wood cores taken from six vineyards covering four geographically distinct regions in New Zealand (Table 1). Six reference isolates (Tegli *et al*., 2000), isolated from five regions in Italy, were kindly provided by Laura Mugnai, University of Florence, Italy (Table 1). *P. chlamydospora* isolates were maintained as spore suspensions and stored at –80ºC in the Lincoln University Culture Collection.

For genomic DNA extraction, the spore suspension of each isolate was streaked onto potato-dextrose agar (PDA) plates (Difco Laboratories, Sparks, MD, USA) and incubated for 4–7 d at 22ºC under diurnal conditions. Approximately 10–15 0.5 cm^2 mycelial plugs derived from the PDA plates were added to 100 ml potato-dextrose broth (PDB) (Difco Laboratories) and placed in a KS250 basic automatic shaker (IKA Labortechnik, Staufen, Germany) at 150 rpm, at approximately 20ºC for 7–10 d. Mycelium was harvested, wrapped in aluminium foil, snap-frozen in liquid nitrogen and stored at –80ºC.

DNA extraction

DNA was extracted from 0.1 g of ground frozen mycelium using the Nucleon™ PhytoPure™ Plant DNA extraction kit (Amersham Lifescience, Buckinghamshire, England) according to manufacturer's instructions. DNA precipitation was facilitated by placing the solution at –20ºC for 10 min. The DNA pellet was rehydrated in 50 µl of water and allowed to resuspend at 4ºC overnight. The quantity of genomic DNA was estimated using 1% agarose gel electrophoresis and concentration was adjusted to 10 ng ml^{-1} prior to amplification with the RAPD, RAM or UP-PCR primers.

RAPD, RAM and UP-PCR analyses

RAPD and RAM analyses were performed as described by Tegli *et al*. (2000). RAM was performed as described for the RAPD method except that 100 pmoles of microsatellite primers were used in each reaction. Amplification was performed in an Eppendorf® Mastercycler® Gradient apparatus (Eppendorf®, Hamburg, Germany). The UP-PCR amplification mixtures were as described for RAPDs

Table 1. Origin of *Phaeomoniella chlamydospora* isolates used in this study.

Isolate	Origin	Year of isolation	Rootstock	Row	Vine	Vine position
A1	Auckland, NZ	2000	3309	30	27	Basal Top
A2	"	"	3309	30	28	Basal Bottom
A3	"	"	3309	30	3	Basal Top
A4	"	"	3309	30	18	Basal Top
A5	"	"	Schwarzman	2	79	Basal Top
A6	"	"	Schwarzman	2	31	Basal Top
A7	"	"	101-14	18	19	Basal Bottom
A8	"	"	101-14	3	29	Basal Bottom
A9	"	"	101-14	3	31	Basal Top
A10	"	"	Schwarzman	2	11	Basal Top
A11	"	"	3309	30	5	Basal Bottom
A12	"	"	101-14	3	31	Basal Bottom
A14	"	"	101-14	18	18	Basal Bottom
A15	"	"	101-14	7	6	Basal Bottom
A16	"	"	101-14	1F	3	Basal Bottom
A17	"	"	3309	30	18	Basal Bottom
A18	"	"	3309	30	5	Basal Top
A19	"	"	101-14	7	59	Basal Bottom
A20	"	"	3309	30	16	Basal Top
A21	"	"	101-14	3	29	Basal Top
A22	"	"	Schwarzman	2	31	Basal Top
A23	"	"	3309	30	5	Basal Bottom
A24	"	"	101-14	3	28	Basal Bottom
A26	"	"	101-14	7	61	Basal Top
B1	Blenheim (1), NZ	"	101-14	88	19	Base
B2	"	"	Schwarzman	28	25	Bottom
B3	"	"	Schwarzman	28	25	Top
Bcb1	Blenheim (2), NZ	"	3309	10	5	Right
Bcb2	"	"	3309	10	2	Bottom
Pch6	Blenheim (3), NZ	"	3309	Unknown	Unknown	Unknown
Pch8	"	"	5C	"	"	"
Pch 21	"	"	SO4	"	"	"
Pch 34	"	"	5C	"	"	"
Pch35	"	"	3309	"	"	"
Pch 66	"	"	SO4	"	"	"
G1	Gisborne, NZ	"	101-14	5	6/7	Top
G2	"	"	Riparia	2	5/7	Top
G3	"	"	101-14	2	5/7	Bottom
Linc.Vine	Canterbury, NZ	1999	3309	Unknown	Unknown	Unknown
Bb13	Tuscany, Italy	1995	Unknown	P13	Unknown	Unknown
Bb32	"	"	"	P32	"	"
113.I.95	Lombardy, Italy	"	"	I	"	"
191.95	Umbria, Italy	"	"	N	"	"
1091.95	Veneto, Italy	"	"	Ven2	"	"
1121.95	Sicily, Italy	"	"	Sic	"	"

except that the reaction was carried out in 2.5 mM $MgCl_2$. The UP-PCR cycling parameters were as follows: denaturation at 94ºC for 5 min, then 5 cycles of 94ºC for 50 s, annealing at the respective temperature (Table 2) for 2 min and primer extension at 72ºC for 1 min, followed by 34 cycles of 94ºC for 50 s, annealing at the respective temperature (Table 2) for 90 s, and primer extension at 72ºC for 1 min, with a final extension at 72ºC for 10 min. All amplification products were separated by 1% agarose gel electrophoresis. The sequence of each RAM, RAPD and UP-PCR primer and the respective annealing temperatures are shown in Table 2.

AFLP analysis

The procedure for AFLP was modified from that of Pei and Ruiz (2000). Genomic DNA (200 ng) was digested with 5 U of *Eco*RI and *Tru*9I according to manufacturer's instructions. *Eco*RI and *Mse*I adapters were ligated to the digested DNA and pre-amplification was performed using either Eco-A/Mse-C or Eco-GA/Mse-C primer pairs. Each 25 µl pre-amplification mixture contained 5 ng of digested and ligated genomic DNA, 15 pmol of each primer, 200 µM each of dATP, dTTP, dGTP and dCTP, 1× PCR buffer (Roche Diagnostics, Mannheim, Germany), 2 mM $MgCl_2$, and 1.25 U Taq DNA polymerase (Roche Diagnostics). Pre-amplification was performed in an Eppendorf® Mastercycler® Gradient (Eppendorf) thermocycler using the profile of 20 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min. The resulting amplification products were diluted 1 in 10 and used in the second amplification reaction. In the second amplification, *Eco*RI and *Mse*I primers with 2 and 3 bp 3' extensions respectively, were used in combination (Eco-AA + Mse-CAT; Eco-GA + Mse-CAT). Each 25 µl reaction mixture contained 1 µl of diluted pre-amplification product, 15 pmol of each primer, 200 µM each of dATP, dTTP, dGTP and dCTP, 1× PCR buffer (Roche Diagnostics), 2 mM $MgCl_2$, and 1.25 U Taq DNA polymerase (Roche Diagnostics). Amplification was performed using the profile: cycle 1, 94°C for 30 s, 65°C for 30 s and 72°C for 1 min; cycles 2–9, as in cycle 1, but the annealing temperature was dropped progressively 1°C in each cycle (64–56°C); cycles 10–34, 94°C for 30 s, 56°C for 30 s and 72°C for 1 min. The PCR products were denatured, separated on 20 cm 5% polyacrylamide gels at 150 V for 3 h in 1× TBE (89 mM Tris borate, pH 8.0, 20 mM EDTA) and visualised by ethidium bromide staining.

Table 2. Nucleotide sequences of RAPD, RAM, and UP-PCR primers used in this study.

Primer	Primer sequence	Annealing temperature (ºC)
RAPD		
OP-B12	$^{5'}$CCTTGACGCA$^{3'}$	36
OP-B14	$^{5'}$TCCGCTCTGG$^{3'}$	36
OP-B18	$^{5'}$CCACAGCAGT$^{3'}$	36
OP-B19	$^{5'}$ACCCCCGAAG$^{3'}$	36
RAM		
CGA	$^{5'}$DHB$(CGA)_5{}^{3'}$	61
GT	$^{5'}$YHY$(GT)_5$G$^{3'}$	58
UP-PCR		
AA2M2	$^{5'}$CTGCGACCCAGAGCGG$^{3'}$	50
AS4	$^{5'}$TGTGGGCGCTCGACAC$^{3'}$	50
AS15	$^{5'}$GGCTAAGCGGTCGTTAC$^{3'}$	52
AS15inv	$^{5'}$CATTGCTGGCGAATCGG$^{3'}$	52
L15	$^{5'}$GAGGGTGGCGGTTCT$^{3'}$	52
L15/AS19	$^{5'}$GAGGGTGGCGGCTAG$^{3'}$	52
3-2	$^{5'}$TAAGGGCGGTGCCAGT$^{3'}$	52
L21	$^{5'}$GGATCCGAGGGTGGCGGTT$^{3'}$	55
L45	$^{5'}$GTAAAACGACGGCCAGT$^{3'}$	51

RAPD, RAM, UP-PCR and AFLP data analysis

Banding patterns produced by amplification with each of the respective primers were recorded as polaroid photographs and bands scored as present (1) or absent (0). In order to fully replicate the analyses, each DNA isolation, extraction and amplification was carried out on two independently derived spore suspensions for each *P. chlamydospora* isolate. Only strongly amplified, reproducible bands were scored. These data were used to compile a presence/absence binomial matrix. Pairwise similarities were calculated between samples using Jaccard's coefficient (Sneath and Sokal, 1973) of similarity S_J= a/(n-c), where: a, is the number of 1-1 matches; c, the number of 0-0 matches; n, the total number of bands compared in a pairwise manner. Jaccard's coeffecient was then used to construct a similarity matrix using the neighbour joining method as described by Tegli *et al*. (2000). For both AFLP and UP-PCR techniques, the PAUP* (Swofford, 1998) computer programme was used to determine relationships between individual isolates and these relationships were visualised as a neighbour joining dendrogram.

Mycelial compatibility groups (MCGs)

A small subset of isolates from different branches of the dendrograms were paired against each other on agar plates (Table 3). Mycelial plugs (0.5 cm^2) from the outer edge of 10-d-old cultures grown on PDA were paired either on modified Patterson's medium (MPM) (0.68 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.15 g KCl, 0.5 g yeast extract [Difco], 1 g NH_4NO_3, 18.4 g D-glucose, 0.2 ml Vogel's trace element solution, 15 g agar [Difco], and 6 drops of red food colouring [McCormick's] l^{-1}), as described by Kohn *et al*. (1991), or on PDA. Mycelial plugs were placed 1 cm apart in 5.5 cm Petri dishes, one pairing per dish (Table 3), and incubated at 22°C for 10–14 d under diurnal light.

Mycelial pairings were scored as incompatible if an aversion line was observed in the interaction zone between two strains 14 d after inoculation, or compatible when no reaction line was observed. Microscopic assessment of the interaction zone was performed by excising 5 mm agar discs encompassing the two opposing colony fronts, mounting in a drop of water and viewing at 100 and 400 × magnification under the light microscope.

Results

Analysis of banding patterns

RAPD and RAM

No genetic variation was detected using RAM analysis (data not shown). RAPD primer OP-B14 produced one genetic group while primers OP-B12, OP-B18 and OP-B19 each produced two genetic groups that differed by only one fragment (Fig. 1). RAPD primers OP-B12, OP-B14, OP-B18, and OP-B19 produced 3, 2, 5, and 3 bands respectively. The product sizes ranged from approximately 517 to 2,036 bp. The combined results from the RAPD analysis are summarised in Table 4. When results from all four RAPD primers were combined, six genetically distinct groups were identified.

UP-PCR

The UP-PCR primers typically produced more bands than either the RAPD or the RAM primers. Five of the nine UP-PCR primers, AA2M2, AS15, L15, L21, and L45, did not detect variation amongst the 45 isolates tested. The remaining four UP-PCR primers, AS4, AS15inv, L15/AS19, and 3-2, produced two genetically distinct groups (Fig. 2) that differed by one band. Scorable bands ranged in size from approximately 517 bp to 3,000 bp. Primers

Table 3. Pairings of isolates tested for mycelial compatibility.

Isolate	A11	A18	A8	A24	A12	Pch8
A11	A11:A11	A11:A18	A11:A8	A11:A24	A11:A12	A11:Pch8
A18		A18:A18	A18:A8	A18:A24	A18:A12	A18:Pch8
A8			A8:A8	A8:A24	A8:A12	A8:Pch8
A24				A24:A24	A24:A12	A2:Pch8
A12					A12:A12	A12:Pch8
Pch8						Pch8:Pch8

Table 4. Combined results from RAPD analysis indicating inter-vineyard, intra-vineyard and intra-vine variation between New Zealand and Italian isolates of *Phaeomoniella chlamydospora*[a].

Isolate	Row	Vine	OP-B12	OP-B14	OP-B18	OP-B19	RAPD group[b]
Auckland							
A10	2	11	B	A	A	B	1
A6	2	31	A	A	B	A	2
A22	2	31	A	A	B	B	3
A5	2	79	B	A	A	B	1
A24	3	28	A	A	A	A	4
A8	3	29	A	A	A	A	4
A21	3	29	B	A	A	B	1
A9	3	31	A	A	A	A	4
A12	3	31	A	A	A	A	4
A15	7	6	A	A	A	A	4
A19	7	59	A	A	A	A	4
A26	7	61	A	A	A	A	4
A14	18	18	A	A	A	B	5
A7	18	19	A	A	A	A	4
A3	30	3	B	A	A	A	6
A11	30	5	B	A	A	B	1
A18	30	5	B	A	A	A	6
A23	30	5	B	A	A	B	1
A20	30	16	A	A	A	B	5
A4	30	18	A	A	A	B	5
A17	30	18	A	A	A	A	4
A1	30	27	A	A	A	A	4
A2	30	28	A	A	A	A	4
A16	1F	3	A	A	A	A	4
Blenheim							
B1	88	19	A	A	A	A	4
B2	28	25	A	A	A	A	4
B3	28	25	A	A	A	A	4
Blenheim							
Bcb2	10	2	A	A	A	A	4
Bcb1	10	5	A	A	A	A	4
Blenheim							
Pch 6	-	-	A	A	A	A	4
Pch 8	-	-	A	A	A	A	4
Pch21	-	-	A	A	A	B	5
Pch 34	-	-	B	A	A	A	6
Pch 35	-	-	A	A	A	A	4
Pch 66	-	-	A	A	A	A	4
Gisborne							
G2	2	5,7	A	A	A	A	4
G3	2	5,7	A	A	A	A	4
G1	5	6,7	A	A	A	A	4
Lincoln							
Linc. Vine	-	-	A	A	A	A	4
Italy							
1091.951	-	-	A	A	A	A	4
191.95	-	-	B	A	A	B	1
Bb13	-	-	B	A	A	A	6
Bb32	-	-	A	A	A	A	4
113.I.95	-	-	B	A	A	A	6
1121.95	-	-	A	A	A	A	4

[a] For each individual primer, the letters A and B indicate the two groups that *P. chlamydospora* isolates have been categorised into.
[b] Genetically different groups identified when information from the individual primers were combined.

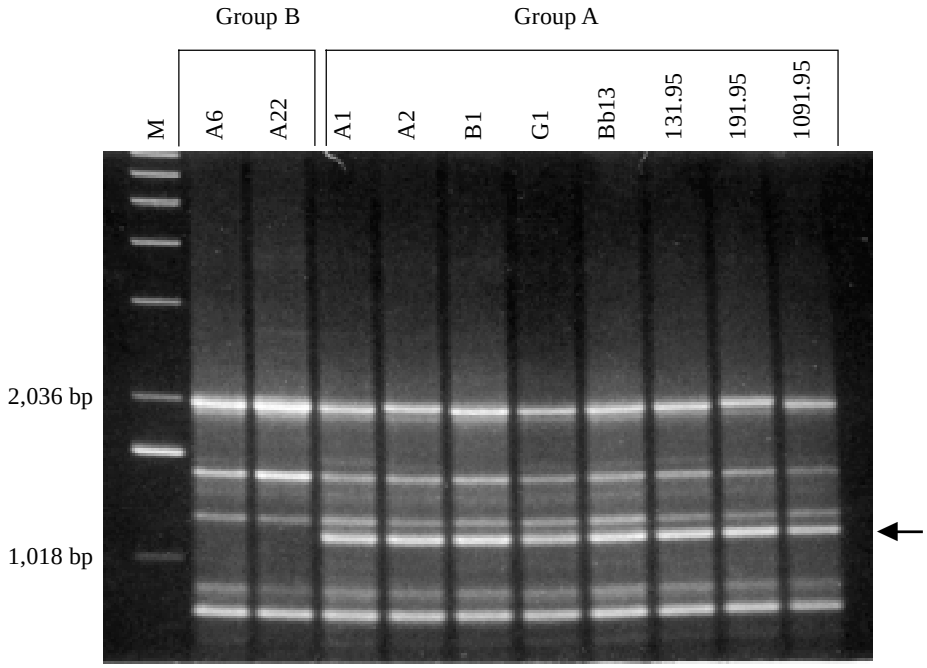

Fig. 1. Banding patterns of *Phaeomoniella chlamydospora* isolates generated by RAPD primer OP-B18 and separated by 1% agarose gel electrophoresis. M designates the 1 kb DNA ladder; size is shown at the left of the gel. Arrow indicates the polymorphic band.

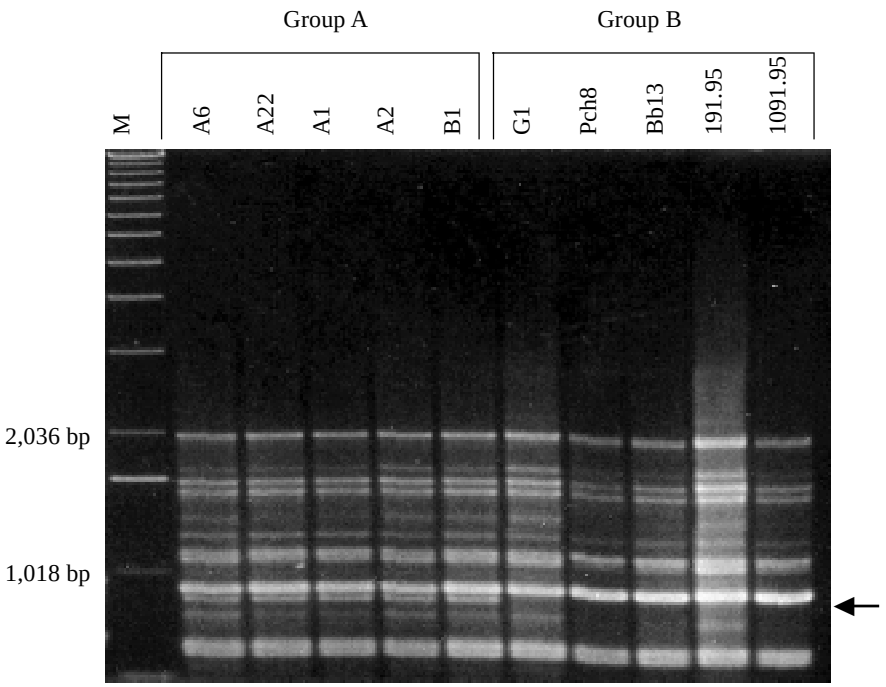

Fig. 2. Banding patterns of *Phaeomoniella chlamydospora* isolates generated by UP-PCR primer AS4 and separated by 1% agarose gel electrophoresis. M designates the 1 kb DNA ladder; size is shown at the left of the gel. Arrow indicates polymorphic band.

AS4, L15/AS19, 3-2, and AS15inv produced 9, 8, 10, and 8 band fragments respectively. For primer 3-2, group 2 was composed of only isolates A21 and A24.

AFLP

The two AFLP primer pairs produced the greatest number of polymorphic bands per primer with both primer pairs producing seven polymorphic bands (Fig. 3). In combination, these primers produced several isolate-specific banding patterns (data not shown). Resolution was adequate for bands between approximately 600 and 3,000 bp. Primer pairs AA/CAT and GA/CAT produced 13 and 23 scorable bands respectively.

Determination of genetic relationships between *P. chlamydospora* isolates

Dendrograms were used to summarise the data provided by the UP-PCR and AFLP analyses. Combined binomial tables (data not shown) generated from both the UP-PCR and the AFLP analyses were used to produce two neighbour joining dendrograms. The neighbour joining dendrogram produced from UP-PCR results (Fig. 4) identified nine genetically different groups, which had two distinct clusters originating from the main branch of the dendrogram. The neighbour joining dendrogram produced from AFLP results (Fig. 5) identified 21 genetically different groups. Isolates from the New Zealand vineyards were dispersed throughout both the UP-PCR and the AFLP dendrograms. All the Italian isolates were found on branches that also contained New Zealand isolates, suggesting that they were genetically similar to the New Zealand isolates.

The largest set of isolates originated from a single Auckland vineyard and all the molecular methods revealed variation both within the vineyard and within individual vines (31, 29, 5 and 18, which contained isolates A6/A22, A8/A21, A11/A18/A23, and A4/A17 respectively).

Determination of genetic variation using mycelial compatibility groupings

All isolates tested were compatible and placed in a single MCG, further suggesting that all isolates were genetically similar. Under light micros-

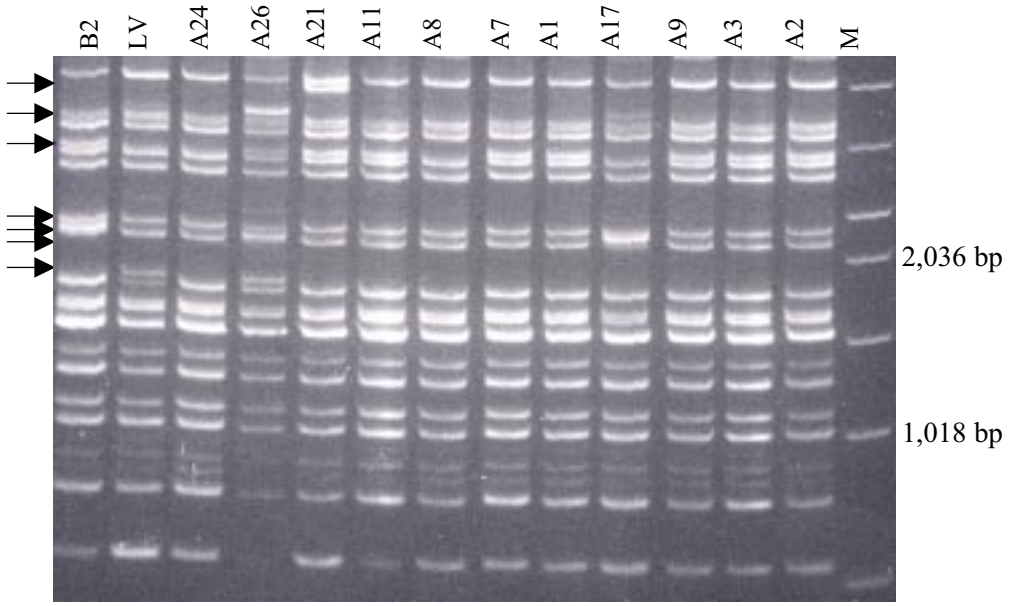

Fig. 3. Banding patterns of *Phaeomoniella chlamydospora* isolates generated by AFLP primer pair GA/CAT and separated by 1% agarose gel electrophoresis. M designates the 1 kb DNA ladder; size is shown on the right of the gel. Arrows indicate polymorphic bands.

copy, there was no evidence of hyphal incompatibility, such as absence of lysis, hyphal tip bursting or development of a demarcation line.

Discussion

In this study, a range of molecular techniques was used to analyse genetic variation within a representative population of 39 New Zealand *P. chlamydospora* isolates. The inclusion of six Italian reference strains and previously described RAPD and RAM methodology (Tegli *et al*., 2000) allowed direct comparison between New Zealand and Italian isolates. Analysis of genetic variation within the 45 isolates was also performed using the techniques of UP-PCR and AFLP. Overall, this study demonstrated a low degree of genetic variation among *P. chlamydospora* isolates from the six New Zealand vineyards.

Although molecular techniques have been useful in research on fungi, the choice of an appropriate method is important to obtain accurate and informative results. In this study, the greatest amount of variation was detected with AFLP analysis. The two AFLP primers revealed 21 genetically different groups, compared to nine and six groups

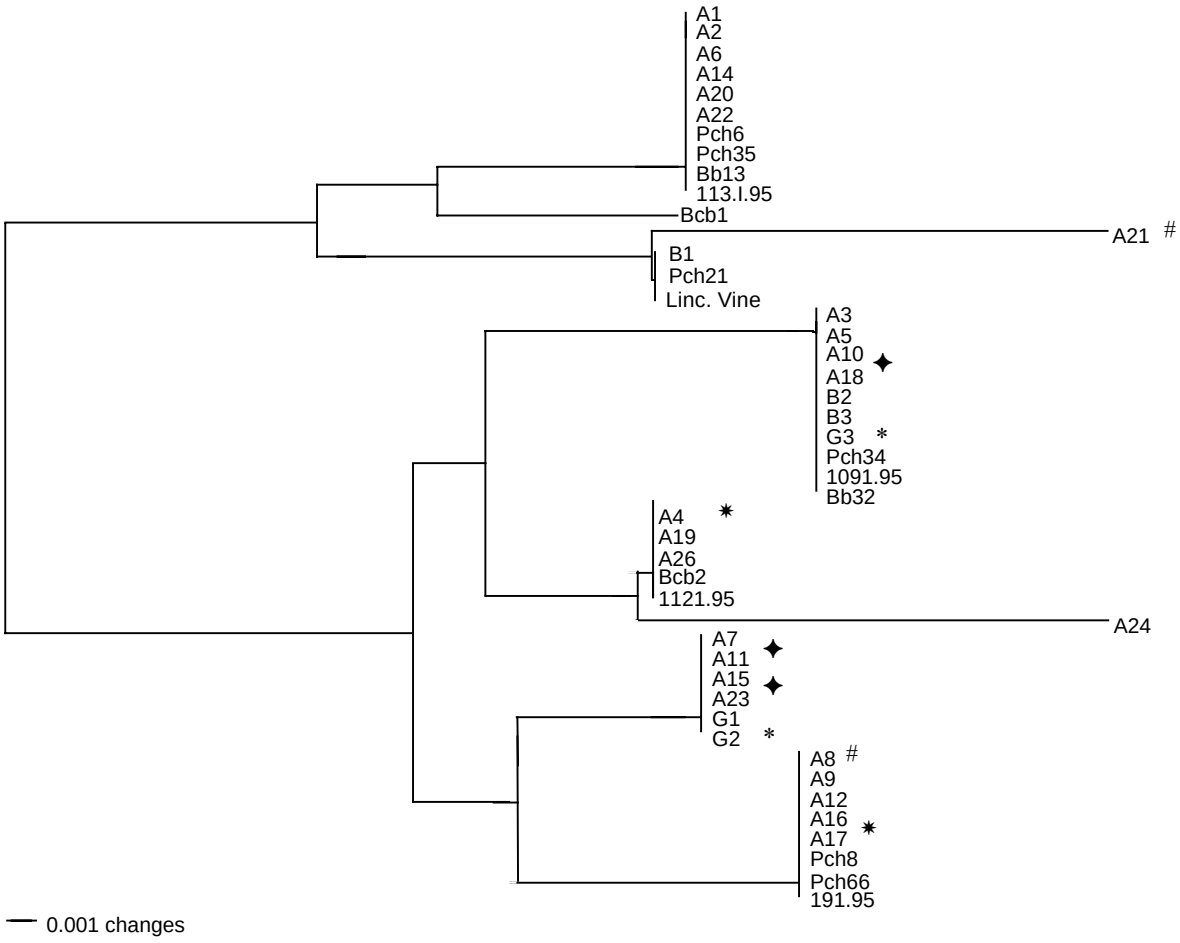

Fig. 4. Neighbour joining dendrogram generated by cumulative binomial tables using UP-PCR primers. Matching symbols on the right of isolates indicate that the isolates were from the same vine. The value indicates the proportion of changes for a given number of bands used in the analysis (Paterson A., personal communication, 2001)

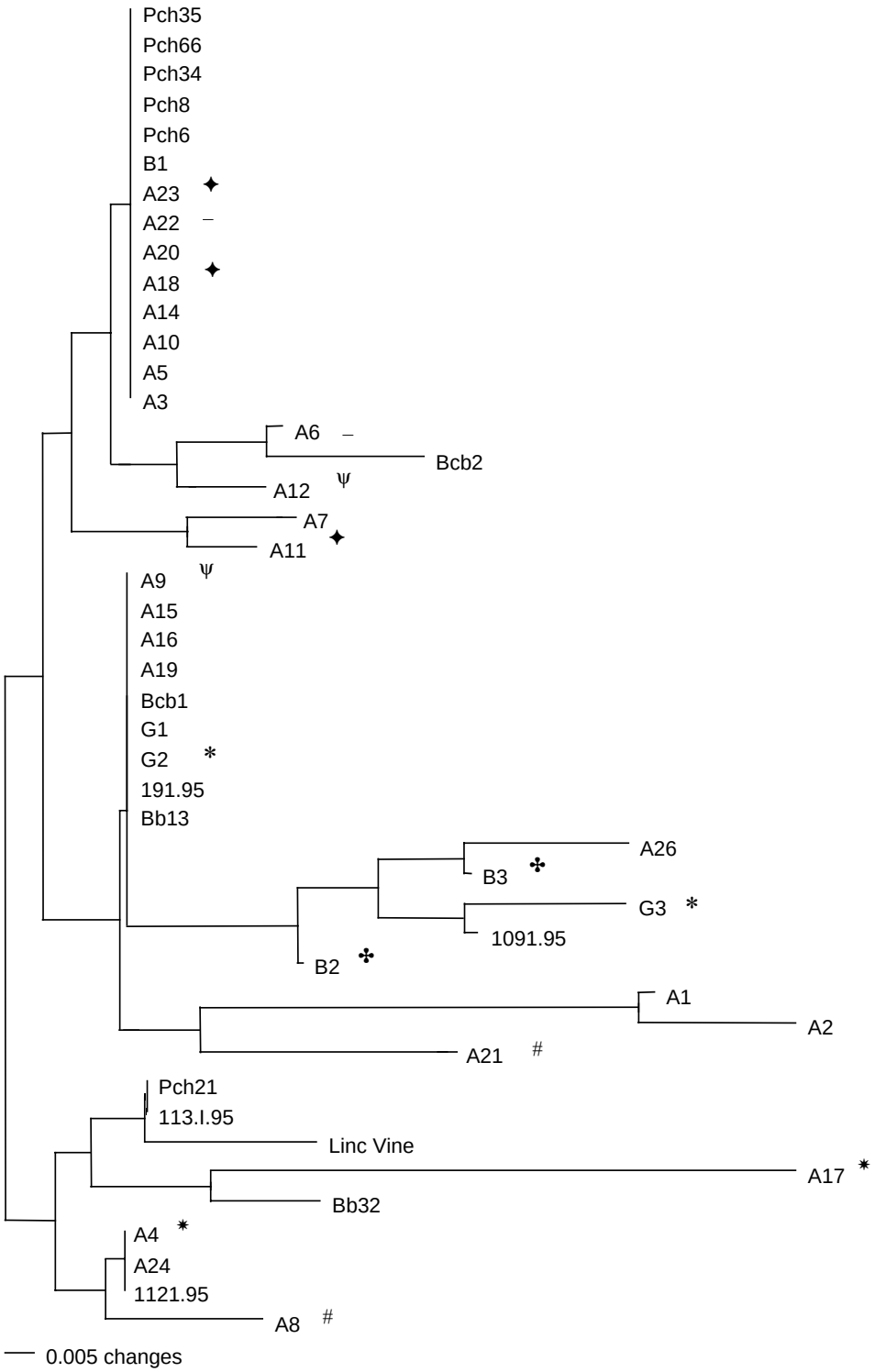

Fig. 5. Neighbour joining dendrogram generated by cumulative binomial tables using AFLP primers. Matching symbols to the right of isolates indicate that the isolates were from the same vine.

identified by nine UP-PCR and four RAPD primers respectively. In addition, RAPD, RAMS and UP-PCR banding patterns differed by only one band, indicating a low level of polymorphism within the amplified region. These results illustrate the advantage of AFLP and its ability to sample an entire genome, allowing the detection of greater polymorphisms between isolates. In contrast, the UP-PCR and RAMS methods may have sampled similar regions of the genome, therefore detecting lower levels of polymorphism. Other genetic variation studies involving *Puccinia striiformis* f. sp. *tritici* (Steele *et al*., 2001) and *Claviceps africana* (Tooley *et al.,* 2000) also found the AFLP method to be superior to the other molecular techniques because of its ability to reveal more polymorphic bands with fewer primers.

Although UP-PCR produced more bands than did RAPDs, it was unable to detect a greater level of variation per primer than RAPDs (Tegli *et al*., 2000). The banding patterns of New Zealand isolates produced by RAPD and RAMs were similar to those of the Italian isolates but it was not possible in the current study to reproduce the results of Tegli *et al*. (2000). This may have been due to factors such as different PCR machines, scoring criteria, amplification enzymes, or a combination of such factors (Tommerup *et al*., 1995; Brown, 1996). In the current study, RAMS analysis revealed no genetic variation between *P. chlamydospora* isolates, and individual RAPD primers produced only two to five bands with the genetic groups differing by only one band.

The current study suggested that New Zealand isolates of *P. chlamydospora* were genetically similar to those from Italy, which has implications for the number and/or origin of introductions into younger vineyards. Dendrogram results from UP-PCR and AFLP data identified five and sixteen groups respectively, consisting of only New Zealand isolates. These genetically distinct *P. chlamydospora* isolates could have been introduced from infected plant material sourced from a number of other countries, such as Australia, South Africa, USA, or other regions of Italy. Isolates from Italy and other grape-growing regions of the world should be tested to determine whether all the New Zealand isolates are related to other genetic groups from vineyards around the world. *P. chlamydospora* isolates from Italy and New Zealand were distributed throughout both dendrograms, suggesting that there was no relationship between genotypic variation and the geographic origin of the isolates examined. Such a relationship concurred with the results of Tegli *et al*. (2000) and further supports the theory of asexual reproduction for this fungal species. The small genetic distances among all genotypes within the dendrogram also provide evidence for the belief that reproduction in this fungus is predominantly asexual (Tegli, 2000).

The dendrograms produced by UP-PCR and AFLP did not correlate well with each other. This may reflect the contrasting methodologies for band generation. AFLP was the more informative technique, producing a greater number of polymorphic bands and a correspondingly greater number of genetic groups. Indeed, the existence of two main branches in the UP-PCR data may be due to the fact that each primer produced a maximum of two groups. A similar discrepancy between the RAPD and the AFLP data has also been found by a number of other authors (Gonzalez *et al*., 1998; Ganter *et al*., 2000).

Several sources of fungal inoculum may be available in the vineyard, and these are important to identify in order to limit the spread of this pathogen. Inter-vineyard and intra-vine genetic variation was revealed by RAPD, UP-PCR and AFLP analysis, supporting the hypothesis that multiple introductions of the pathogen have occurred into New Zealand vineyards. Adjacent vines in the Auckland vineyard had isolates with the same banding profiles and this may indicate that *P. chlamydospora* can spread to neighbouring vines by either aerial or soil dispersal. Conidia may spread through grafting cuts or wounds produced during different times of the season, i.e., pruning, mechanical trimming and/or frost damage. In addition, although it has not been proved that *P. chlamydospora* can spread via soil inoculum (Morton, 1997; Pascoe, 1998; Mugnai *et al*., 1999; Rooney *et al*., 2001), these findings support such a theory.

Interestingly, the level of variation in New Zealand vineyards was similar to that in Italian vineyards, even though the former are much younger than the latter. It has been suggested that *P. chlamydospora* populations found on older, more established grapevines would have a greater degree of genetic variation than those in younger vineyards (Tegli *et al*., 2000), but no such correla-

tion was found in the present study. This suggests that the cause of the increased incidence of Petri disease in vineyards worldwide is a recent phenomenon. In addition, when the dendrograms produced by this study were compared with those of Tegli *et al*. (2000), distances between isolates were less than 0.05, suggesting that, globally, genetic variation between *P. chlamydospora* isolates is low.

A small subset of *P. chlamydospora* isolates was tested for mycelial compatibility. These isolates were chosen from different branches of the dendrogram to provide isolate pairs exhibiting the greatest amount of genetic variation. Only compatible reactions were observed suggesting isolates were genetically similar. Although all isolates tested belonged to one MCG, the combined RAPD, RAMs and UP-PCR data showed that the *P. chlamydospora* belonged to more than one genetic group. Similar findings of multiple genetic groups within one MCG have also been reported with *Sclerotium* spp. isolates (Nalim *et al*., 1995; Cilliers *et al*., 2000; Tyson *et al*., 2002).

In summary, of the four molecular methods used in this study, AFLP was the most useful in the genetic characterisation of *P. chlamydospora*. As in previous Italian studies (Tegli *et al*., 2000), genetic variation among isolates of *P. chlamydospora* was low. Surprisingly, the overall amount of variation was similar to that found in Italy, despite the widely disparate ages of the two viticulture industries. The occurrence of the same genetic group in more than one location also indicates movement of *P. chlamydospora* between locations. Further research on the occurrence of *P. chlamydospora* isolates in different regions of the world may provide further information on introduction frequencies, national spread and insight into the mechanisms of inoculum dispersal.

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