Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in Argentina: ecological associations to diversity, population structure and reproductive mode

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Abstract

Background and Aims: The North American insect pest grape phylloxera was introduced into Europe in the 19th century and devastated the *Vitis vinifera*-based vineyards. It has since become widely distributed among the world's vineyards. Although it is present in Argentina, it has not caused any major damage. This work aims: to characterise the genetic diversity of Argentinean phylloxera; to determine population structure and reproductive mode; to compare Argentinean genotypes with samples from seven other countries; and to examine relationships between the infestation level and genetic profile with ecological factors.

Methods and Results: One hundred and twenty-nine samples of phylloxera from Argentinean provinces were analysed with 21 microsatellite markers. Seventeen multilocus genotypes were identified. Unweighted pair group method with arithmetic mean analysis identified two major groups. Principal coordinates analysis grouped together Peruvian, California biotypes A and B and Argentinean phylloxera, showing dissimilarity with other foreign samples. Associations between infestation level, berry colour and climatic region, and associations between genetic clusters and climatic region were established.

Conclusions: Endemic Argentinean phylloxera genotypes showed genetic diversity and were different from other samples assessed. Parthenogenesis is proposed as the main reproductive mode, but rare sexual reproduction is not ruled out. No particular structure was observed in the phylloxera populations.

Significance of the Study: This is the first detailed report on the genetic characterisation of phylloxera populations in Argentina using microsatellites markers. It provides the foundation for further studies.

Keywords: *Argentina, microsatellite marker, parthenogenesis, phylloxera,* Vitis *spp.*

Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* Fitch (Hemiptera: Sternorrhyncha), is a damaging grapevine pest that is widely distributed in most viticultural regions of the world (Powell et al. 2013). This insect is native to North America where it coevolved with American *Vitis* species that are tolerant to phylloxera feeding (Wapshere and Helm 1987). In its native range, phylloxera feeds mostly on the leaves, producing abnormal tissue growth and tumour-like gall structures. In contrast, in Eurasian *V. vinifera*, feeding is predominantly on the roots, and the leaves are usually not attacked, although it has been observed feeding on leaves in Europe and some South American countries (Vidart et al. 2013, Professor M.A. Walker, unpublished data, 2006). Phylloxera root feeding induces the formation of hyperplasic and hypertrophied tissues, which result in gall-like structures (nodosities on young non-lignified roots and tuberosities on mature lignified roots), which act as carbon sinks. These structures disrupt the water and nutrient uptake by the vine causing loss of vigour (Stellwaag 1928). Feeding sites also provide entry avenues for fungal pathogens, leading to root necrosis (Omer et al. 1995, Granett et al. 1998). As a consequence, root growth is stunted, causing loss of vigour and yield and ultimately vine death (Davidson and Nougaret 1921, Boubals 1966). According to Granett et al. (1987) *V. vinifera* own-rooted Californian vineyards decline to a level of non-productivity within 5–10 years after the pest is established. In the 1860s, the pest was unintentionally introduced into Europe where it devastated own-rooted *V. vinifera* vineyards. The situation triggered a serious economic crisis, in some cases with demographic implications (Loubere 1974, Gallego 1981). Since then, phylloxera has spread across most of the world's viticultural areas, including South America, New Zealand, Australia, South Africa and China (Skinkis et al. 1995, Powell and Herbert 2005, Sun et al. 2011). Gironés de Sánchez (2007) reports that phylloxera was introduced into Argentina in 1878 via infested plants received from Marseille, France, which were then planted throughout the province of Buenos Aires. She also states that efforts to prevent the dispersion from the primary sites failed leading to discoveries in Río Negro province in 1924, in San Juan province in 1929 and in Mendoza province by the year 1936. Currently, phylloxera can be found in all Argentinean viticultural regions (Del Toro et al. 2007). Even though 90% of vineyards are own-rooted *V. vinifera*, phylloxera has surprisingly not become a major problem. In own-rooted vineyards,

doi: 10.1111/ajgw.12337

damage is severe only when plants are under biotic or abiotic stress (Del Toro et al. 2007). One hypothesis for this is that the phylloxera strains present in Argentina are less aggressive and consequently causes less damage. The other plausible explanation is that phylloxera is controlled by the predominant flood irrigation, which encourages wide-spread rooting, allows roots to escape infestation and at the same time drowns phylloxera reducing their populations in the rhizosphere. Nonetheless, Korosi et al. (2009) showed that phylloxera can survive submerged under water for a maximum of 5 days at 25°C. In Argentina, this may radically change, given the introduction of drip irrigation systems in the 1990s. There is also the potential introduction of exotic genotypes or the change of endemic genotypes to more aggressive strains. A clear example of this was the appearance of the so-called biotype B in California vineyards, a more aggressive phylloxera biotype capable of overcoming the resistance of the Ganzin 1 (*V. vinifera* cv. Aramon × *V. rupestris*) rootstock (Granett et al. 1985).

The phylloxera life cycle is both complex and not well understood. It includes sexual, asexual, winged and wingless reproductive forms (Forneck and Huber 2009, Bao et al. 2015). In Argentina, individuals with rudimental wings sometimes appear, but winged phylloxera have not been seen (Del Toro et al. 2007). Winged individuals capable of flying and laying viable eggs have been observed on excised roots conserved in Petri dishes (Miss Celeste Arancibia, unpublished data, 2014–17). The life cycle is assumed to be similar to California and Australian phylloxera populations, which are anholocyclic (parthenogenic females) and composed of root-feeding individuals hibernating as quiescent first or second instars (Corrie et al. 2002, Forneck and Huber 2009). Knowledge of the diversity and population structure of Argentinean phylloxera is scant. Information does not yet exist regarding the biotypes present in different viticultural regions nor are studies available in which DNA-based markers were used to decipher diversity or to determine the mode of reproduction. In other countries, co-dominant simple sequence repeat (SSR) markers are being used to determine the relationship of phylloxera genotypes. Numerous studies have used SSR markers to understand genetic structure and reproduction mode and to identify host-associated clones (Corrie et al. 2003, Umina et al. 2007, Griesser and Forneck 2009, Herbert et al. 2010, Sun et al. 2011, Islam et al. 2013, Bao et al. 2015, Čajić 2016). A large number of microsatellite markers (Riaz et al. 2014) are currently available to analyse Argentinean grape phylloxera. Agüero et al. (2017) identified two fingerprint profiles from the analysis of 21 samples, one of them grouping close to a reference sample of phylloxera from AXR#1 rootstock.

In this study, we employed SSR markers to evaluate phylloxera collected from own-rooted vineyards throughout the major viticultural regions of Argentina, which are either irrigated by drip systems or with traditional flooding. The main objectives were: (i) to characterise the genetic diversity within Argentinean phylloxera; (ii) to determine the structure and reproductive mode of phylloxera present in Argentina; (iii) to compare Argentinean phylloxera with known phylloxera genotypes in the USA, Europe and other South American countries and (iv) to find relationships between phylloxera infestation levels and genotype profiles and ecological factors, such as climate, soil texture, trellis system and irrigation method. Results are discussed in response to these objectives. This is the first detailed report of phylloxera population biology in Argentina that utilises DNA-based SSR markers. This work will provide the foundation for other studies, tracking and monitoring seasonal changes in the population structure of phylloxera, devising and establishing measures to control movement of different genotypes among regions and identifying additional types.

Materials and methods

Sample collection

Phylloxera samples were collected from vineyards throughout the main grapevine regions between 29°9′57.5″ and 39°3′51.4″ south latitude, and 69°35′ and 67°18′20″ west longitude, from July to November 2013. Samples consisted of phylloxera-infested pieces of roots from a wide range of red and white berried own-rooted *V. vinifera* cultivars (Aspirant Bouchet, Balsamina, Barbera, Bonarda, Cabernet Sauvignon, Cereza, Chardonnay, Corbina, Criolla Chica, Criolla Grande, Flame Seedless, Gibbi, Malbec, Merlot, Muscat of Alexandria, Pedro Giménez, Pinot Noir, Pinot Gris, Rieslina, Redglobe, Sauvignon Blanc, Sémillon, Superior, Syrah, Tannat, Tempranillo, Torrontés Riojano and Ugni Blanc). Insects, generally visible with the naked eye, were collected from mature-lignified and young roots. Roots were harvested from up to 50 cm deep in the soil, placed in plastic bags and kept in cold storage until processed in the laboratory. The number of samples from each region was proportional to the cultivated area. From a total of 129 samples, 88 were taken from different areas of Mendoza province (West Mendoza, South Mendoza and East Mendoza), 18 from San Juan province, 17 from Río Negro province and six from the La Rioja province. Samples collected from each of these areas were named: population West Mendoza (WM), South Mendoza (SM), East Mendoza (EM), San Juan (SJ), La Rioja (LR) and Río Negro (RN). Vineyards from Catamarca and Salta provinces were also examined but no phylloxera were found.

Genotyping

The Chelex method (Lin and Walker 1996) was used to extract DNA from four to six nymphs or adult insects (otherwise eight to ten eggs), feeding near or on the same tuberosity/nodosity, although in this occasion samples were mechanically disrupted using an electric drill with a plastic drill bit. Polymerase chain reactions of 10 μL were carried out with 21 fluorescently labelled forward primers using standardised conditions previously published (Corrie et al. 2002, Riaz et al. 2014) (Table 1). Each DNA sample was amplified individually and up to three markers were combined depending on the amplicon size and fluorescent labels of the markers. Samples were run on an ABI 3500 capillary electrophoresis analyser with GeneScan-500 Liz Size Standard (Life Technologies, Carlsbad, CA, USA). Allele sizes were determined using GeneMapper 4.1 software (Applied Biosystems, Foster City, CA, USA). Four additional samples from an earlier study were used as a reference to keep scoring consistent among different data sets (Riaz et al. 2014).

Data analysis

The average number of alleles for each marker was determined, and a clonal diversity index was calculated to determine clonal richness, according to Dorken and Eckert (2001) as $R = (G - 1)/(N - 1)$, where G is the number of genotypes and N is the sample size. Multilocus genotypes

Table 1. Microsatellite allelic data of 17 unique multilocus genotypes identified within the Argentinean phylloxera populations.

Population	No. multilocus genotypes																
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
LR	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
SJ	0	12	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0
WM	2	9	0	5	0	0	1	1	1	0	0	0	0	0	1	1	0
EM	0	34	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1
SM	0	11	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0
RN	3	1	1	5	0	0	0	0	0	1	0	0	0	0	0	0	0
Total	5	69	4	11	2	2	1	1	1	1	1	1	1	1	1	1	1

SSR	Allele size, in base pairs																
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
PhyII_6	110	110	110	110	110	110	110	110	110	–	110	110	110	110	110	110	110
	116	116	116	116	116	116	116	116	116	–	116	116	116	116	116	116	116
PhyII_10	136	136	136	136	136	136	136	136	136	136	136	136	136	136	134	136	136
	136	136	136	136	136	136	136	136	136	136	136	136	136	136	136	136	136
PhyII_13	117	117	117	117	117	117	117	117	117	117	117	117	117	117	117	117	117
	132	132	132	132	132	134	132	132	132	117	132	132	132	117	132	132	132
PhyII_16	112	112	112	112	112	112	112	112	112	112	112	112	112	112	112	112	112
	114	114	114	114	114	114	114	114	114	114	114	114	114	114	114	114	114
PhyII_23	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
	106	106	106	110	110	106	110	106	106	106	106	106	106	106	106	106	106
PhyII_31	96	96	96	96	96	96	96	96	96	96	96	96	96	96	96	96	96
	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111
PhyII_34	109	109	109	109	109	109	109	109	109	109	109	109	109	109	109	109	109
	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119
PhyII_36	120	120	120	120	120	120	120	120	120	120	120	120	120	120	120	120	120
	126	126	126	126	126	126	126	126	126	126	126	126	126	126	126	126	126
PhyIII_15	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134
	134	137	137	134	134	137	134	134	134	134	137	137	137	137	137	137	137
PhyIII_30	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119
	122	131	131	122	122	131	122	122	122	131	131	131	131	131	131	131	131
PhyIII_42	142	146	146	142	146	146	142	146	142	142	146	146	146	146	146	146	146
	146	146	148	146	146	146	146	146	146	146	150	150	146	146	146	146	150
PhyIII_46	104	104	104	104	104	104	104	104	–	104	104	104	104	104	104	104	104
	107	107	107	107	107	107	107	107	–	107	107	107	107	107	107	107	107
PhyIII_49	123	123	123	123	123	123	123	123	123	123	123	123	123	123	123	123	123
	123	123	123	123	123	123	123	123	123	123	123	123	123	123	123	123	123
PhyIII_53	238	238	238	238	238	238	238	238	238	238	238	238	238	238	238	238	238
	238	238	238	238	238	238	238	238	238	238	238	238	238	238	238	238	238
PhyIII_55	123	126	126	123	123	126	123	123	123	123	126	126	126	126	126	126	126
	129	129	129	129	129	129	129	129	129	126	129	129	129	129	129	129	129
PhyIII_61	110	110	110	110	110	110	110	110	110	110	110	110	110	110	110	110	110
	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119	119
PhyIII_63	132	132	132	132	132	132	132	132	132	132	132	132	132	132	132	132	132
	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135
PhyIII_65	100	100	100	100	100	100	79	100	100	100	100	100	100	100	100	100	100
	100	100	100	100	100	100	100	100	100	100	100	100	107	100	100	100	107
PhyIII_87	134	134	134	137	137	134	137	137	137	–	134	134	134	134	134	134	134
	137	137	137	143	143	137	143	143	143	–	137	137	137	137	137	137	137
PhyIV_4	233	233	233	233	233	233	233	233	233	233	–	233	233	233	233	238	233
	238	238	238	238	238	238	238	238	238	238	–	238	238	238	238	238	238
Dvit1	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122
	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130

–, missing data; EM, east Mendoza; LR, La Rioja; RN, Río Negro; SJ, San Juan; SM, South Mendoza; SSR, simple sequence repeat; WM, West Mendoza.

(MLGs) were identified with GenAlEx 6V (Peakall and Smouse 2006). Samples with missing data in monomorphic loci were classified within a previously defined MLG when the remaining markers were identical or as a unique MLG when alleles deferred. Samples with missing data in polymorphic loci were not included in the analysis when the remaining markers were identical, or were classified as a unique MLG when alleles differed. To establish the MLG modal class, a generalised linear model was applied assuming a binomial distribution and a logit link function for the proportion of the class in each population using InfoStat software (Grupo Infostat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina) (Di Rienzo et al. 2014). Mean differences were analysed with the Di Rienzo, Guzmán y Casanoves (DGC) test (Di Rienzo et al. 2002). A hierarchical clustering unweighted pair group method with arithmetic mean (UPGMA) was performed to analyse MLG groupings by Nei's distance (Nei 1978) and 10 000 bootstraps with R software (RStudio Team 2016) and Poppr package (Kamvar et al. 2014). Only one sample per repeated MLG was used in order to create the dendrogram. Genotypes belonging to California biotypes A and B were included in the construction of the tree, since we previously found that they grouped closely to some Argentinean samples. From now on, we will refer to them as California A and B biotype

samples. In this study, the term biotype is used as proposed by Forneck et al. (2016). In addition, three Peruvian foliar samples from an earlier study (Lund et al. 2017) were utilised as an outgroup. The results obtained from the hierarchical clustering were supported by a principal coordinates analysis (PCoA).

Analysis of molecular variance and F_{ST} coefficients were calculated to analyse the structure within the population of Argentinean samples, after removing samples with missing data to avoid bias. These two calculations, plus the probability of sex (P_{sex}) and deviations from Hardy–Weinberg equilibrium were performed with GenAlEx 6V (Peakall and Smouse 2006).

For comparison with international data, 189 samples from roots and leaves (161 samples from the USA, three from Brazil, four from Uruguay, seven from Austria, six from France and eight from Hungary) were added (Lund et al. 2017) and analysed through PCoA. The PCoA was carried out with DARWIN software (version 5.0.158) (Perrier and Jacquemoud-Collet 2006).

Test of independence for ecological variables associated with clusters of phylloxera genotypes and infestation level. Analysis of symptoms (feeding sites, tuberosities and nodosities with insects) and insect density relied upon the visual inspection of infested root pieces. High (H) corresponded to the occurrence of symptoms at a density of one or more per cm of root length. On these roots a high number of insects was observed, symptoms were obvious and the roots were badly damaged. Medium (M) corresponded to a density of one symptom every 3 cm length, and damage was less prominent. Finally, intensity was considered low (L) when the symptoms were more than 3 cm apart or few isolated insects were seen feeding on the root. In this case, the recognition of the pest was not easy by naked eyed and the general sanitary status of the roots was good.

The association of DNA-based genetic clusters derived from the UPGMA analysis or infestation level with the following variables: climatic region (warm or cold region), grape colour (pink-red or white berries), population (EM, WM, SM, SJ, LR and RN), vine age (over or under 26 years, which was the median), irrigation system (drip or flood), soil texture (sandy, clay or loam) and trellis system (vertically shoot positioned or overhead trellis) was assessed with Pearson's chi-square contingency tables or the Fisher–Irwin bilateral test, when observations were less than five per cell, using InfoStat software. Soil texture classification was based on FAO texture qualifiers where: clayic is clay; arenic is sand and siltic and loamic are combined in loam (International Union of Soil Sciences 2015). Bonferroni's correction for multiple comparisons was applied in cases of significant 2 × 3 contingency tables. Region categories were based on the mean of monthly temperature from January 2004 to December 2014, obtained from the Servicio Meteorológico Nacional, Argentina. San Juan (18°C), La Rioja (18°C) and East Mendoza (17°C) were classified as warm regions while South Mendoza (13°C), West Mendoza, (15°C) and Río Negro (16°C) as cold regions. Twenty-one cultivars were investigated in warm regions and 14 in cold regions, nine of which were common to both regions.

Results

Genetic diversity of Argentinean phylloxera

Of the 21 loci included in this study, 19 displayed polymorphism and the average number of alleles per locus was 2.29, ranging from 1 to 4 (Table S1). The highest clonal diversity (R) was found in La Rioja (R = 0.67), followed by Río Negro (R = 0.4), West Mendoza (R = 0.35), South Mendoza (R = 0.15), San Juan (R = 0.13) and East Mendoza (R = 0.11). Seventeen MLGs were identified, of which 6 were present in more than one population and 11 were unique (Table 1). The number of samples in each MLG varied from one to 69. The MLG modal class was named MLG B and gathered samples from all populations, followed by MLG D with 11 samples (five from West Mendoza, five from Río Negro and one from La Rioja population). The incidence of MLG B, relative to other MLGs in each population, was significantly higher in East Mendoza, South Mendoza and San Juan populations (Table S2). Two major divergent groups were identified by UPGMA analysis (Figure 1). The top group consisted of samples from Argentina and California biotype B, while the bottom group consisted of samples from Peru and California biotype A. In the top group, two branches diverged from the first node into two distinctive clusters designated as Large Cluster and Small Cluster. In the Large Cluster, MLGs B, C and F grouped with unique MLGs K, N, L, O and P and samples with missing data from West Mendoza, East Mendoza, South Mendoza, San Juan and La Rioja. Unique genotypes M and Q were located in separate branches and MLG J clustered alone.

The Small Cluster included the following groups: (i) MLG D and three samples with missing data from Río Negro and MLG G clustered close; (ii) MLG A, MLG I and two samples with missing data from Río Negro; and (iii) MLG E, MLG H, one sample from La Rioja and one from San Juan. The California biotype B sample clustered alone.

The groupings obtained in the UPGMA dendrogram corresponded with the PCoA, with three main groups identified (Figure 2). The California biotype B sample and the Small Cluster grouped on the negative side of the horizontal axis, while the California biotype A and Peruvian samples grouped on the positive side of both axes, and the Large Cluster on the negative side of the vertical axis. When all of the international samples were examined with Argentinean samples using PCoA analysis, three distinct groups were observed. The Peruvian, California biotypes A and B and Argentinean samples grouped together showing high dissimilarity with the other two groups. No common MLGs between Argentinean and foreign samples were found (Figure 3).

Structure and reproductive mode

The low F_{ST} value among populations (0.021) indicates lack of population structure. Moreover, analysis of molecular variance (AMOVA) revealed that only 1% of the total genetic variance was attributed to the variance among Argentinean populations [degrees of freedom (d.f.) = 5, sum of squares (SS) = 14.128, mean squares (MS) = 2.826, estimated variance (Est. var.) = 0.091], while 99% of the total variation was explained by the variance within individuals (d.f. = 92, SS = 738, MS = 8.022, Est. Var. = 8.022) and 0% among individuals (d.f. = 86, SS = 25.965, MS = 0.302, Est. Var. = 0.000).

Highly significant P_{sex} values, for all the repeated MLGs, showed the unlikely occurrence of sexual events (Table S3). Moreover, deviations from the Hardy–Weinberg equilibrium were significant in 16 loci out of 18 in East Mendoza, 16 out

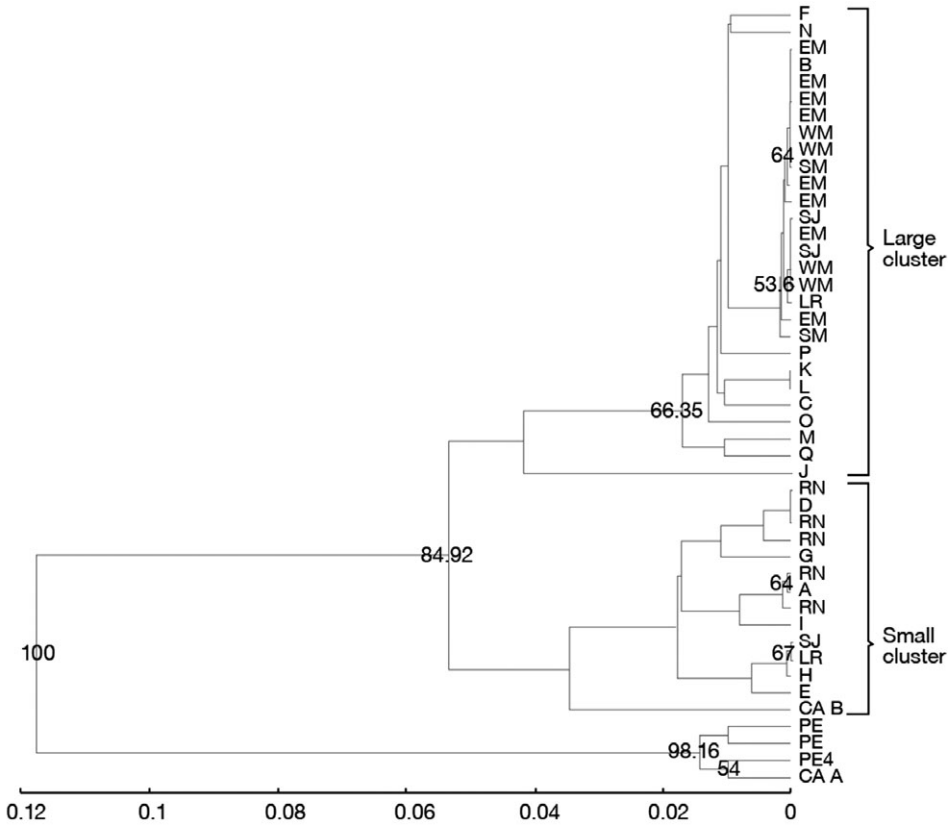

Figure 1. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram showing the relationship between Argentinean, Peruvian and California biotype A and B genotypes. Samples with missing data were designated with the name of the region where they were found. EM, East Mendoza; LR, La Rioja; RN, Río Negro; SJ, San Juan; SM, South Mendoza; WM, West Mendoza. Letters from A to Q are multilocus genotypes (MLGs).

of 17 in San Juan and Río Negro, 15 out of 19 in West Mendoza and 11 out of 17 in La Rioja population (Table 2).

Association of phylloxera and other variables

Tests of independence for the association between two variables at $\alpha = 0.05$, were significant for infestation level–climate region ($\chi^2 = 6.14$, d.f. = 2, $P = 0.0465$) and infestation level–grape colour ($\chi^2 = 9.33$, d.f. = 2, $P = 0.0094$). In both cases, Medium was the significant category ($P = 0.002314$ and $P = 0.013997$, respectively) (Table 3). The genetic cluster–climatic region association was also significant (Irwin Fisher bilateral test value = −0.57, $P < 0.0001$). In this case, cold region associated with Small Cluster and warm region with Large Cluster. Trellis system and climatic region showed a significant association ($\chi^2 = 7.2$, d.f. = 1, $P = 0.0073$); the cold region was associated with vertically shoot positioned and the warm region with the overhead trellis system (Table 4). The rest of the combinations studied were not significant.

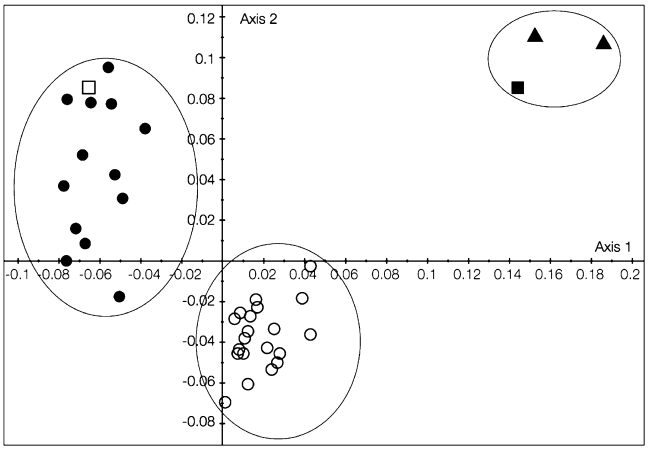

Figure 2. Principal coordinates analysis (PCoA) of California biotypes A (■) and B (□), Peru (▲) and Argentinean Small Cluster (●) and Large Cluster (○) samples. Axis 1 accounts for 38.99% of the variation and while the axis 2 accounts for 30.79 %.

Discussion

This study found that the genetic diversity of Argentinean phylloxera was higher than expected (17 MLGs). The highest number of alleles per locus was found in the West Mendoza and Río Negro populations and could be the result of multiple introductions of phylloxera into Argentina since the late 19th century. Alternatively, increases in the genetic diversity of phylloxera could be related to colder regions, as has been observed in other aphid species with rare sexual reproduction (Delmotte et al. 2001, Llewellyn et al. 2003, Vorburger et al. 2003). This rare sexual reproduction could explain the higher clonal richness index obtained for the colder Río Negro and West Mendoza regions.

The most frequent MLG was MLG B, and the generalised linear model showed higher a proportion of this MLG in the San Juan, East Mendoza and South Mendoza populations. These findings lead us to speculate that it could be a genotype with a broad ecological tolerance that can grow well both in cold and warm regions. In Australia, G1 and G4 genotypes have also a wider geographical distribution than other genotypes (Umina et al. 2007). Up to three different MLGs, some unique, were found within the same block and on the same cultivar in some vineyards in San Juan, West Mendoza, Río Negro and East Mendoza. Again, this could be because of new phylloxera introductions when replanting individual grapevines (missing, dead or unhealthy) or to uncommon sexual recombination.

The hierarchical clustering showed two major Argentinean phylloxera fingerprint profiles. These results are consistent with our preliminary study on Argentinean phylloxera

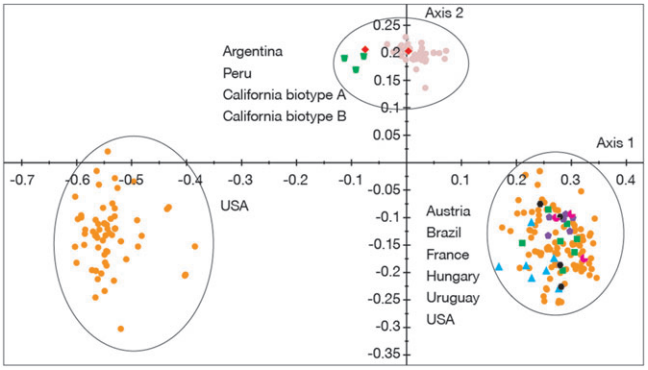

Figure 3. Principal coordinate analysis (PCoA) of genotypes from Argentina (●), Peru (▼), Austria (▲), Hungary (■), France (⬟), Brazil (◔), Uruguay (⬢) and USA (●) and California biotype A and B (◆). Axis 1 accounts for 39% of the variation, while axis 2 accounts for 12.97%.

carried out with a smaller set of samples (Agüero et al. 2017). The fact that the Large Cluster comprises more genotypes compared to that of the Small Cluster might derive from the association between Large Cluster and warm region, a vast region that includes the largest number of vineyards. The dissimilarity between the Large and Small Cluster and the similarity of the latter with California biotype B was confirmed by PCoA analysis. One possible explanation for two genetic clusters in Argentinean phylloxera could be the occurrence of two initial independent introductions that spread over the area asymmetrically followed by mutations or sexual reproduction events over an extended time period. Unfortunately, most data regarding the source of plant material are untraceable. It has been widely demonstrated, that phylloxera from different geographical areas and different genotypes have diverse biological performance at different temperature values (Fergusson-Kolmes and Dennehy 1993, Omer et al. 1999, Corrie et al. 2003). If this was the case for Argentinean phylloxera, the genotypes included in the Small Cluster might have adapted to the lower temperature in the cold region, while Large Cluster genotypes could perform better under the environmental conditions that characterise warmer regions.

Argentinean phylloxera showed more similarity with California and Peruvian phylloxera than with phylloxera from the rest of the world used in this study, and this was clearly seen on the PCoA analyses. In fact, it is reasonable to think that Argentinean phylloxera could have originated from California phylloxera because of its similarity to California biotypes A and B. This is in agreement with previous results that showed samples from Argentina and California grouping together. The lack of shared genotypes between Argentinean and other international samples indicates that those found in Argentina might be different. This observation points to the need for testing the feeding behaviour of local phylloxera on different rootstocks in controlled experiments to distinguish biotypes. In other countries, it has been well documented that different phylloxera genotypes have different aggressiveness on different rootstocks (Granett et al. 1985, Kocsis et al. 1999, Corrie et al. 2003). A better understanding of Argentinean phylloxera behaviour and life tables would also be a valuable tool in predicting the impact of climate change on its population structure.

In general, the Argentinean phylloxera population is not structured and this is revealed by the non-significant F_{ST} value. Allele frequencies among populations are not significantly different and there is an excess of heterozygosity in the population. The molecular variance analysis showed that main differences were observed within every individual compared to the general population and this is reflected in the detection of 17 MLGs.

As previously found in other regions (Vorwerk and Forneck 2006, 2007, Islam et al. 2013, Forneck et al. 2015, Riaz et al. 2017), the parthenogenetic nature of the insect in Argentina is confirmed by P_{sex} values, the excess of heterozygous individuals and the significant deviations from the Hardy–Weinberg equilibrium. The possible occurrence, however, of sporadic sexual reproduction cannot be discarded.

This study does not support the association of high infestation with clay soils and drip irrigation. It was interesting, however, to find a strong association between region and infestation level, which could explain the genotypic differences. Interestingly, the tests of independence exposed a significant association between trellis system and climate region. Overhead systems allow the development of vigorous vines that could improve the plant defences against

Table 2. Chi-square values for Hardy–Weinberg expectations over individual loci within phylloxera populations.

Population	La Rioja probability	San Juan probability	West Mendoza probability	East Mendoza probability	South Mendoza probability	Río Negro probability
PhyII_6	0.014*	0***	0***	0***	0***	0***
PhyII_10	NA	NA	0.915 ns	NA	NA	NA
PhyII_13	0.014*	0***	0***	0***	0.002**	0.001***
PhyII_16	0.014*	0***	0***	0***	0***	0***
PhyII_23	0.261 ns	0.001**	0***	0***	0***	0.001**
PhyII_31	0.014*	0***	0***	0***	0***	0***
PhyII_34	0.014*	0***	0***	0***	0***	0***
PhyII_36	0.014*	0***	0***	0***	0***	0***
PhyIII_15	0.414 ns	0***	0.059 ns	0***	0***	0.79 ns
PhyIII_30	0.112 ns	0***	0***	0***	0***	0.001**
PhyIII_42	0.824 ns	0.709 ns	0.243 ns	0.882 ns	0.897 ns	0.006**
PhyIII_46	0.014*	0***	0***	0***	0***	0***
PhyIII_55	0.112 ns	0***	0***	0***	0***	0.004**
PhyIII_61	0.014*	0***	0***	0***	0***	0***
PhyIII_63	0.014*	0***	0***	0***	0***	0***
PhyIII_65	NA	NA	0.915 ns	0.882 ns	NA	NA
PhyIII_87	0.112 ns	0***	0***	0***	0***	0.005**
PhyIV_4	0.014*	0***	0***	0***	0***	0***
Dvit1	0.014*	0***	0***	0***	0***	0***

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NA, not applicable; ns, not significant.

Table 3. Adjusted residuals of tests of independence for infestation level versus climate region and grape colour.

	Adjusted residuals of the independence tests		
	Low†	**Medium**	**High**
Climate region			
Cold region	1.28	−2.46†	1.43
Warm region	−1.28	2.46†	−1.43
Grape colour			
Red/pink	−1.45	3.05†	−1.88
White	1.45	−3.05†	1.88

†Infestation level; indicates statistical significance at adjusted P value = 0.017 (Bonferroni's correction). n = 83.

Table 4. Adjusted residuals of tests of independence for trellis system versus climate region.

Climate region	Adjusted residuals of the independence tests	
	Overhead trellis	**Vertical shoot position trellis**
Cold region	−2.68	2.68
Warm region	2.68	−2.68

n = 83.

phylloxera infestations, particularly in the larger more frequently irrigated vines. In addition, this type of trellis system decreases soil exposure and daily soil temperature variation, which would also improve the ability of the root system to withstand phylloxera.

The association between grape colour and infestation level suggests that red and pink cultivars maintain the infestation at a medium level while white cultivars exhibit a higher susceptibility. A possible explanation could be related to the production of compounds derived from the phenolic pathways implicated in plant defence mechanisms. Many secondary plant metabolites are involved in plant–herbivorous insect interactions, and they are important factors in the plant resistance/susceptibility towards various species of aphids (Awmack and Leather 2002, Goławska and Łukasik 2009). These hypotheses are being tested with feeding tests using root piece-bioassays.

Conclusions

Two main groups of phylloxera genotypes were identified in Argentina both of which showed some affinity to reference samples of the California biotype B. Parthenogenesis is proposed as the main reproductive mode, but the occurrence of rare sexual reproduction is not ruled out. No particular structure was observed in phylloxera populations and more variability was observed among the individuals. We also established an association of medium infestation levels with red/pink berried *V. vinifera* cultivars, and higher infestation with white fruited cultivars. The results from this study provide evidence that phylloxera in Argentina are diverse, and there is immediate need to study the biology of feeding behaviour of local genotypes on the rootstocks used in Argentina.

Acknowledgements

We wish to thank CONICET, Instituto de Biología Agrícola Mendoza, Facultad de Ciencias Agrarias, SECTYP Universidad Nacional de Cuyo, Department of Viticulture and Enology, University of California Davis, California Grapevine Rootstock Improvement Commission and Catena Institute of Wine for assistance and financial support. We also thank the Servicio Meteorológico Nacional (Argentina) for the climate data and Dr Daciana Papura from INRA Bordeaux for providing the French samples.

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Manuscript received: 18 January 2017
Revised manuscript received: 12 June 2017
Accepted: 21 August 2017

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website: http://onlinelibrary.wiley.com/doi/10.1111/ajgw.12337/abstract.

Table S1. Allele frequencies per locus in each Argentinean phylloxera population.

Table S2. Proportion of multilocus genotype B found in each Argentinean phylloxera population.

Table S3. Probability of sex (P_{sex}) values for multilocus genotypes found more than once in populations of Argentinean phylloxera.