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Evidence for host-associated clones of grape phylloxera *Daktulosphaira vitifoliae* (Hemiptera: Phylloxeridae) in Australia

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Abstract

Grape phylloxera, *Daktulosphaira vitifoliae* Fitch, is an important pest of grapevines (*Vitis vinifera* L.) (Vitaceae). Using microsatellite DNA markers it was demonstrated strong associations can exist between *D. vitifoliae* asexual lineages and vine host type within a vineyard. Also, in excised root bioassays, *D. vitifoliae* collected from three regions where different genotypic classes predominated showed host-specific differences in life table parameters of reproductive rate and intrinsic rate of increase. Lastly, comparisons of mitochondrial DNA (cytochrome oxidase I) sequences revealed that *D. vitifoliae* in Australia have paraphyletic origins and fall into two clades partially related to vine host usage. These findings indicate introduction of separate lineages of *D. vitifoliae* which have close host associations and as such, have important implications for management of this pest in Australia.

Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae) infests the roots and leaves of its host plants, members of the genus *Vitis*. It is a devastating pest of the European grapevine species *V. vinifera* L. (Vitaceae) worldwide, e.g. Europe, North America, Australia, where feeding on the root system results in vine decline and death. The insect is native to North America and indigenous on *Vitis* species (Downie *et al.*, 2000). Some *Vitis* species have resistance to root feeding by this insect, and these have been used as rootstocks for continued commercial production of *V. vinifera* in grape phylloxera-infested soils. The ability of *D. vitifoliae* populations to adapt to rootstocks is unknown, although the presence of pest variants that can overcome resistance has already been documented (Granett *et al.*, 1985) and is well known in a range of other agriculture contexts (Kim & McPheron, 1993).

Studies on native populations of *D. vitifoliae* have demonstrated that they vary in their ability to infest different vine genotypes (Fergusson-Kolmes & Dennehy, 1993; Hawthorne & Via, 1994; Downie, 1999), suggesting the potential for host adaptation within this species. More recently, the presence of genetic variability within *D. vitifoliae* populations from commercial vineyards has been demonstrated using DNA typing in California (Fong *et al.*, 1995), Europe (Kocsis *et al.*, 1999; Forneck *et al.*, 2000) and Australia (Corrie *et al.*, 1997, 2002). However, no association between such genetic variability and host plant performance has yet been established. In fact, research on grape phylloxera from its native range reveals that there is only a weak genetic structuring associated with host type when grape phylloxera populations are sourced from sympatric *Vitis* species (Downie, 2000; Downie *et al.*, 2001). Genetic divergence within the insect's native range was suggested to be driven primarily by geographic factors (Downie *et al.*, 2001).

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Recently, microsatellite DNA markers have been developed for this insect species and used to demonstrate that, in Australia, populations on vine roots are largely clonal and that clonal lineages are non-randomly distributed between vineyard regions and among vineyards within a region (Corrie *et al*., 2002). The purpose of the present study was to determine if genetically-based host associations exist among populations of *D. vitifoliae* within a vineyard. By intensive sampling of insects from various vine types in three different vineyards, it was shown that certain phylloxera clonal lineages are found on particular vine types. Also, grape phylloxera collected from regions where different genotypes predominate displayed differences in performance on excised roots of different vine hosts. In order to establish if these host associations had a common genetic origin and in order to relate our information to a recently derived phylogeny of *D. vitifoliae* genotypes (Downie *et al*., 2001), partial DNA sequence analysis of a mitochondrial gene (cytochrome oxidase I) was also performed.

Materials and methods

Biological assays

Daktulosphaira vitifoliae were sampled from vineyards located in Nagambie (NA-2), King Valley (WL-1) and Rutherglen (RU-1) in Victoria, Australia (Corrie *et al*., 1997, 2002). Both the NA-2 and WL-1 samples were collected from roots of *V. vinifera. Daktulosphaira vitifoliae* in the NA-2 and WL-1 vineyards consist of the single clonal lineages, G1 and G4 respectively (Corrie *et al*. 2002). The RU-1 sample was collected from the leaves of a single vine of the rootstock Schwarzmann (*V. riparia* Michaux × *V. rupestris* Scheele). Neither G1 nor G4 were present in this leaf population; however, a range of genotypic classes do occur in this particular vineyard (A.M. Corrie, unpublished data). In the laboratory, insects were maintained on excised roots from the vine hosts from which they were collected. Decreasing health of roots and overcrowding influence grape phylloxera in culture, with populations switching from parthenogenetic reproduction to the production of winged alates and sexual morphs within two to three generations (Forneck *et al*., 2001). Because of this and other problems associated with rearing the insect over more than one generation, maternal effects were not considered in this design and host/genetic effects could thus not be separated.

A laboratory based bioassay with excised root pieces (Granett *et al*., 1987) was used to determine life-table parameters. Lignified root pieces were collected from field grown vines and roots 2–5 mm in diameter were cut into 4 cm sections. They were then washed with sterile distilled water and dipped into 300 mg l^{-1} chlorothalonil solution (Bravo^{TM}), blotted dry, wrapped on the proximal end with cotton wool (moistened with sterile water) and placed into plastic Petri dishes lined with filter paper. One root piece was placed into each Petri dish and covered with a lid (with a 1 cm diameter hole covered with mesh). The bioassay dishes were stored at 23°C ± 1°C, in sealed containers to restrict light exposure.

Experiments were initiated by placing 10 two- to five-day-old eggs of the appropriate *D. vitifoliae* sample (NA-2, WL-1 or RU-1) on an excised root piece with a paintbrush. The three vine types used were; Cabernet Sauvignon (*V. vinifera*) and the rootstocks ARG1 (*V. vinifera* var. Aramon × *V. rupestris* Ganzin) and Schwarzmann (*V. riparia* × *V. rupestris*). There were 10 replicates of each insect sample with the vine types *V. vinifera* and Schwarzmann and 20 replicates of each insect sample with ARG1, where a replicate consisted of a Petri dish containing one excised root piece inoculated with 10 eggs.

Data were collected over three months. Evaluations commenced nine days after inoculation, and were performed twice a week thereafter. At each evaluation, the location of individual insects on the excised root piece, developmental stage and number of eggs produced per adult asexual female were recorded. When feeding on roots, grape phylloxera are sedentary and therefore the location of individuals on the root piece rarely changes once feeding has commenced. Any eggs produced were removed prior to hatching to prevent an effect of overlapping generations on insect counts. Consequently only one generation was screened. At day 89 (the end of the experiment), only 28 insects were still alive across all 120 replicates and the experiment was discontinued. These 28 insects were no longer reproducing and therefore were not contributing to the next generation and to measurements of fitness.

Insects settled and fed on both the lignified roots and primary root tissue (callus and/or fibrous roots) that arose from the exposed end of the lignified roots during the time of bioassay. The location of individual insects and hence the type of root tissue fed on was noted, but only data from those individuals feeding on the lignified roots were used for comparative analysis of life-table parameters. This was because although *D. vitifoliae* can inhabit both the primary and secondary root system of *Vitis* spp., grapevine damage is most often associated with its effects on secondary roots. There are alternative bioassay systems for primary roots (Forneck *et al*., 2002; Kellow *et al*., 2002).

Life-table parameters computed included the intrinsic rate of increase (r) and the net reproductive rate (R_0) (Birch, 1948). A Leslie projection matrix, incorporating survival and age specific fecundity, was used to calculate r (Leslie, 1945). Initial host plant establishment was expressed as survival to adulthood by one or more insects on a root piece (replicate). Only replicates in which adults became established were used to determine r and R_0. Comparisons between the different grape phylloxera samples and host plants were made using two-way analysis of variance (ANOVA). Differences between pairs of means were determined with the posthoc Newman-Keuls procedure. SPSS version 10.0 was used to perform all statistical analyses (SPSS Incorporated, USA).

Spatial distribution of genotypic classes

Daktulosphaira vitifoliae were collected from the root system of various vine types at four study sites located in north eastern Victoria, Australia during the period of population growth (February–March 2001). Geographical regions and vineyard codes are as described in Corrie *et al*. (2002). Study sites consisted of adjacent plantings of vines on two different root systems (rootstocks). All rootstocks used in the study were grafted to *V. vinifera*. Two study sites were within one vineyard at Milawa (ML-1), separated by a distance of 600 m (fig. 1a,b). One site was at Glenrowan (GR-1) and one at Rutherglen (RU-1) (fig. 1c,d). Table 1 provides a summary of vine types and sample numbers at each site,

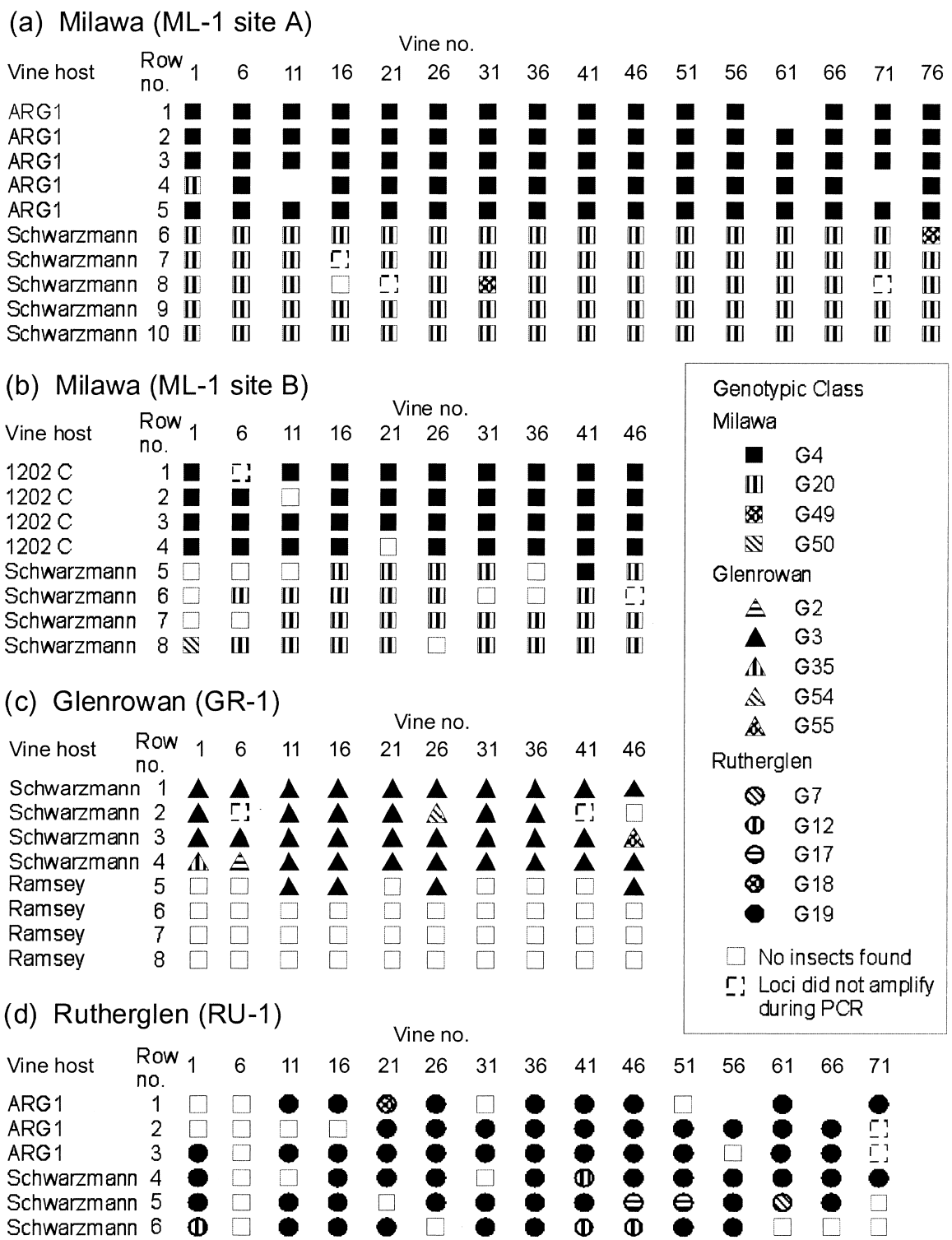

Fig. 1. Spatial distribution of *Daktulosphaira vitifoliae* genotypic classes sampled from the roots of various vine types at four study sites in northeastern Victoria: (a) Milawa, ML-1 site A, (b) Milawa, ML-1 site B, (c) Glenrowan, GR-1, (d) Rutherglen, RU-1. Insects were sampled from every fifth vine in each row and from adjacent rows of vines. Symbols designate the location of insect samples of each genotypic class (table 3). Vines from which no insects were found or the loci failed to amplify during PCR are also indicated. An absent symbol identifies the location of a vine missing from a sample site. For numbers of insects collected from each vine type at each study site refer to table 1. The distances between samples were: (a) and (b) 10.7 m along rows, 2.5 m between rows; (c) 10 m along rows, 3.0 m between rows; (d) 8.0 m along rows, 3.0 m between rows.

Table 1. Vineyard code, vine types and genotypic classes of samples from four sites in north-east Victoria.

Geographic region	Vineyard code	Vine type	No. of vines sampled	No. of vines with insects	Genotypic class													
					G2	G3	G4	G7	G12	G17	G18	G19	G20	G35	G49	G50	G54	G55
Milawa (site A)	ML-1	Schwarzmann	80	79	–	–	–	–	–	–	–	–	74	–	2	–	–	–
		ARG1	77	77	–	–	76	–	–	–	–	–	1	–	–	–	–	–
Milawa (site B)	ML-1	Schwarzmann	40	30	–	–	1	–	–	–	–	–	27	–	–	1	–	–
		1202 C	40	38	–	–	37	–	–	–	–	–	–	–	–	–	–	–
Glenrowan	GR-1	Schwarzmann	40	39	1	33	–	–	–	–	–	–	–	1	–	–	1	1
		Ramsey	40	4	–	4	–	–	–	–	–	–	–	–	–	–	–	–
Rutherglen	RU-1	Schwarzmann	45	34	–	–	–	1	4	2	–	27	–	–	–	–	–	–
		ARG1	43	33	–	–	–	–	–	–	1	30	–	–	–	–	–	–

The number of genotypic classes sampled from each vine type at each location is indicated. Genotypic classes are as defined in table 3.

whilst fig. 1 illustrates the sampling design including spatial distribution of the vines. Vine types sampled included the rootstocks ARG1 (*V. vinifera* var. Aramon × *V. rupestris* Ganzin), 1202 C (*V. vinifera* var. Mourvèdre × *V. rupestris* Martin), Schwarzmann (*V. riparia* × *V. rupestris*) and Ramsey (*V. champini* Planch thought to be a natural hybrid between *V. mustangensis* Buckley and *V. rupestris* (Moore, 1991)). Phylloxera samples were collected by digging around the trunk of the grapevine to a maximum depth of 70 cm and excising infested roots. Care was taken to ensure that roots sampled originated from below the graft union. Adults on established gall sites were usually collected to ensure that the insects sampled were feeding on the vine type. Only one insect per vine was collected to reduce the problem of vine associated clonal growth, although on some vines no insects could be found. Insects were stored in 100% ethanol at –20°C. Sampling at random within a vineyard was not possible because this would have yielded few phylloxera, given the tight spatial association between these insects and vine roots.

Insects were DNA typed using four microsatellite loci (DVIT1, DVIT2, DVIT3, and DVIT4) and assigned to a genotypic class based on the DNA pattern obtained. Single insect DNA extractions, polymerase chain reaction (PCR) amplification and characterization of the microsatellites have been outlined previously (Corrie *et al*., 2002).

Mitochondrial DNA sequence analysis

A partial sequence of the cytochrome oxidase I region was obtained from representatives of each genotypic class using the primers C1-J-1751 (5′-GGATCACCTGATATAGGATTCCC-3′) and C1-N-2191 (5′-CCCGGTAAAATTAAAATATAAACTTC-3′) as detailed by Simon *et al*. (1994). Polymerase chain reaction was carried out in a 25 μl volume using the pre-mixed Ready-To-Go™ PCR bead kit (Amersham Biosciences, UK) according to the manufacturers instructions. For amplification, initial denaturation at 94°C for 4 min was followed by 94°C 30 s, 50°C 30 s, and 72°C 30 s for 30 cycles. Products were cleaned using Wizard® PCR Prep Kit (Promega, USA). All templates were sequenced in both directions using the ABI PRISM™ Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturers instructions (Perkin Elmer Applied Biosystems, USA). Electrophoresis was performed by Genetic Technologies (Melbourne, Australia) using the ABI PRISM™ 377 automated sequencer (Perkin Elmer Applied Biosystems, USA).

Forward and reverse sequences were aligned and checked using the program Sequencer™, version 3.1.2 (Gene Codes Corporation) and the consensus sequences submitted to the GenBank nucleotide database (accession numbers AY228512 – AY228541). Additional COI sequences, from GenBank (accession numbers AF307369, AF307374, AF307426, AF307416, AF307417, AF307357, AF307359, AF307379, AF307396, AF307429, AF307431), were also incorporated into the study to enable comparison with a previous phylogenetic study by Downie *et al*. (2001). These sequences were gained from insects collected from commercial vineyards in California, Oregon and Washington, USA and from the native range of *D. vitifoliae* in North America (Downie *et al*., 2001). Two members of the Phylloxeridae family were used as the outgroup species (Genbank accession numbers AF307447, AF307446). Naming of samples from North America was as described in Downie *et al*. (2001). The name is comprised of a state, location and host plant abbreviation, i.e. VIN, *V. vinifera*, ARI, *V. arizonica*, AXR, AxR#1 (*V. vinifera* × *V. rupestris*), VULP, *V. vulpina*, CIN, *V. cinerea*, RIP, *V. riparia*. A 426 bp fragment of all COI sequences was aligned using ClustalX (Thompson *et al*., 1997). The data set was analysed with the distance (neighbourhood-joining) 2-parameter method (Kimura, 1980) with bootstrapping undertaken to test for robustness (1000 replications). The number of samples restricted the analysis to neighbourhood-joining. However, the tree generated was consistent with previous analysis performed by Downie *et al*. (2001) and Downie (2002). Both nucleotide diversity computations and phylogeny analysis were performed with MEGA version 2.1 (Kumar *et al*., 2001).

Results

Biological assays

The majority of *D. vitifoliae* established on *V. vinifera* roots but few established on the rootstock Schwarzmann and there was also reduced establishment success on the vine type ARG1 (table 2). No individuals from the NA-2 and WL-1 samples developed past the second instar stage on the rootstock Schwarzmann while five RU-1 colonies became established on this host. These differences in establishment

Table 2. Means of the life table parameters of net reproductive rate (R_0) and intrinsic rate of increase (r) for three *Daktulosphaira vitifoliae* populations on excised roots of *Vitis vinifera*, ARG1 and Schwarzmann rootstocks.

Vine type	Replicates	No. replicates in which one or more insects reached adulthood (n)	*D. vitifoliae* population	R_0 (SE)	r (SE)
V. vinifera	10	9	RU-1	53.4 (13.0) a	0.40 (0.05) a
(cv. Cabernet	10	10	NA-2	111.0 (13.0) b	0.45 (0.04) a
Sauvignon)	10	9	WL-1	163.1 (22.5) c	0.59 (0.04) b
ARG1	20	11	RU-1	73.1 (16.0) a	0.42 (0.05) a
	20	9	NA-2	251.0 (51.3) b	0.50 (0.03) a
	20	14	WL-1	273.3 (27.6) b	0.51 (0.05) a
Schwarzmann	10	5*	RU-1	106.3 (48.9)	0.45 (0.05)
	10	0	NA-2	–	–
	10	0	WL-1	–	–

*Data were not included in ANOVA analysis. In three replicates insects were only observed feeding on the primary root system which was generated on the lignified root pieces during the bioassay.

Initial establishment is expressed as the number of replicates where one or more insects reached adulthood (n). The life table parameters r and R_0 were only determined for these replicates. Comparisons between the different populations and two of the vine types, *V. vinifera* and ARG1, were made using ANOVA. Within vine types the means of the individual parameters are compared and those followed by a different letter are significantly different at $P < 0.05$ (post hoc Newman-Keuls test). The standard error (SE) is provided in brackets.

were significant (contingency analysis, $G = 13.2$, df = 2, $p = 0.004$ by permutation).

ANOVAs were performed on R_0 and r estimated for replicates that established on *V. vinifera* and ARG1. Both vine type ($F_{1, 62} = 10.840$, $P < 0.01$) and grape phylloxera population ($F_{2,62} = 11.871$, $P < 0.01$) had an influence on the net reproductive rate but there was no significant interaction between vine host and grape phylloxera population ($F_{2,62} = 1.680$, $P = 0.195$). RU-1 exhibited the lowest mean values, WL-1 the highest (table 2). Posthoc tests (Newman-Keuls) indicated significant differences among the means of all three populations on *V. vinifera*, and between RU-1 and both NA-2 and WL-1 on ARG1. The grape phylloxera population used also influenced the intrinsic rate of increase r ($F_{2,62} = 3.706$, $P < 0.05$), but there was no distinction between the two vine types ($F_{1,62} = 0.002$, $P = 0.966$), nor any interaction between the two parameters ($F_{1,62} = 2.387$, $P = 0.101$). Post hoc tests revealed that WL-1 had a significantly higher mean r than both NA-2 and RU-1 on *V. vinifera*, yet there were no population differences on ARG1. Although a similar trend was observed for both r and R_0, the lack of distinction between the three populations for r, particularly on ARG1, is indicative of an influence of factors other than fecundity on population growth (i.e. fitness). The timing of reproduction and survivorship rate has an important influence on growth of populations with overlapping generations and are incorporated into r.

Spatial distribution of genotypic classes

Fourteen genotypic classes of grape phylloxera were identified from the 334 insect samples and genotypic class composition differed between vineyards (tables 1 and 3). One class predominated at the Rutherglen (RU-1) and Glenrowan (GR-1) sites (G19 and G3 respectively), whilst the genotypic classes G4 and G20 were common at Milawa (ML-1, site A and B). All other genotypic classes identified were sampled less than five times each. The G1 genotypic class was not sampled from the study sites although it had previously been detected in the GR-1 vineyard, but on rootstocks not examined in this study (Corrie *et al.*, 2002).

There were distinct differences in the distribution of genotypic classes within a vineyard. The distribution of the two most common classes at Milawa (ML-1) followed the distribution of vine types closely (fig. 1a,b). G4 was sampled almost exclusively from ARG1 at site (A) and from 1202 C at site (B) with only one exception, whilst G20 was collected almost entirely from Schwarzmann rootstock vines at both sites. The only G4 individual sampled from Schwarzmann rootstocks was at the first instar stage and was not sampled from an established feeding site.

Most samples from the Glenrowan (GR-1) site were identified as G3. This genotypic class was sampled from the roots of both Schwarzmann and Ramsey vines, but there was a considerable difference in levels of infestation between the two vine types (table 1, fig. 1c). Insects could be sampled from the roots of only four Ramsey vines compared to 39 adjacent Schwarzmann vines.

Lastly, at Rutherglen (RU-1), there were a number of genotypic classes scattered throughout the site. However, no vine type association was evident based either on genotypic class distribution or levels of infestation on the Schwarzmann and ARG1 vine types (fig. 1d).

Mitochondrial DNA (COI) sequence analysis

Among the 14 genotypic classes of grape phylloxera examined, three mitochondrial haplotypes were identified based on variation at 20 positions within the 426 bp of cytochrome oxidase I (COI) partial gene sequence (table 3). No sequence variation was detected within any genotypic class. Most sequences obtained from representatives from each genotypic class were identified as haplotype B, including all those sampled from the GR-1 site and the majority of those from the RU-1 site. Only a single nucleotide change differentiated haplotype B from D.

Table 3. Genotypic classes and mitochondrial DNA haplotypes of *Daktulosphaira vitifoliae* sampled from various vine types and vineyards located in Victoria, Australia.

Genotypic class	Vineyard code	Size of alleles at each locus (bp's)				No. of insects sequenced	Mitochondrial haplotype
		DVIT1	DVIT2	DVIT3	DVIT4		
G1	NA-2	128/136	259/289	175/190	159/159	1	C
G2	GR-1	134/136	261/261	175/175	156/156	1	B
G3	GR-1	134/136	259/261	175/175	156/156	4	B
G4	ML-1, WL-1	128/136	259/289	175/190	159/168	4	A
G7	RU-1	134/134	259/259	175/175	156/164	1	B
G12	RU-1	134/136	259/261	175/175	164/164	2	B
G17	RU-1	134/134	259/261	175/175	156/164	2	B
G18	RU-1	134/134	261/261	175/175	164/164	1	B
G19	RU-1	134/136	259/259	175/175	164/164	3	B
G20	ML-1	132/136	259/259	175/175	159/164	5	D
G35	GR-1	134/136	259/259	175/175	156/156	1	B
G49	ML-1	132/136	259/259	175/175	159/159	2	D
G50	ML-1	132/136	259/259	175/190	159/164	1	D
G54	GR-1	136/136	259/261	175/175	156/156	1	B
G55	GR-1	136/136	261/261	175/175	156/156	1	B

Genotypic classes and vineyard codes are as described in Corrie *et al*. (2002). The DNA fragment sizes (alleles) for the microsatellite loci DVIT1, DVIT2, DVIT3 and DVIT4 are shown for each genotypic class. DNA sequence from the gene cytochrome oxidase I (426 bp fragment) was used to define the mitochondrial haplotypes.

However, with 17 nucleotide differences, there was clear distinction between haplotypes A and B. The genotypic class G1, identified as haplotype C, was not collected from vines in the spatial distribution survey but was represented in the biological assay (sample NA-2) and so was included in the phylogenetic analysis. The estimated nucleotide diversity for all grape phylloxera samples from Australia was 0.021 ± 0.005.

The four Australian haplotypes were compared to haplotypes recently defined by Downie (2002) and the same nomenclature was used for those identical in sequence for a 426 bp region of CO1. Downie (2002) sampled 20 haplotypes from commercial vineyards located in several countries, although only six were observed multiple times (haplotypes A–F). Of these six haplotypes, A-D were identified in the present study. The Australian haplotypes shared identity with grape phylloxera sampled from commercial vineyards in Europe (haplotypes B, C and D), South America (A and C), New Zealand (A and C) and North America (A, B, and C).

Phylogenetic analysis revealed two clades (fig. 2). The overall topology was consistent with the phylogenetic tree generated by Downie *et al*. (2001) and Downie (2002). Clade 1 contained the majority of samples including the Australian haplotypes B, C and D along with some samples from commercial grapevines of North America. Haplotype C, along with the samples from Washington (WA zil VIN) and Oregon (OR sh VIN), were separated from haplotypes B and D. There was also strong support for the grouping of haplotype C with insects sampled from native *V. riparia* grapevines in New York State. A similar grouping was observed by Downie *et al*. (2001) and Downie (2002); however in these studies, there was insufficient support to define this group as a separate clade. Haplotype A appeared in a separate clade (clade 2) with a COI sequence identical to one sample from a commercial vineyard in California (CA son AXR). Insects sampled from the southeastern regions of the USA were also located within clade 2. Although the relationship between these insects and haplotype A was only moderately supported by bootstrap analysis, the result agrees with those of Downie *et al*. (2001) and Downie (2002).

Previously, Corrie *et al*. (2002) concluded that the majority of *D. vitifoliae* populations in Australia are comprised of functionally parthenogenetic lineages, i.e. essentially asexual with rare recombination. Evidence included the absence of key expected recombinant genotypes, high levels of microsatellite heterozygosity, and the geographical distribution and sampling frequency of genotypic classes, including the sampling in several regions of only one genotypic class. The absence of mitochondrial sequence variation within a genotypic class (table 3) supports the previous conclusion of Corrie *et al*. (2002) and members of each genotypic class are described as belonging to a clonal lineage.

Discussion

The spatial distribution of *D. vitifoliae* genotypes within three of the four sites examined, clearly indicates the association of specific asexual phylloxera lineages with particular vine genotypes. At the Milawa (ML-1) vineyard, there are tight associations at both sites between the distribution of genotypic classes and three different vine types. These associations cannot simply be the result of selective spatial introduction of genotypic classes since grape phylloxera has been present in this vineyard for more than 80 years and all three vine types have been exposed to the resident populations since their planting about 20 years ago. Over this time there would have been substantial movement of grape phylloxera within the vineyard since dispersal studies carried out under Australian conditions have demonstrated first instars move an average of 20 m per year (King & Buchanan, 1986). At the Glenrowan (GR-1) site, the specific host associations are illustrated by a predominance of genotypic class G3 on the Schwarzmann rootstock and an almost complete absence of phylloxera on the neighbouring Ramsey rootstock. This lack of infestation on Ramsey is site specific, since studies conducted at other

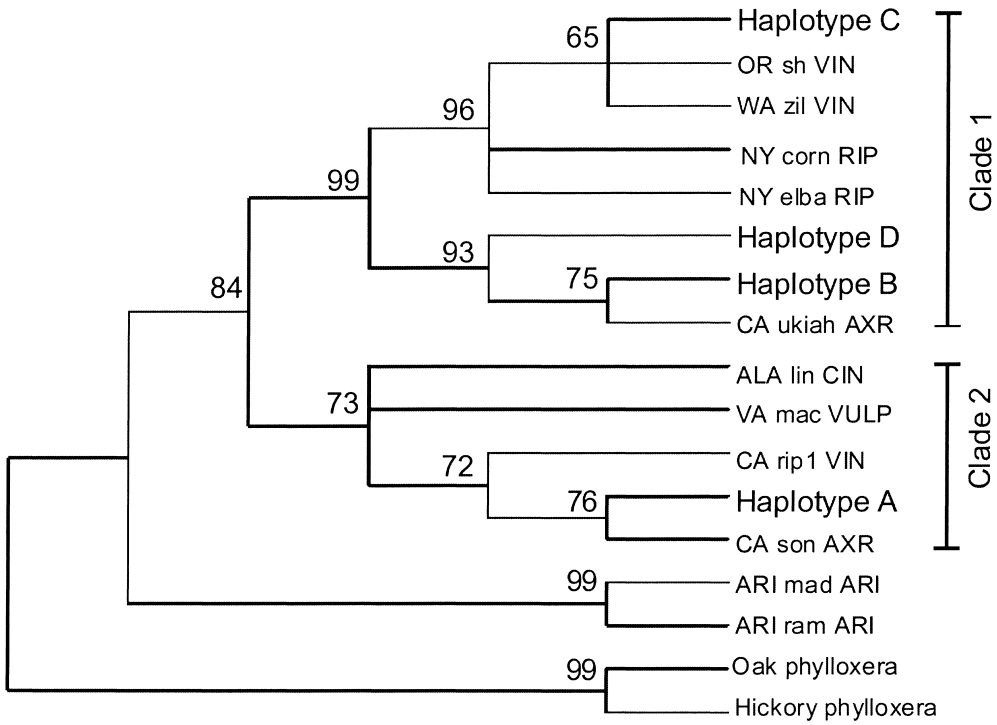

Fig. 2. Relatedness among *Daktulosphaira vitifoliae* mitochondrial DNA haplotypes sampled from Australia (haplotype A, B, C and D) and from wild grapevines in New York State (NY corn RIP, NY elba RIP), Alabama (ALA lin CIN), Arizona (ARI mad ARI, ARI ram ARI), Virginia (VA mac VULP) and from commercial vineyards in California (CA ukiah AXR, CA rip1 vin, CA son AXR), Oregon (OR sh VIN) and Washington (WA zil VIN), USA. The tree from neighbourhood-joining analysis with the percentage of replications (bootstrap; 1000 replications) is shown.

sites in Australia show much higher levels of infestation on this vine type (Whiting *et al.*, 1987; Buchanan, 1990).

Interestingly, at the Rutherglen (RU-1) site there was no apparent association between genotypic class and vine type, despite the presence of a range of genotypic classes. One explanation for this is that all of the grape phylloxera at Rutherglen have a similar evolutionary origin as indicated by the analysis of their COI sequences. This contrasts with the situation at the Milawa (ML-1) vineyard where the genotypic classes collected from different host plants belong to different clades. All of the grape phylloxera collected at the Glenrowan (GR-1) site were identified as haplotype B. However, as discussed before, the host association at this site was most clearly illustrated by the presence of one predominant genotypic class on one vine type and the virtual absence of phylloxera on the other.

In the biological assays, differential performance of all three phylloxera samples was observed on roots of the three vines. The sample WL-1 generally outperformed the other two samples on *V. vinifera* and ARG1, but was unable to develop to adulthood on the vine type Schwarzmann. This population is comprised of one clonal lineage, G4 (Corrie *et al.*, 2002), which was also detected in the two intra-vineyard spatial distribution study sites located at Milawa (ML-1). Clone G4 was found exclusively on ARG1 (ML-1 site A) and 1202 C (ML-1 site B) rootstocks at these sites, with other genotypes of phylloxera, mainly G20, sampled from adjacent Schwarzmann vines. Both host plants from which G4 was sampled from in the ML-1 vineyard have *V. vinifera* in their parentage, being *V. vinifera* and *V. rupestris* hybrids. Together with the relatively high fitness of the WL-1 population on *V. vinifera* and the *V. vinifera* × *V. rupestris* hybrid ARG1 in the biological assays, these results suggest that G4 is host associated with *V. vinifera* derived vines.

The bioassays also showed better performance of the NA-2 phylloxera sample on *V. vinifera* relative to the RU-1 sample. The NA-2 population is comprised of one genotypic class, G1 (Corrie *et al.*, 2002). G1 and G4 are the most widespread phylloxera lineages in Australia. They are also the lineages associated with the most recent infestations of previously uninfested vineyards in this country. It was earlier proposed that both of these lineages possess characteristics that enable them to colonize vineyards comprised of *V. vinifera* (Corrie *et al.*, 2002). The present bioassay results provide some support for this. Even so, the phylogenetic analyses indicate that G1 and G4 have different evolutionary origins, so that similar behavioural characteristics may result from convergent evolution.

The results of the phylogenetic analyses support a paraphyletic origin for grape phylloxera in Australia, being classed into two clades, each comprised of animals from Australia and North America. This analysis is consistent with a comprehensive study on the phylogenetic origins of grape phylloxera in North America (Downie *et al.*, 2001). Representatives from several branches of these studies were used in the present analysis. Genetic similarity was also observed between the four haplotypes of grape phylloxera isolated from vineyards in Australia and sampled from

commercial vineyards worldwide (Downie *et al*., 2001; Downie, 2002). The grouping of haplotype A with representative Californian clones is supportive of a common origin for these lineages. Similar inferences can also be made for haplotype C and samples of grape phylloxera isolated from recently infested vineyards in Oregon and Washington (Downie *et al*., 2001) and others from vineyards located in Europe, Argentina and New Zealand (Downie, 2002). Nevertheless, more detailed genetic information is required to ascertain whether global distribution of mitochondrial haplotypes is indicative of the spread of individual clones as well as a lack of interaction of these via, for example, sexual recombination (i.e. gene flow) with other grape phylloxera post introduction to commercial vineyards. The availability of co-dominant nuclear DNA markers for *D. vitifoliae* will greatly assist such studies.

Host specialization has been proposed as contributing to the maintenance of high levels of genetic diversity in some aphid populations (Hales *et al*., 1997). The presence of genetically diverse phylloxera with strong vine host associations suggests that populations of this insect may be comprised of host 'specialists'. Genetic markers have been used to discriminate between colonizers of different host plants in other aphid species such as the grain aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) (De Barro *et al*., 1995a; Sunnucks *et al*., 1997; Haack *et al*., 2000; Lushai *et al*., 2002), green bug, *Schizaphis graminum* (Rondani) (Aphididae) (Anstead *et al*., 2002) and the pea aphid, *Acyrthosiphon pisum* (Harris) (Aphididae) (Bournoville *et al*., 2000). Genetic information, combined with biological data, has been used to investigate whether host associations reveal host specialization (De Barro *et al*., 1995b; Anstead *et al*., 2002) whilst similarly facilitating the investigation of processes that drive host adaptation and speciation (see Via, 1999; Hawthorne & Via, 2001). Now that there is clear evidence for host associations under field conditions, asexual lineages of grape phylloxera can be selected for more extensive fitness comparison and selection experiments – for example reciprocal transfer (De Barro *et al*., 1995b). These will help determine if the observed host associations are indeed the result of the presence of host adapted genotypes.

The presented findings have implications for the breeding and selection of resistant rootstocks and the management of *D. vitifoliae* in infested vineyards. Because grape phylloxera populations are comprised of genetically diverse lineages that differ in their host association, this variability needs to be considered when developing resistant rootstocks. Regional assessments of rootstock performance are already known to be important. However, the patchy distribution of lineages and clades in vineyards as evidenced from this study and Corrie *et al*. (2002), means that such assessments may not expose the vines under trial to all the available genotypes. If true, more controlled trials, possibly using excised roots or tissue cultured vines, are required to assess the resistance of vine types to representatives of the full range of phylloxera types known to be present in vineyards.

In Australia, quarantine is used to prevent spread of grape phylloxera from infested to uninfested vineyards. The implications of this study suggest it may also be important to practice vineyard hygiene and thereby reduce movement of insects between infested vineyards since the susceptibility of particular vine types may depend on the specific phylloxera lineage present in the vineyard. While rootstock resistance may be stable in the presence of certain genotypes, the introduction of lineages with different evolutionary histories may well lead to increased infestation levels. This in turn can lead to increased risk of breakdown in rootstock resistance and/or increased risk of breakdown in quarantine containment and spread to uninfested vineyards.

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