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# Genetic diversity and insecticide resistance of *Myzus persicae* (Hemiptera: Aphididae) populations from tobacco in Chile: evidence for the existence of a single predominant clone

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**Abstract**

The tobacco-feeding race of *Myzus persicae* (Sulzer), formerly known as *M. nicotianae* Blackman, was introduced into Chile during the last decade. In order to evaluate the genetic diversity and insecticide resistance status of Chilean tobacco aphid populations, a field survey was conducted in 35 tobacco fields covering a 300 km latitudinal survey. The populations sampled were characterized using microsatellite markers and morphometric multivariate analysis. Insecticide resistance levels were assessed through a microplate esterase assay and the mutation status of the *kdr* gene. All samples collected corresponded to the same anholocyclic aphid genotype, and showed morphological variation within the range expected for the tobacco-feeding race of *M. persicae*. Esterase activity showed the level and variability expected for an R1 clone lacking mutations in the sodium channels (susceptible *kdr*), thus corresponding to a type slightly resistant to organophosphate and carbamate, and susceptible to pyrethroid insecticides.

## Introduction

Polyphagous aphids (Hemiptera: Aphididae) are frequent agricultural pests around the world, among which the peach–potato aphid, *Myzus persicae* (Sulzer), is of primary importance in several crops (Blackman & Eastop, 2000). This notoriously polyphagous aphid species is known to develop populations adapted to specific host-plants, such as the populations of *M. persicae* colonizing tobacco, which have been previously described as a sibling species, *Myzus nicotianae* Blackman (Blackman, 1987a). However, recent studies have characterized both aphids genetically and biochemically using molecular markers (RAPD–PCR, mitochondrial gene sequencing, microsatellites, etc.) and biochemical markers (esterases, cuticular hydrocarbons, etc.), all those failing to reveal detectable differences between these hypothetically distinct species (Margaritopoulos *et al*., 1998; Clements *et al*., 2000a,b; Zitoudi *et al*., 2001). Furthermore, hybridization between the tobacco-feeding race and *M. persicae* sensu stricto occurs in Mediterranean Europe, particularly in areas where the primary host, peach, *Prunus persica* (L.) Batsch (Rosaceae) is present near tobacco fields (Kephalogianni *et al*., 2002; Margaritopoulos *et al*., 2002).

Field populations of *M. persicae* sensu stricto have shown a variable genetic diversity in different countries. Thus, almost every sample corresponded to a different genotype in Spain (Martínez-Torres *et al*., 1997), in Australia (Wilson *et al*.,

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2002) and in France (Fenton *et al.*, 2003; Guillemaud *et al.*, 2003), while just a single predominant clone was detected in Scotland (Fenton *et al.*, 1998, 2003) and in Canada (Al-Aboodi & ffrench-Constant, 1995). These studies have mentioned the availability of the primary host (*P. persica*) as a necessary factor for sexual reproduction and for the occurrence of higher genetic variability (Wilson *et al.*, 2002; Fenton *et al.*, 2003; Guillemaud *et al.*, 2003). The tobacco-feeding race of *M. persicae* in Greece showed this pattern, with higher genetic variability and heterozygosity occurring in areas where the primary host was present (Zitoudi *et al.*, 2001; Margaritopoulos *et al.*, 2002). However, the predominance of sexual reproduction will also depend on life cycle attributes of the *M. persicae* genotypes present (Martínez-Torres *et al.*, 1997; Fenton *et al.*, 1998; Zitoudi *et al.*, 2001; Margaritopoulos *et al.*, 2002, 2003; Guillemaud *et al.*, 2003).

The ability to develop insecticide resistance is a well-known attribute of *M. persicae* on several crops (Unruh *et al.*, 1996; Foster *et al.*, 1998; Foster *et al.*, 2002; Mazzoni & Cravedi, 2002), including the race on tobacco (Koziol & Semtner, 1984; Harlow & Lampert, 1990; Wolf *et al.*, 1994). The mechanisms involved in such insecticide resistance include: elevated carboxylesterase expression (E4 or FE4), insensitive acetylcholinesterase (MACE) and insensitive sodium channels (knockdown resistance by mutation in the *kdr* gene) (Devonshire *et al.*, 1998; Field & Foster, 2002). Selection pressures and genetic population structure may change in space and time leading to variation in the resistance levels of field populations of *M. persicae*. In particular, when only a minor proportion of genotypes of the source population is successfully established in a new geographical region (i.e., a founder effect), the insecticide resistance attributes of the recently introduced population might be strongly influenced by the available genetic variability of the established immigrants (Roush & McKenzie, 1987; Roush & Daly, 1990).

Although *M. persicae* has been for a long time recognized as one of the most important agricultural pests in Chile (Zúñiga, 1969; Klein & Waterhouse, 2000), the tobacco-feeding race had not been observed or reported until 1998, when severe outbreaks started to take place on tobacco in central Chile (Fuentes-Contreras *et al.*, 2003). Field applications of the organophosphate insecticide acephate resulted in poor control of the tobacco-feeding race of *M. persicae* suggesting the presence of insecticide resistance, as previously reported for this aphid species on sugarbeet (Stevens & Dewar, 1996; Casals & Silva, 1999). In the present study, the genetic diversity of the tobacco-feeding populations of *M. persicae* in Chile, a rather isolated biogeographical area where this aphid has been recently introduced, was addressed and correlated with known insecticide resistance mechanisms described for *M. persicae*. A field survey was performed along the tobacco-growing belt in Chile (a latitudinal transect of nearly 300 km) that included 35 samples from different localities. The samples were identified using morphometric multivariate analysis (Margaritopoulos *et al.*, 2000; Kephalogianni *et al.*, 2002) and genetically characterized studying the occurrence of polymorphisms at 14 microsatellite loci previously described (Sloane *et al.*, 2001; Wilson *et al.*, 2002). Levels of insecticide resistance were addressed through a microplate esterase assay (Devonshire *et al.*, 1992) and the evaluation of the mutation status of the *kdr* gene (Foster *et al.*, 1999; Martínez-Torres *et al.*, 1999).

## Materials and methods

### *Field sampling and laboratory cultures*

A total of 35 tobacco fields were sampled from San Vicente (34°30′S) to Chillán (36°36′S), along a *c.* 300 km latitudinal gradient across the tobacco growing area in Chile (fig. 1). Over 80% of Burley and Virginia tobacco is grown in this area (ODEPA, 2002). Since extensive applications of imidacloprid to the seedbeds prevented the appearance of aphids on tobacco during spring (Fuentes-Contreras *et al.*, 2003), field collections were performed during summer (January–March) 2000 and 2001. Aphids were collected alive from tobacco, *Nicotiana tabacum* L. (Solanaceae), recording its body colour under field conditions. A single individual from each field was used to establish a laboratory lineage (a putative clone). All the samples were maintained in growth chambers at 22 ± 1°C and 16:8 L:D photoperiod, these conditions ensuring that aphids reproduced by parthenogenesis. In order to keep putative clones from each field separate fom one another, the laboratory cultures were maintained on excised tobacco leaves (Burley BY64) inside individual Petri dishes.

### *Morphometric analyses*

Winged aphids from all localities were identified using the morphometric keys based on multivariate techniques described by Blackman (1987a). Following this initial identification, a group of nine localities was randomly selected for further morphometric analyses: three northern localities from areas with extensive commercial peach

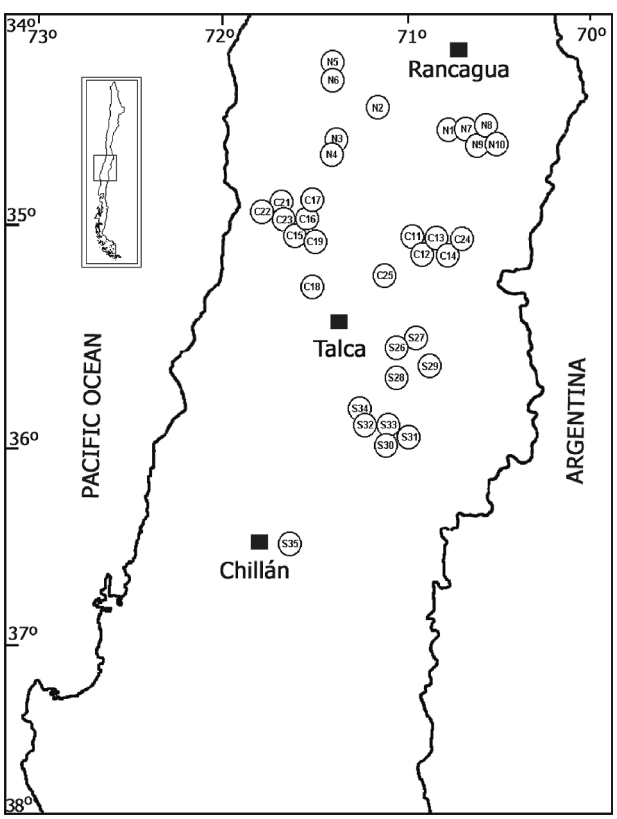


Fig. 1. Collection localities of *Myzus persicae* in the tobacco growing area of Chile (Regions of O'higgins, Maule and Bio Bio).

orchards near to tobacco fields (San Vicente 2, La Higuerilla, and Laboratorio), three central localities with mainly domestic peach trees near tobacco fields (Mercedes 3, Palquibudi and Panguilemo), and finally three southern localities with no peach trees near tobacco fields (La Puntilla 1, San Víctor Álamos 5, and Chillán). Using winged and wingless aphids the following characters were measured: length of the last rostral segment (urs), length of the third antennal segment (ant III), length of the base of the sixth antennal segment (base VI), length of the processus terminalis (pt), length of hind femur (hf), length of second segment of the hind tarsus (ht II), length of siphunculi (ls), maximal width of swollen part of siphunculi (mws), and length of cauda (lc) (Margaritopoulos *et al.*, 2000; Kephalogianni *et al.*, 2002). At least ten individuals (wingless and winged) of each selected locality were included in the analysis. Voucher specimens have been deposited at the Museo Nacional de Historia Natural (Santiago, Chile).

*Microsatellite loci*

For microsatellite analyses, three individual aphids were collected from each of the 35 putative clones maintained in the laboratory (total sample = 105). Patterns of allelic diversity of 105 individual aphids from the 35 localities were examined at 14 microsatellite loci (Myz2, Myz3, Myz9, Myz25, M35, M37, M40, M49, M55, M62, M63, M77, M86, and M107), previously described for *M. persicae* (Sloane *et al.*, 2001; Wilson *et al.*, 2002; R.L. Blackman & G. Malarky, personal communication). Genomic DNA was extracted from single adult wingless aphids using the 'salting-out' method according to Sunnucks *et al.* (1996). In brief, each single individual was homogenized in TNES buffer (50 mM Tris-HCl pH 7.5, 400 mM NaCl, 20 mM EDTA, 0.5% SDS) and incubated at 37°C overnight in the presence of proteinase *K* (100 µg ml$^{-1}$). Proteins were extracted using 5 M NaCl, and the DNA precipitated in ethanol and resuspended in 40 µl of sterile ultra-pure water.

Table 1. Mean length (± SE) in mm of nine characters of winged and wingless *Myzus persicae* collected from tobacco in different localities of northern (N), central (C) and southern (S) areas of Chile.

| Locality | Area | urs | base VI | pt | ant III | lc | hf | ht II | msw | ls |
|---|---|---|---|---|---|---|---|---|---|---|
| Winged | | | | | | | | | | |
| La Higuerilla | N(7) | 0.122 | 0.142 | 0.561 | 0.529 | 0.157 | 0.637 | 0.108 | 0.041 | 0.341 |
| | | (0.001) | (0.002) | (0.011) | (0.014) | (0.003) | (0.016) | (0.002) | (0.000) | (0.001) |
| Laboratorio | N(8) | 0.125 | 0.150 | 0.639 | 0.554 | 0.190 | 0.698 | 0.109 | 0.041 | 0.381 |
| | | (0.001) | (0.004) | (0.015) | (0.005) | (0.005) | (0.007) | (0.002) | (0.001) | (0.007) |
| San Vicente 2 | N(9) | 0.126 | 0.143 | 0.550 | 0.542 | 0.140 | 0.643 | 0.116 | 0.042 | 0.358 |
| | | (0.002) | (0.002) | (0.004) | (0.012) | (0.006) | (0.011) | (0.003) | (0.001) | (0.004) |
| Mercedes 3 | C(14) | 0.123 | 0.142 | 0.594 | 0.535 | 0.156 | 0.625 | 0.113 | 0.042 | 0.367 |
| | | (0.001) | (0.002) | (0.008) | (0.007) | (0.005) | (0.028) | (0.002) | (0.001) | (0.007) |
| Palquibudi | C(15) | 0.125 | 0.145 | 0.605 | 0.560 | 0.166 | 0.661 | 0.103 | 0.042 | 0.351 |
| | | (0.001) | (0.003) | (0.013) | (0.012) | (0.006) | (0.015) | (0.002) | (0.001) | (0.008) |
| Panguilemo | C(18) | 0.126 | 0.137 | 0.564 | 0.523 | 0.180 | 0.628 | 0.104 | 0.040 | 0.336 |
| | | (0.002) | (0.003) | (0.007) | (0.008) | (0.004) | (0.014) | (0.002) | (0.001) | (0.006) |
| La Puntilla 1 | S(28) | 0.121 | 0.144 | 0.560 | 0.524 | 0.143 | 0.607 | 0.112 | 0.042 | 0.339 |
| | | (0.001) | (0.003) | (0.008) | (0.013) | (0.006) | (0.020) | (0.003) | (0.001) | (0.006) |
| San Víctor Álamos 5 | S(33) | 0.123 | 0.144 | 0.594 | 0.530 | 0.156 | 0.655 | 0.110 | 0.043 | 0.362 |
| | | (0.001) | (0.004) | (0.016) | (0.018) | (0.006) | (0.019) | (0.002) | (0.001) | (0.007) |
| Chillán | S(35) | 0.121 | 0.142 | 0.536 | 0.491 | 0.169 | 0.588 | 0.101 | 0.039 | 0.315 |
| | | (0.000) | (0.003) | (0.008) | (0.008) | (0.005) | (0.015) | (0.000) | (0.001) | (0.008) |
| Wingless | | | | | | | | | | |
| La Higuerilla | N(7) | 0.121 | 0.119 | 0.464 | 0.405 | 0.176 | 0.600 | 0.105 | 0.044 | 0.475 |
| | | (0.001) | (0.003) | (0.017) | (0.011) | (0.005) | (0.016) | (0.002) | (0.001) | (0.014) |
| Laboratorio | N(8) | 0.118 | 0.110 | 0.434 | 0.388 | 0.166 | 0.578 | 0.102 | 0.046 | 0.460 |
| | | (0.001) | (0.003) | (0.013) | (0.016) | (0.007) | (0.022) | (0.003) | (0.001) | (0.016) |
| San Vicente 2 | N(9) | 0.120 | 0.121 | 0.461 | 0.419 | 0.207 | 0.604 | 0.105 | 0.049 | 0.464 |
| | | (0.001) | (0.002) | (0.010) | (0.012) | (0.005) | (0.016) | (0.002) | (0.001) | (0.019) |
| Mercedes 3 | C(14) | 0.120 | 0.117 | 0.491 | 0.415 | 0.188 | 0.600 | 0.106 | 0.044 | 0.463 |
| | | (0.001) | (0.003) | (0.021) | (0.019) | (0.007) | (0.023) | (0.002) | (0.001) | (0.021) |
| Palquibudi | C(15) | 0.118 | 0.111 | 0.453 | 0.388 | 0.183 | 0.562 | 0.101 | 0.047 | 0.437 |
| | | (0.002) | (0.002) | (0.009) | (0.008) | (0.005) | (0.022) | (0.002) | (0.001) | (0.017) |
| Panguilemo | C(18) | 0.123 | 0.118 | 0.464 | 0.424 | 0.194 | 0.587 | 0.103 | 0.049 | 0.483 |
| | | (0.001) | (0.002) | (0.014) | (0.012) | (0.004) | (0.022) | (0.002) | (0.001) | (0.016) |
| La Puntilla 1 | S(28) | 0.116 | 0.112 | 0.442 | 0.350 | 0.183 | 0.546 | 0.102 | 0.048 | 0.437 |
| | | (0.000) | (0.003) | (0.019) | (0.012) | (0.007) | (0.026) | (0.002) | (0.001) | (0.026) |
| San Víctor Álamos 5 | S(33) | 0.120 | 0.117 | 0.457 | 0.416 | 0.160 | 0.596 | 0.105 | 0.048 | 0.488 |
| | | (0.001) | (0.002) | (0.007) | (0.018) | (0.004) | (0.020) | (0.002) | (0.001) | (0.020) |
| Chillán | S(35) | 0.119 | 0.122 | 0.539 | 0.453 | 0.186 | 0.660 | 0.107 | 0.052 | 0.521 |
| | | (0.000) | (0.002) | (0.016) | (0.008) | (0.008) | (0.014) | (0.001) | (0.001) | (0.013) |

Numbers between brackets after the area indicate the sample location in the map (fig. 1). The characters are: length of the last rostral segment (urs), length of the base of sixth antennal segment (base VI), length of processus terminalis (pt), length of third antennal segment (ant III), length of cauda (lc), length of hind femur (hf), length of second segment of the hind tarsus (ht II), maximal width of swollen part of siphunculi (mws), and length of siphunculi (ls).

The polymerase chain reaction (PCR) amplifications of microsatellite loci were prepared in 15 µl aliquots, including 0.5 U of *Taq* DNA polymerase (Gibco-BRL, USA), $Mg^{++}$-free reaction buffer, 2 mM $MgCl_2$, 200 µM dNTPs, 10 pmol of each primer (BiosChile-IGSA, Chile), and about 10 ng of aphid DNA. The PCR reactions were carried out in a Perkin-Elmer 9700 thermocycler using the following steps: an initial denaturation for 2 min at 94°C, and 40 cycles consisting of denaturation for 40 s at 94°C, annealing for 45 s with temperature dependent on locus (47–60°C) (Sloane *et al.*, 2001; Wilson *et al.*, 2002), and elongation at 72°C for 45 s. For the last cycle, the elongation time was extended by 4 min.

The PCR reaction was mixed with 4× loading buffer (Sambrook *et al.*, 1989), denatured for 3 min at 95°C, loaded on a 6% polyacrylamide–urea gel, and subjected to electrophoresis in 0.5× TBE at 1.5 kV. After electrophoresis, the gel was silver stained as follows. In brief, the gel was fixed in 10% ethanol, oxidized in 1% nitric acid, washed with ultrapure water, and incubated for 20 min in a solution of silver nitrate (1 g $l^{-1}$). After incubation, the gel was washed, and developed in a sodium carbonate/formaldehyde solution (30 g $l^{-1}$ $Na_2CO_3$ and 540 µl $l^{-1}$ of 37% formaldehyde). The reaction was stopped with 10% acetic acid and washed with water. The gel was air dried overnight at room temperature. The size of the alleles of each locus was estimated using a sequencing size ladder corresponding to the sequence of pGEM®-3Zf(+) vector (Promega, USA).

### *Life cycle categories*

Life cycle categories were evaluated by transferring aphids from the 35 localities under standard long-day rearing conditions (see field sampling and laboratory cultures) to short-day and low temperature conditions (10:14 LD photoperiod, 15 ± 1°C) for three generations. For this, single wingless aphids (adults and fourth instar nymphs) were placed under short-day conditions. These aphids produced a first generation, from which five to seven first-born nymphs were selected to establish sexual morphs in the second generation (gynoparae and males). The whole life span progeny (second generation) produced by the first generation aphids was examined, in order to detect even a late and reduced production of males that may appear in some androcyclic clones (Blackman, 1987b; Margaritopoulos *et al.*, 2002; Margaritopoulos & Tsitsipis, 2002). Although no gynoparae or males were found in the second generation, these aphids were further maintained under short-day conditions for a third generation.

### *Esterase activity*

Total esterase activity was evaluated using the microplate assay described by Devonshire *et al*. (1992). A minimum of five adult or fourth instar aphids (200–650 µg fresh weight) from each of the 35 localities, were individually analysed to evaluate total esterase activity. Aphids were individually homogenized in 100 µl of phosphate buffer (0.02 M, pH 7.0) in the presence of Tween 20 (0.05% v/v). Aliquots of 25 µl of each homogenate were transferred to a microplate, containing 25 µl of phosphate buffer (0.02 M, pH 7.0) with Triton X-100 (0.1% v/v). The reaction started with the addition of 150 µl of 1-naphthyl-acetate (0.3 mM), and incubation lasted for 5 min at room temperature. After the incubation period, 25 µl of diazo blue/lauryl sulphate reagent were added and the microplates were left for 20 min in the darkness. Finally, absorbance at 620 nm was measured with a microplate reader (Packard Spectracount). *Myzus persicae* sensu stricto from pepper was used to calibrate the microplate esterase assays, providing meaningful results (data not shown).

### *Sequence of the* kdr *gene*

Aphids from the nine localities selected for morphometric analyses were characterized in terms of the presence/absence of the *kdr* mutation (CTC to TTC; leucine to phenylalanine) in the IIS6 fragment of the sodium channel gene (Martínez-Torres *et al.*, 1999). The characterization was performed by sequencing the DNA fragment after its amplification by PCR. The PCR amplification was performed using published primers (Foster *et al.*, 1999; Martínez-Torres *et al.*, 1999) specific for the genus *Myzus*. The 330 bp DNA fragment was run on an agarose gel, purified using the SNAP™ gel purification kit (Invitrogen), and sent for sequencing (Gen y Tec, Santiago, Chile).

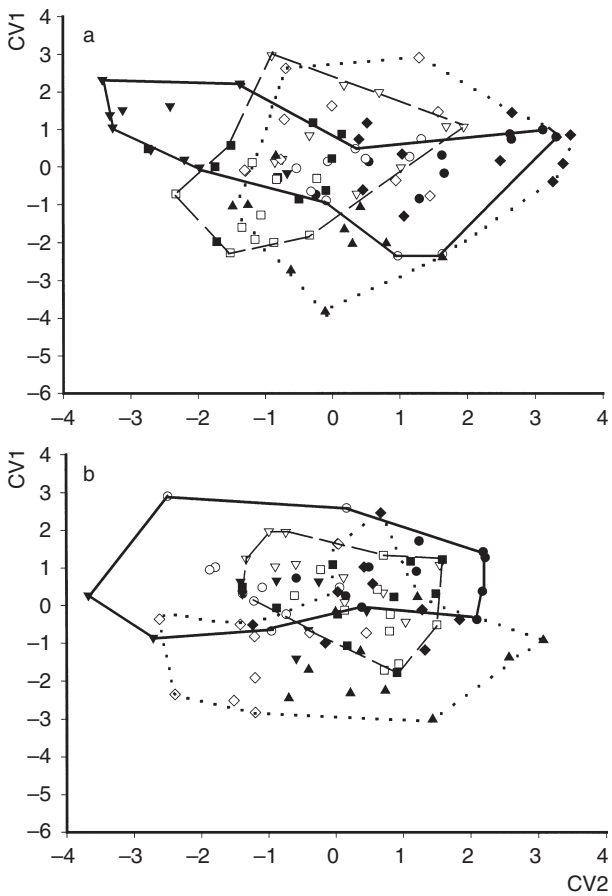


Fig. 2. Morphological variation of winged (a) and wingless (b) aphids of *Myzus persicae* collected in nine localities from northern (N), central (C) and southern (S) areas of Chile: plot of the mean scores of the first two canonical variates. Solid line represents the aphids from northern (N), dashed line represents the aphids from central (C), and dotted line represents the aphids from southern (S) areas. ●, San Vicente 2 (N9); ○,La Higuerilla (N7); ▼, Laboratorio (N8); ∇, Mercedes 3 (C14); ■, Palquibudi (C15); □, Panguilemo (C18); ♦, La Puntilla 1 (S28); ◇, San Víctor Álamos 5 (S33); ▲, Chillán (S35).

## Results

Winged aphids from all 35 localities were identified following the morphometric key (Blackman, 1987a) as the tobacco-feeding race of *M. persicae*. Only red morphs were observed on tobacco in all localities sampled. Morphological variation found in the nine selected localities is shown for winged and wingless aphids in table 1. No significant difference in any morphometric variable analysed was found between samples of winged and wingless aphids from northern (peach orchards), central (scattered peach trees) and southern (no peach trees) areas. Canonical variate analyses of these samples revealed that morphological variation between these areas overlapped extensively for winged and wingless aphids (fig. 2). However, regardless of the sampling area (peach availability) significant differences were detected between localities in the canonical variate analysis of winged (Wilk's lambda = 0.894; $F_{72, 451}$= 3.06; $P <$ 0.001) and wingless aphids (Wilk's lambda = 0.121; $F_{72, 451}$= 2.61; $P < 0.001$). This was best described for the variables urs, pt and lc in winged and wingless aphids, while ht II and msw were significant only for winged and wingless aphids, respectively (table 1).

Microsatellite analysis was carried out on the 14 loci evaluated, with the allele sizes shown in table 2. A single multilocus genotype was observed in all the 105 individuals from the 35 localities included in the study (table 2). The allele sizes from the respective loci lie within the range previously reported for *M. persicae*. Life cycle evaluations showed the complete absence of sexual morphs in the second and third generation. Therefore, the predominant clone was anholocyclic under the laboratory conditions used in our experiments (10:14 LD photoperiod, 15 ± 1°C).

Esterase levels found in aphids sampled from all localities are shown in table 3. No significant differences were found between northern (peach), central (few peach) and southern (no peach) areas, evaluated either as total absorbance (Kruskal-Wallis test: $H_{2,35}$ = 1.214, $P$ = 0.54) or absorbance per µg fresh weight (Kruskal-Wallis test: $H_{2,35}$ = 2.769, $P$ = 0.25). Finally, all localities sequenced (N = 9) presented leucine in position 1014 of the IIS6 fragment of the sodium channel gene; indicating that the *kdr* mutation was absent in the samples analysed.

## Discussion

A single predominant red genotype of the tobacco race of *M. persicae* was found to be widespread in Chile. The morphometric variability found between localities for urs, pt and lc was consistent with morphometric variability observed in Europe (Margaritopoulos *et al.*, 2000; Kephalogianni *et al.*, 2002), although in the case of Chile no significant differences were found between areas latitudinally ordered through availability of the primary host.

Regardless of the presence of the primary host of *M. persicae*, sexual reproduction seems to be absent in the predominant clone found in the tobacco growing areas of Chile. The presence of only red forms in the field, with morphological variability uncorrelated with the distribution of the primary host, are consistent with the anholocyclic attributes observed under short-day laboratory conditions. All these sources of evidence are further reinforced by the complete absence of genetic variability revealed by microsatellite markers. These results seems to be similar to the situation found in southern Italy, where *M. persicae* on peach were holocyclic, whereas *M. persicae* on tobacco were unable to produce sexual morphs (anholocyclic). The results suggest that tobacco-adapted and non-tobacco-adapted forms of *M. persicae* may co-exist in the same region, not only because they colonize different host plants, but also because they have different life cycles (Margaritopoulos *et al.*, 2003).

The presence of a single predominant anholocyclic clone of the tobacco race of *M. persicae* means that over wintering should occur on weeds, winter crops and/or tobacco voluntary regrowth after harvest. Semtner *et al*. (1998) reported that the tobacco-feeding race of *M. persicae* was able to grow and reproduce on several plant species of at least five families, exhibiting fair to good performance on *Raphanus* and two species of *Brassica* (Brassicaceae). *Brassica* crops and weeds are widespread in Chile during the winter season, even after the voluntary regrowth of Virginia

Table 2. Allele combinations of the tobacco-feeding Chilean clone of *Myzus persicae*, in relation to allele sizes and number of alleles previously reported for fourteen microsatellite loci for *M. persicae* (Sloane *et al.*, 2001; Wilson *et al.*, 2002).

| Locus | Allele combinations in Chilean samples | Range of allele size in other studies | Number of alleles in other studies |
|---|---|---|---|
| Myz2 | 191/193 | 177–199 | 5 |
| Myz3 | 125/125 | 109–125 | 3 |
| Myz9 | 214/218 | 206–218 | 7 |
| Myz25 | 123/123 | 120–123 | 2 |
| M35 | 203/203 | 178–203 | 8 |
| M37 | 155/157 | 153–161 | 4 |
| M40 | 128/130 | 123–140 | 6 |
| M49 | 121/121 | 121–199 | 17 |
| M55 | 119/119 | 119–129 | 4 |
| M62 | 125/127 | 125–143 | 6 |
| M63 | 174/174 | 163–207 | 7 |
| M77 | 139/140 | 138–140 | 3 |
| M86 | 115/117 | 97–141 | 9 |
| M107 | 134/142 | 133–145 | 7 |

For microsatellite analyses three individual aphids were collected from each of the 35 putative clones maintained in the laboratory.

Table 3. Mean values of esterase activity expressed as absorbance and absorbance per μg fresh weight of *Myzus persicae* collected from tobacco from different localities in northern (N), central (C) and southern (S) areas of Chile.

| Locality | Area (no.) | Absorbance | Absorbance per μg |
|---|---|---|---|
| Cerrillos 1 | N(1) | 0.53 | 1.91 |
| Huique 1 | N(2) | 0.47 | 1.91 |
| Santa Cruz 1 | N(3) | 0.58 | 1.86 |
| Nancagua 2 | N(4) | 0.61 | 1.56 |
| Peralillo 1 | N(5) | 0.72 | 2.27 |
| Peralillo 2 | N(6) | 0.57 | 2.47 |
| La Higuerilla | N(7) | 0.25 | 1.18 |
| Laboratorio | N(8) | 0.68 | 1.73 |
| San Vicente 2 | N(9) | 0.34 | 1.47 |
| Los Cerrillos 2 | N(10) | 1.02 | 3.16 |
| Mean (SD) | | 0.58 (0.21) | 1.95 (0.57) |
| Platina Porvenir | C(11) | 0.68 | 1.77 |
| San Hernán | C(12) | 0.42 | 1.28 |
| Mercedes 1 | C(13) | 0.31 | 1.14 |
| Mercedes 3 | C(14) | 0.63 | 1.87 |
| Palquibudi | C(15) | 0.46 | 1.48 |
| San Enrique | C(16) | 0.36 | 1.42 |
| San Gerardo (R. Claro) | C(17) | 0.43 | 1.20 |
| Panguilemo | C(18) | 0.75 | 2.41 |
| Mesamavida | C(19) | 0.47 | 1.98 |
| Mira Río | C(20) | 0.42 | 2.05 |
| Alisos 1 | C(21) | 0.41 | 1.81 |
| Alisos 2 | C(22) | 0.62 | 1.69 |
| Hualañé | C(23) | 0.40 | 1.97 |
| Los Aromos | C(24) | 0.48 | 1.83 |
| Perejil 3 | C(25) | 0.53 | 1.00 |
| Mean (SD) | | 0.49 (0.13) | 1.66 (0.40) |
| La Batalla | S(26) | 0.46 | 1.83 |
| La Isla | S(27) | 0.63 | 2.29 |
| La Puntilla 1 | S(28) | 0.34 | 1.46 |
| Manuel Rodríguez 2 | S(29) | 0.62 | 1.66 |
| San Guillermo 1 | S(30) | 0.44 | 1.46 |
| San Guillermo 2 | S(31) | 0.36 | 1.11 |
| San Víctor Álamos 3 | S(32) | 0.44 | 1.31 |
| San Víctor Álamos 5 | S(33) | 0.81 | 1.42 |
| Manuel Rodríguez | S(34) | 0.42 | 1.23 |
| Chillán | S(35) | 0.80 | 2.35 |
| Mean (SD) | | 0.53 (0.17) | 1.61 (0.43) |
| Total Mean (SD) | | 0.53 (0.16) | 1.75 (0.47) |

Numbers between brackets after the area indicate the sample location on the map (fig. 1).

tobacco plants has been either removed or killed by frost. Mediterranean weather conditions present in the tobacco-growing region of Chile are characterized by rather mild winters with few frosts in the central valley and extensive areas completely free from frosts along the coast. Such winter conditions clearly allow the survival of the anholocyclic tobacco-feeding race of *M. persicae* in Chile, as previously described for *M. persicae* sensu stricto on sugarbeet in Chile (Stevens & Dewar, 1996).

The host range of colonizers (i.e. generalists versus specialists) has been proposed as an important factor in the successful invasion of herbivores into a new environment (Kolar & Lodge, 2001). For example, if the introduced individuals are generalists, they will show an advantage in terms of flexibility to attack other hosts, while if the introduced genotypes are specialists, a high performance is expected if the host species and environmental conditions are similar to those in the region of origin. This phenomenon has been proposed for parthenogenetic species, where the existence of a general-purpose genotype (GPG) could be expected in terms of genetic structure of the invading population (Lynch, 1984; Van Doninck *et al.*, 2002). The presence of a single predominant clone of the tobacco-feeding race of *M. persicae* recently introduced into Chile could represent the introduction of a general-purpose genotype, but a comparative analysis of sexual genotypes of this species growing on other crops is necessary to properly test this hypothesis.

Low levels of genetic diversity have been reported for *M. persicae* sensu stricto in other studies. For instance, a single IGS fingerprinting pattern ('type j') was predominant and widespread in Scotland for several years (Fenton *et al.*, 1998, 2003), while RAPD–PCR revealed no genetic variation among 32 samples of insecticide susceptible and resistant *M. persicae* in Canada (Al-Aboodi & ffrench-Constant, 1995). Previous studies using allozyme electrophoresis also suggested the presence of a few predominant clones of *M. persicae* in the UK (Brookes & Loxdale, 1987). No information was available on the population genetic structure of *M. persicae* sensu stricto in Chile, although other recently introduced and anholocyclic aphid species such as *Sitobion avenae* (Fabricius) are known to persist with low genetic variability (Figueroa *et al.*, 2002).

Esterase activity levels showed that mean absorbance was 0.53 with a standard deviation of 0.17 (variation coefficient = 32.1%), while the absorbance per μg fresh weight was 1.73 with a standard deviation of 0.47 (variation coefficient = 27.2%). These values correspond with the mean and variability expected for a slightly resistant (R1) esterase genotype (Devonshire *et al.*, 1992). Since susceptible *kdr* genes were also found in all samples evaluated, it might, therefore, be concluded that resistance levels of the tobacco-feeding race of *M. persicae* in Chile are rather low in contrast with the situation observed in the USA (Koziol & Semtner, 1984; Harlow & Lampert, 1990; Wolf *et al.*, 1994). The presence of MACE resistance was not evaluated in our samples, but since carbamate aphicides such as pirimicarb and triazamate are not used on tobacco in Chile, it is unlikely that this resistance mechanism is present. Control failures observed in Chile after acephate application might be related to poor coverage of the leaves in fully grown tobacco plants when using backpack sprayers, rather than to organophosphate resistance as previously described for sugarbeet (Stevens & Dewar, 1996; Casals & Silva, 1999).

The level of insecticide resistance found under field conditions is dependent on the selection pressure of insecticides applied, as well as the population genetic component of the aphids present in the region under study. The recent introduction of a single predominant clone of the tobacco-feeding race of *M. persicae* in Chile seems to have been produced by a strong 'founder effect' followed by asexual reproduction, where insecticide resistance characteristics (slightly resistant R1 and *kdr* susceptible genotypes) observed in Chile represent a small proportion of the genetic and phenotypic variability described for this race in other areas of the world (USA, Mediterranean Europe and Asia). Fitness costs associated with high levels of insecticide resistance (R2 and R3 genotypes) may restrict the successful invasion of new environments where the aphids have to spend the winter parthenogenetically (Foster *et al.*, 1996, 1997). Since no sexual reproduction appears to occur in Chile with *M. persicae* sensu stricto from other crops, the

development of insecticide resistance in the tobacco race is likely to depend solely on the selection pressures of insecticides applied to this crop.

At present, the neonicotinoid insecticide imidacloprid mixed with the pyrethroid cyfluthrin (Confidor Supra) are extensively used in tobacco as seedbed treatments, supplemented if necessary with acephate applications during the season. This management strategy involving insecticides with different modes of action is expected to mitigate the development of resistance in the tobacco-feeding race of *M. persicae* in Chile.

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