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Changes in the genetic composition of *Myzus persicae nicotianae* populations in Chile and frequency of insecticide resistance mutations

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Research Paper

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Abstract

Myzus persicae is a cosmopolitan aphid that is highly polyphagous and an important agricultural pest. The subspecies *M. persicae nicotianae* has been described for highly specialized phenotypes adapted to tobacco (*Nicotiana tabacum*). In Chile, the population of *M. persicae nicotianae* was originally composed of a single red genotype that did not possess insecticide resistance mutations. However, in the last decade, variation in the colour of tobacco aphids has been observed in the field. To determine whether this variation stems from the presence of new genotypes, sampling was carried out across the entire distribution of tobacco cultivation regions in Chile. The aphids collected were genotyped, and the frequency of *kdr* (L1014F), super-*kdr* (M918T), modification of acetylcholinesterase (MACE) and nicotinic acetylcholine receptor β subunit (nAChRβ) mutations associated with insecticide resistance was determined. A total of 16 new genotypes of *M. persicae nicotianae* were detected in Chile: four of them possessed the MACE mutation, and none of them possessed the *kdr*, super-*kdr* or nAChRβ mutation. The previously described red genotype was not detected in any of the sampled fields over two seasons. These results raise questions about the mechanisms underlying changes in the genetic structure of *M. persicae nicotianae* populations in Chile. Future research aimed at addressing these questions could provide new insight into aphid evolution and agricultural practices.

Introduction

The green peach aphid *Myzus persicae* (Sulzer) is a highly polyphagous insect that can utilize a variety of weeds and crops belonging to more than 40 plant families (Blackman and Eastop, 2000, 2017). The ability of *M. persicae* to use several host plants with different chemical defences stems from genetically inherited mechanisms that facilitate the response to plant allelochemicals, which often results in interclonal variation in fitness (e.g., reproduction rates, longevity and feeding behaviour) between host plant species (Vorburger *et al.*, 2003; Turcotte *et al.*, 2011; Cabrera-Brandt *et al.*, 2015). *Myzus persicae nicotianae* is a specialized subspecies adapted to tobacco (*Nicotiana tabacum* L.) (Margaritopoulos *et al.*, 2000; Blackman and Eastop, 2017). Unlike *Myzus persicae persicae*, this tobacco-adapted subspecies can overcome the physical and chemical defences of tobacco plants via morphological, genetic, transcriptomic and metabolic traits (Blackman *et al.*, 2007; Margaritopoulos *et al.*, 2007*b*; Cabrera-Brandt *et al.*, 2010; Vučetić *et al.*, 2010; Bass *et al.*, 2013; Peng *et al.*, 2016; Pan *et al.*, 2019*a*, 2019*b*; Singh *et al.*, 2020).

Various groups of insecticides have been used to control *M. persicae* in several crops (Foster *et al.*, 2017), which has resulted in strong selection for insecticide resistance (Bass *et al.*, 2014). The tobacco aphid *M. persicae nicotianae* has developed several insecticide resistance mechanisms, including (i) elevated carboxylesterase levels, which confer resistance to organophosphates, carbamates and pyrethroids (Margaritopoulos *et al.*, 2007*b*; Kati *et al.*, 2014); (ii) modification of acetylcholinesterase (MACE), which confers resistance to dimethyl carbamates (Margaritopoulos *et al.*, 2007*b*); (iii) *kdr* (L1014F) and super-*kdr* (M918T) mutations in the voltage-gated sodium channel, which confers resistance to pyrethroids and dichlorodiphenyltrichlorethane (DDT) (Margaritopoulos *et al.*, 2007*b*); (iv) a mutation in the nicotinic acetylcholine receptor β subunit (nAChRβ) associated with resistance to neonicotinoid insecticides (Bass *et al.*, 2011; Voudouris *et al.*, 2016) and (v) a metabolic mechanism based on a cytochrome P450 monooxygenase, which confers resistance to neonicotinoids (Puinean *et al.*, 2010; Voudouris *et al.*, 2016). Furthermore, transcriptomic mechanisms involved in the

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aphid detoxification response that suppress the metabolic effects of the insecticide or nicotine have also been described in *M. persicae nicotianae* (Cabrera-Brandt *et al.*, 2014; Peng *et al.*, 2016; Pan *et al.*, 2019*a*, 2019*b*; Singh *et al.*, 2020).

In Chile, populations of *M. persicae nicotianae* were previously reported to be composed of a single predominantly red genotype on tobacco crops (Fuentes-Contreras *et al.*, 2004), a genotype that was later found to also be widespread in tobacco fields in the USA, Brazil and Argentina (Zepeda-Paulo *et al.*, 2010). Such ecologically successful aphid genotypes are considered 'superclones' (Vorburger *et al.*, 2003), which are frequently obligate parthenogenetic asexual lineages (Figueroa *et al.*, 2018). This red tobacco aphid 'superclone' exhibited moderate levels of carboxylesterase activity (R1) with no *kdr* (L1014F), super-*kdr* (M918T) or MACE mutations (Fuentes-Contreras *et al.*, 2004; Cabrera-Brandt *et al.*, 2014). However, in the last decade, aphid colonies in tobacco fields in Chile have begun to display colour variations (e.g., green, yellow and red). This colour polymorphism has been observed throughout the geographic range of tobacco plantations in Chile, and the colour of aphids varies between plants, as each plant hosts a single colony (unpublished data). Similar patterns have been previously described in Greece (Margaritopoulos *et al.*, 2000, 2002; Zitoudi *et al.*, 2001; Poupoulidou *et al.*, 2006; Blackman *et al.*, 2007), Italy (Margaritopoulos *et al.*, 2003), Japan (Shigehara and Takada, 2003, 2004; Margaritopoulos *et al.*, 2007*a*, 2007*b*) and the USA (Harlow and Lampert, 1990; Clements *et al.*, 2000*a*, 2000*b*; Srigiriraju *et al.*, 2009), where higher genotype diversity of tobacco aphids has been observed (Zepeda-Paulo *et al.*, 2010). The colour polymorphisms can be explained by the presence of new *M. persicae nicotianae* genotypes resulting from sexual reproduction events, as reported for *M. persicae* in Chile on peaches (Rubiano-Rodríguez *et al.*, 2014, 2019; Rubio-Meléndez *et al.*, 2018) or by new introduction events from neighbouring countries. These putative new genotypes could possess insecticide resistance mutations that affect tobacco aphid control in tobacco fields in Chile. The aim of this study was to evaluate whether phenotypic colour variation is related to the presence of new *M. persicae nicotianae* genotypes in Chile. This study provides an updated assessment of the insecticide resistance status of Chilean tobacco aphid populations by evaluating the presence previously described insecticide resistance mutations in *M. persicae nicotianae*.

Materials and methods

Sampling programme

A total of 32 tobacco fields were sampled from February–March in 2015 and 2016 along a 400-km latitudinal gradient that spans the entire range of the tobacco-growing area in Chile. Tobacco fields (table 1) were divided into three areas of tobacco production: the North comprising five fields with 28 samples (2015) from the northern Valparaíso Region; the Centre comprising 12 fields (2015) and four fields (2016) totalling 121 samples from the southern O'Higgins Region to the centre of the Maule Region and the South comprising six fields (2015) and five fields (2016) totalling 82 samples from the eastern Ñuble Region. A total of 231 aphid samples were obtained (table 1). Aphids were collected from the two most commonly grown tobacco types in Chile (Burley and Virginia). The minimum distance between points from which aphids were collected was 30 m. In each field, each aphid sample was collected from a single colony per tobacco plant, gently lifted from the plant with a paintbrush, placed in 1.7-ml tubes filled with absolute ethanol and stored at 4°C for later analysis.

Microsatellite genotyping

All samples, including three samples corresponding to the single red genotype of *M. persicae nicotianae* (MPNsrg, table 1) previously described by Fuentes-Contreras *et al.* (2004) and stored in our laboratory, were genotyped using six microsatellite loci (*Myz2*, *Myz9*, *Myz25*, *M35*, *M37* and *M40*), which have been described and used extensively for *M. persicae* (Fuentes-Contreras *et al.*, 2004; Margaritopoulos *et al.*, 2007*a*, 2007*b*; Cabrera-Brandt *et al.*, 2010, 2014). The DNA quality and quantity were assessed using a Nanodrop (Nanodrop Technologies, Wilmington, DE, USA) spectrophotometer. Polymerase chain reactions (PCRs) were performed using the M13 universal primer (−21) labelled with fluorescent FAM or VIC at the 5′ end of the forward primer as described previously (Schuelke, 2000). Each amplification was conducted in a 15-µl reaction volume containing 1× Mg^{2+}-free reaction buffer, 25 mM $MgCl_2$, 10 µM dNTPs, 1 µM each of the forward and reverse primers, 1 µM of the M13 primer, 0.5 U Platinum® Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 20 ng $µl^{-1}$ of total DNA in sterile nanopure water. PCRs were carried out with the following thermal cycling programme: initial denaturation at 94°C for 5 min, followed by four touchdown cycles of 30 s of denaturation at 94°C, 30 s of annealing (62, 61, 59 and 57°C each cycle) and 45 s of elongation at 72°C. Next, 26 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 55°C and 45 s of elongation at 72°C were performed. Finally, eight cycles using an annealing temperature of 53°C, followed by 10 min of elongation at 72°C completed the amplification. Positive DNA amplifications were checked following electrophoresis in a 2.0% agarose gel. Automated fragment analysis by sequencing was conducted by Macrogen Inc. (Seoul, South Korea). The allele size and configuration for each individual were obtained using GeneMarker® software (SoftGenetics, State College, PA, USA).

Identification of insecticide resistance mutations

DNA isolation was conducted to characterize the insecticide resistance mutations in each sample. This analysis was performed using the TaqMan assay in a STRATAGENE MX 3000 (Agilent Technologies, Santa Clara, CA) thermocycler. The *kdr* (L1014F) and super-*kdr* (M918T) mutations were identified according to Anstead *et al.* (2004), while MACE (S431F) was identified according to Anstead *et al.* (2008). Finally, the arginine to threonine (R81T) mutation in the nAChRβ was detected according to Voudouris *et al.* (2017). The detection output was interpreted using the S (sensitive) allele and R (insensitive) allele nomenclature, according to Rubio-Meléndez *et al.* (2018).

Data analysis

The clonal heterogeneity indices (adapted from Simpson's index and Hill's Simpson reciprocal index) and clonal evenness index (Simpson's evenness index) were calculated using GENCLONE 2.0 software (Arnaud-Haond and Belkhir, 2007). The observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding

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Table 1. Details of *M. persicae nicotianae* sampling from tobacco fields in central Chile

Field	GPS coordinates (Lat./Long.)	Number of samples	Collection year	Cultivation area
T1	32°43′12.87″S/70°55′42.01″W	9	2015	N
T2	32°42′48.50″S/70°55′33.71″W	3	2015	N
T3	32°42′51.97″S/70°55′23.50″W	7	2015	N
T5	32°42′55.67″S/70°55′18.90″W	4	2015	N
T7	32°43′23.88″S/70°55′24.13″W	5	2015	N
MPNsrg	32°41′42.91″S/70°55′38.38″W	3	2008	N
T6	34°46′49.56″S/71°2′51.76″W	5	2015	C
T8	34°44′40.21″S/71°2′20.92″W	1	2015	C
T9	35°23′32.49″S/71°30′8.76″W	2	2015	C
T10	35°23′32.00″S/71°30′59.31″W	4	2015	C
T11	35°23′44.73″S/71°31′21.59″W	8	2015	C
T12	35°31′40.02″S/70°58′3.20″W	4	2015	C
T13	34°43′22.95″S/70°58′3.20″W	9	2015	C
T14	34°46′39.56″S/71°1′44.98″W	5	2015	C
T15	34°46′31.49″S/71°2′30.11″W	10	2015	C
T16	34°42′1.42″S/70°57′25.31″W	10	2015	C
T17	34°43′32.56″S/70°59′48.95″W	10	2015	C
T18	34°47′12.40″S/71°0′28.16″W	9	2015	C
T2	34°47′34.37″S/71° l′55.60″W	21	2016	C
T3	34°48′18.28″S/71°1′19.94″W	2	2016	C
T4	34°46′40.42″S/71°2′49.57″W	10	2016	C
T5	34°44′43.02″S/71°2′13.38″W	10	2016	C
T19	36°35′37.10″S/71°45′15.90″W	8	2015	S
T20	36°35′24.51″S/71°45′15.54″W	9	2015	S
T21	36°33′43.16″S/71°45′47.57″W	2	2015	S
T22	36°33′27.36″S/71°45′54.45″W	2	2015	S
T23	36°31′49.29″S/71°46′10.87″W	5	2015	S
T24	36°32′21.75″S/71°46′45.90″W	4	2015	S
T6	36°35′37.55″S/71°45′18.77″W	10	2016	S
T7	36°33′52.64″S/71°45′46.35″W	11	2016	S
T8	36°30′54.91″S/71°45′44.97″W	11	2016	S
T9	36°30′47.60″S/71°46′6.18″W	10	2016	S
T10	36°31′5.64″S/71°46′13.25″W	10	2016	S

The sampled tobacco fields with their coordinates, number of samples, year of collection and areas of tobacco production: north (N), centre (C) and south (S). MPNsrg: single red genotype of *M. persicae nicotianae*.

coefficient (F_{IS}) (log likelihood ratio statistic with dememorization number = 10,000, iterations per batch = 10,000 and batches = 100) were calculated according to Brookfield (1996) using the GENEPOP package version 1.2 (Raymond and Rousset, 1995; see http://genepop.curtin.edu.au/). The inbreeding coefficient over all loci was calculated in the same software (log likelihood ratio statistic with dememorization number = 10,000, iterations per batch = 10,000 and batches = 100).

The genetic distance matrix between genotypes was evaluated using POPULATIONS 1.2.32 software, and the resulting tree was built with FIGTREE 1.4.3 software.

The proportion of genetic differentiation among areas due to molecular differences was assessed for the three areas (considering all copies and one single copy per multilocus genotype (MLG)). A hierarchical analysis of molecular variance (AMOVA) was conducted, including variation among and within areas and samples from the same source. Hence, *F*-statistics were calculated according to AMOVA on GenAlEx v6.4 (Peakall and Smouse, 2006) to assess the genetic differentiation for microsatellite loci (which assumes a stepwise mutation model) and with the Codom-Microsat genetic distance using 999 permutations (Excoffier *et al.*, 1992; Peakall *et al.*, 1995).

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Table 2. Allele size of six loci evaluated for aphid body colour and insecticide resistance mutations for all new genotypes and the single red genotype (MPNsrg) of *M. persicae nicotianae* in Chile

	Allele size						Insecticide resistance mutations				
Genotype	*Myz2*	Myz9	Myz25	*M35*	*M37*	*M40*	Colour	MACE	kdr	S-kdr	nAChRβ
MPN1	182,200	226,240	136,138	193,215	168,172	148,150	G	SS	SS	SS	SS
MPN2	182,206	224,224	136,136	193,193	168,172	148,150	G	SS	SS	SS	SS
MPN3	182,206	224,224	136,136	193,213	168,172	148,150	R	SS	SS	SS	SS
MPN4	182,206	224,224	136,136	193,213	170,172	148,150	R	SS	SS	SS	SS
MPN5	194,196	224,230	136,136	193,211	170,180	148,150	G	SS	SS	SS	SS
MPN6	194,196	240,242	136,136	193,211	170,180	148,150	G	SS	SS	SS	SS
MPN7	194,210	224,230	136,136	193,211	000,000	148,150	G	RS	SS	SS	SS
MPN8	196,196	224,226	136,136	193,215	170,172	142,150	G	RS	SS	SS	SS
MPN9	200,206	212,240	136,136	193,199	170,172	148,150	Y	RS	SS	SS	SS
MPN10	200,206	224,240	136,136	193,215	172,172	142,150	G	SS	SS	SS	SS
MPN11	200,206	224,240	136,138	193,213	170,172	148,150	G	SS	SS	SS	SS
MPN12	206,206	224,226	136,136	193,215	170,172	142,150	G	SS	SS	SS	SS
MPN13	206,206	224,226	136,136	193,215	172,172	142,150	G	RS	SS	SS	SS
MPN14	206,210	224,240	136,136	199,213	170,172	148,150	G	SS	SS	SS	SS
MPN15	210,210	240,242	136,136	195,199	170,178	148,150	G	SS	SS	SS	SS
MPN16	210,222	212,240	134,136	199,199	170,170	136,150	G	SS	SS	SS	SS
MPNsrg	208,218	230,234	136,136	213,215	170,172	130,138	R	SS	SS	SS	SS

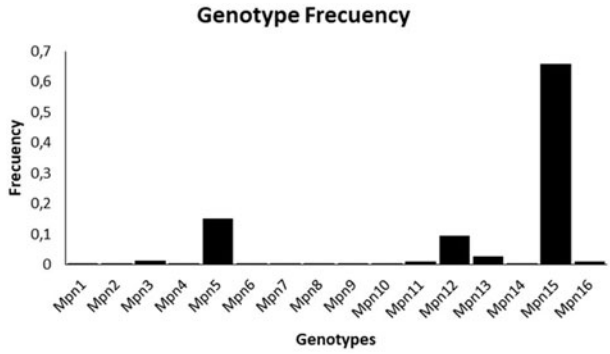

Figure 1 Overall frequencies of each of the 16 genotypes of *M. persicae nicotianae* detected in Chile.

A Bayesian clustering analysis conducted with single copies of each genotype was performed in STRUCTURE version 2 software 3 (Pritchard *et al.*, 2000) using admixture ancestry and correlated allele frequency models. The number of clusters (K) was varied from 1 to 10, and the analysis was repeated five times. Each repetition consisted of a burn-in period of 60,000 iterations and 600,000 Markov chain Monte Carlo iterations. The online program STRUCTURE HARVESTER (Earl and von Holdt, 2012) was used to calculate the most likely number of genetic clusters (K) using the Evanno method (Evanno *et al.*, 2005).

Results

Genetic diversity

Genotyping revealed the presence of 16 MLGs in the entire sample of *M. persicae nicotianae* from Chilean tobacco fields, which were named MPN1 to MPN16 (table 2). The single red genotype previously reported in Chile was not detected (table 2). The most frequent genotypes were MPN15, MPN5 and MPN12 (fig. 1). The population genetic parameters of *M. persicae nicotianae* are shown in table 3; there are no signs of heterozygote deficiency (F is negative), and heterozygosity was near expectation.

The Goldstein genetic distance was not closely related among genotypes or between the newly found genotypes and the single red genotype previously described (fig. 2).

Differentiation among populations

The hierarchical AMOVA revealed no genetic differentiation among areas for analyses performed with all copies ($F_{ST} = -0.006$, $P = 1.000$, table 4) or with a single copy per MLG ($F_{ST} = -0.021$, $P = 0.609$, table 4). Low but significant genetic differentiation was observed within samples of *M. persicae nicotianae* (4.0 and 1.0% for all and single-copy MLGs, respectively). Finally, a large and significant percentage of variation (96 and 99% for all and single-copy MLGs, respectively) was observed among samples of the three areas studied (table 4).

Similarly, the Bayesian analysis conducted using a single copy per MLG revealed no genetic differentiation among areas. Analysis of the population genetic structure considering a single copy per MLG from all areas revealed three genetic clusters ($K = 3$) according to the Evanno method (modal value of ΔK (fig. 3a)). In the northern area of tobacco fields, the three clusters were represented in the same proportions (33.3%), while in the central area, cluster 1 (red) represented 37% of the population, cluster 2 (green) 28% and cluster 3 (blue) 35%. In the southern area, the proportions for clusters 1, 2 and 3 were 11, 18 and 71%, respectively (fig. 3b).

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Table 3. Population genetic parameters of *M. persicae nicotianae*

Area	H_o	H_e	F_{IS}	H''	D^*	ED*	MLGs
N	0.473	0.696	0.471	0.616	0.362	0.403	3
C	0.523	0.703	−0.346	1.012	0.501	0.505	8
S	0.562	0.677	−0.205	1.471	0.632	0.582	11
Total	0.534	0.694	−0.307	1.233	0.536	0.503	16

Areas of tobacco production, north (N), centre (C) and south (S); H_o, observed heterozygosity; H_e: expected heterozygosity; F_{IS}: inbreeding coefficient; H'': Shannon index; D^*: adapted Simpson's index; ED*: Simpson's evenness index; MLGs: number of multilocus genotypes.

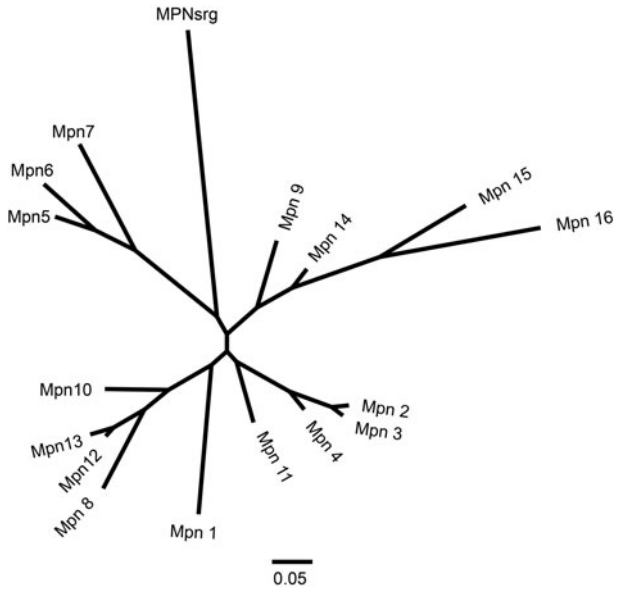

Figure 2 Goldstein genetic distance between 17 *M. persicae nicotianae* genotypes in Chile, including the single red genotype (MPNsrg).

Insecticide resistance mutations

Mutations conferring resistance to insecticides were found only in MLGs (MPN7, MPN8, MPN9 and MPN13) and corresponded to the MACE mutation in the heterozygote state (RS). No MLGs were found to carry the *kdr*, super-*kdr* or nAChRβ mutations and were characterized as susceptible homozygotes (SS) (table 2).

Discussion

In 2004, the presence of the tobacco aphid *M. persicae nicotianae* was reported in Chile for the first time. After a population genetic survey and evaluation of insecticide resistance mechanisms, a single red genotype was found to dominate the entire population and was determined to be slightly resistant to organophosphate and carbamate and susceptible to pyrethroids (R1 level of esterase activity and *kdr* susceptible), despite the intense use of insecticides for its control (Fuentes-Contreras *et al.*, 2004, 2007).

The predominance of this single red genotype was maintained for many years, and the mechanisms involved in its ecological success have been widely studied. The biological features of this genotype have been characterized through the study of its behaviour on tobacco (Troncoso *et al.*, 2005; Vargas *et al.*, 2005; Tapia *et al.*, 2015), performance on different host plants (Olivares-Donoso *et al.*, 2007; Tapia *et al.*, 2008), route of introduction to Chile (Zepeda-Paulo *et al.*, 2010), the effects of individual insecticides (Fuentes-Contreras *et al.*, 2007), transcriptomic responses for moderating the effects of insecticides (Cabrera-Brandt *et al.*, 2014) and the properties of its allelochemical detoxification metabolism that have allowed it to overcome tobacco's chemical defences (Cabrera-Brandt *et al.*, 2010).

Nevertheless, the Chilean population of *M. persicae nicotianae* has experienced drastic changes. Despite identifying 16 new MLGs of *M. persicae nicotianae*, there were no signs of the single red superclone in any of the sampled Chilean tobacco fields. These new genotypes have phenotypic and genetic differences from the founder genotype, which include colour variation (green, yellow and red) and the presence of MACE mutations in four of the new genotypes, a mutation that confers resistance to dimethyl carbamates. Although pyrethroids and neonicotinoids have been intensively sprayed on tobacco fields to control the tobacco aphid during the last decade in Chile, none of the new genotypes have *kdr* (L1014F), super-*kdr* (M918T) or nAChRβ mutations (Fuentes-Contreras *et al.*, 2007). Similar changes in the frequencies of genotypes and insecticide resistance mutations have been described for *M. persicae nicotianae* in tobacco fields in Greece (Margaritopoulos *et al.*, 2007*a*, 2007*b*, 2021; Kati *et al.*, 2014).

The higher genetic diversity and detection of MACE mutations raise questions about the origin of these new genotypes. Multiple introduction events and sexual reproduction may account for an increase in the genetic diversity of aphid populations following demographic bottlenecks (Nibouche, *et al.*, 2014; Bebber, 2015; Figueroa *et al.*, 2018). Introductions from different countries are likely to occur, as was shown for the red genotype by Zepeda-Paulo *et al.* (2010). Furthermore, sexual reproduction or hybridization events between *M. persicae nicotianae* and *M. persicae persicae* have been reported in Greece (Blackman *et al.*, 2007; Margaritopoulos *et al.*, 2007*a*) in areas where tobacco is cultivated near peach (*Prunus persica*) orchards. Indeed, *M. persicae persicae* reproduces sexually on peach in Chile (Rubiano-Rodríguez *et al.*, 2014; Rubio-Meléndez *et al.*, 2018), which makes hybridization with *M. persicae nicotianae* a likely occurrence. This idea is supported by the lack of genetic differentiation between aphids collected from different tobacco-growing areas in Chile, which form a single large population that might be connected by gene flow from aphid populations on peach. This is consistent with the finding that most of the alleles present in the new *M. persicae nicotianae* genotypes are present in *M. persicae persicae* collected from secondary and primary hosts. Second, MACE is present in *M. persicae persicae* on peach and other crops, where the dimethyl carbamate insecticide pirimicarb is used, but this chemical has not been applied to tobacco. However, *kdr* has been found to be present at high frequencies in aphids on peach and other

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Table 4. Molecular variance AMOVA for *M. persicae nicotianae* aphids from samples collected in three areas of tobacco production in Chile: north (N), centre (C) and south (S)

Source of variation	*df*	Sum of squares	Variance components	Percentage variation	Fixation indices	P-value
Three areas (all copies per genotypes)						
Among areas	2	6874.042	0.000	0	F_{ST}: −0.006	1.000
Among samples within areas	228	1,393,869.785	2994.013	96	F_{IS}: 0.960	0.001
Within samples	231	28,976.000	125.437	4	F_{it}: 0.960	0.001
Three areas (single copies per genotypes)						
Among areas	2	109,441.538	0.000	0	F_{ST}: −0.021	0.609
Among samples within areas	19	1,200,370.735	31,439.658	99	F_{IS}: 0.991	0.014
Within samples	22	6558.000	289.091	1	F_{it}: 0.990	0.014

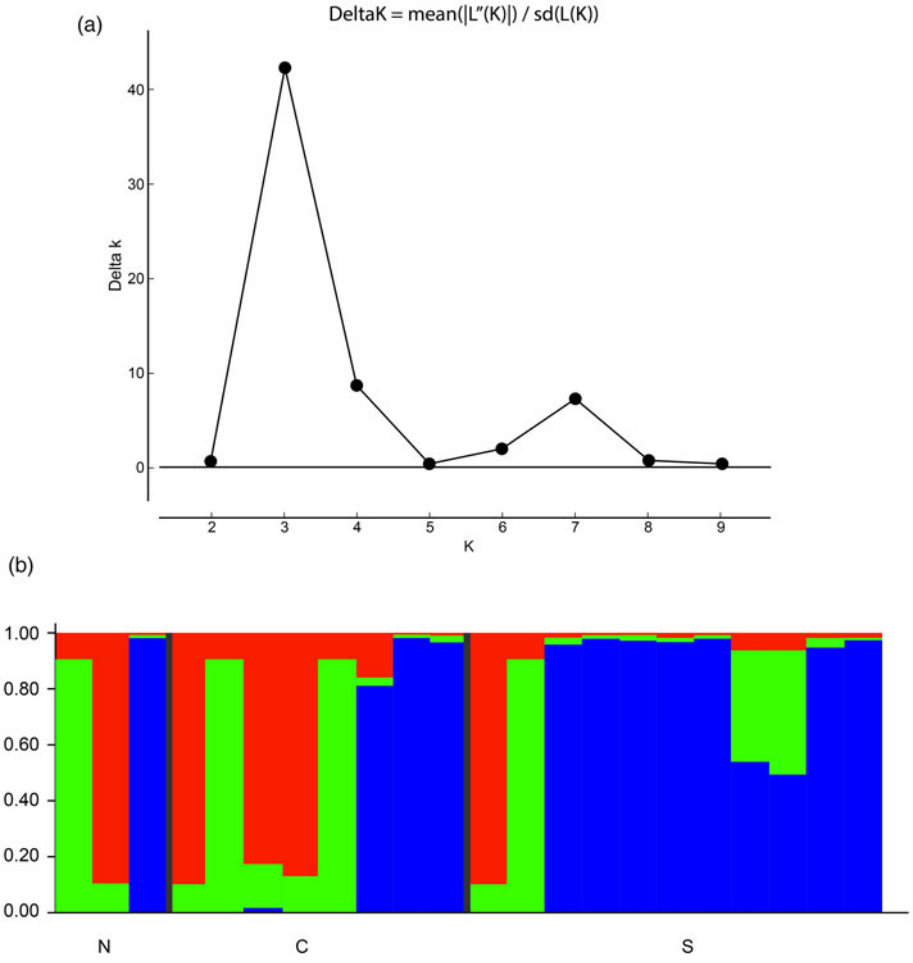

Figure 3 Bayesian assignment analysis of individuals to clusters. (a) Δ*K* for different numbers of clusters was *K* = 3. (b) Assessment of population genetic structure by Bayesian cluster analysis. The MLGs were grouped according to the areas of tobacco production (north (N), centre (C) and south (S)), and each of the MLGs was represented by vertical bars divided by colours according to their coefficients of ancestry to each cluster.

crops but was previously noted to be absent from tobacco populations (Cabrera-Brandt *et al.*, 2014; Rubio-Meléndez *et al.*, 2018). Furthermore, the presence of other super-*kdr* mutations detected in *M. persicae persicae*, such as the super-*kdr* M918L mutation, is not associated with the presence of the *kdr* L1014F mutation (Fontaine *et al.*, 2011; Panini *et al.*, 2014: Mingeot *et al.*, 2021). These super-*kdr* M918L mutations have not yet been described in *M. persicae nicotianae*, even in Greece, where recent studies have established their absence (Margaritopoulos *et al.*, 2021).

One question raised by our findings relates to why the single red genotype, which was previously found to be widespread in Chile, apparently disappeared. This is a difficult question to answer given that the single red genotype was not detected in any of the tobacco fields sampled throughout Chile's tobacco-growing regions over the two consecutive seasons of this study. The last preserved samples of the single red genotype are from laboratory colonies used in Cabrera-Brandt *et al.* (2014) and Tapia *et al.* (2015). Furthermore, the genetic distance of the single red genotype is high with respect to the 16 new genotypes; thus, it

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is unlikely that the new genotypes evolved from the single red genotype because of the number of mutational steps that would have been required. Because the intrinsic rate of growth (r_m) of the single red genotype is lower on tobacco than on sweet pepper, which is considered its optimal host (Nikolakakis *et al.*, 2003; Tapia *et al.*, 2008; Cabrera-Brandt *et al.*, 2010), the single red genotype might have been competitively displaced towards other secondary hosts by these new genotypes. Furthermore, global climate change has affected environmental conditions in Chile over the past decade; specifically, there has been a sustained increase in the number of hot days (over 30°C) per year (Dirección Meteorológica de Chile; www.meteochile.gob.cl). Such changes in climatic variables are known to affect aphid population dynamics in many crops (Bell *et al.*, 2015).

Zepeda-Paulo *et al.* (2010) reported low-genetic diversity in the populations of *M. persicae nicotianae* in Argentina and Brazil; in Chile, the diversity was zero because of the presence of a single *M. persicae nicotianae* genotype in tobacco crops. Therefore, the increase in the genetic diversity of *M. persicae nicotianae* observed in recent times on Chilean tobacco crops may have also occurred in these neighbouring countries. Additional studies are needed to characterize insecticide resistance mechanisms and ecological traits, such as the performance and behaviour of these new genotypes on different host plants, and compare them with other *M. persicae nicotianae* populations to evaluate the generalizability of the patterns observed in Chile.

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