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Inferring the population structure of *Myzus persicae* in diverse agroecosystems using microsatellite markers

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Abstract

Diverse agroecosystems offer phytophagous insects a wide choice of host plants. *Myzus persicae* is a polyphagous aphid common in moderate climates. During its life cycle it alternates between primary and secondary hosts. A spatial genetic population structure may arise due to environmental factors and reproduction modes. The aim of this work was to determine the spatial and temporal genetic population structure of *M. persicae* in relation to host plants and climatic conditions. For this, 923 individuals of *M. persicae* collected from six plant families between 2005 and 2008 in south-eastern Spain were genotyped for eight microsatellite loci. The population structure was inferred by neighbour-joining, analysis of molecular variance (AMOVA) and Bayesian analyses. Moderate polymorphism was observed for the eight loci in almost all the samples. No differences in the number of alleles were observed between primary and secondary hosts or between geographical areas. The proportion of unique genotypes found in the primary host was similar in the north (0.961 ± 0.036) and the south (0.987 ± 0.013), while in the secondary host it was higher in the north (0.801 ± 0.159) than in the south (0.318 ± 0.063). Heterozygosity excess and linkage disequilibrium suggest a high representation of obligate parthenogens in areas with warmer climate and in the secondary hosts. The F_{ST}-values pointed to no genetic differentiation of *M. persicae* on the different plant families. F_{ST}-values, AMOVA and Bayesian model-based cluster analyses pointed to a significant population structure that was related to primary and secondary hosts. Differences between primary and secondary hosts could be due to the overrepresentation of parthenogens on herbaceous plants.

Keywords: hemiptera, aphids, host plants, parthenogenesis, molecular diversity, climate

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Introduction

The complex environmental matrix of diverse agroecosystems offers phytophagous insects a wide array of host plants with variable degrees of suitability for development and reproduction. In this scenario, it is likely that different genotypes perform differently according to their intrinsic aptitudes and the characteristics of their host plants. Ecotypes associated with particular host plant species have been reported in many phytophagous arthropods, leading eventually to the formation of new species living in sympatry (Feder *et al.*, 1998; Berlocher & Feder, 2002). Aphids are a very interesting group for studying the effect of host plants on population structure because of their polyphagy, host alternation and variations in their reproduction modes. *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) are among the aphid species in which population differentiation and the ecological and genetic factors driving divergence between host-races have been most extensively investigated (Carroll & Boyd, 1992; Via, 1999; Via *et al.*, 2000; Via & Hawthorne, 2002; Del Campo *et al.*, 2003; Barker, 2005; Frantz *et al.*, 2006).

M. persicae is a ubiquitous and polyphagous aphid of great economic importance, frequently found in many agroecosystems in areas with a moderate climate (Blackman & Eastop, 2000). The life cycle of *M. persicae* generally involves the alternation of several parthenogenetic generations in herbaceous plants with migration to the primary host for sexual reproduction. However, there are many variations in the general pattern of cyclical alternation of holocyclic morphs between primary and secondary hosts, for example: (1) obligate parthenogenetic morphs, which reproduce cyclically by parthenogenesis and lack the capacity to reproduce sexually, also known as anholocyclic; (2) morphs that reproduce by obligate parthenogenesis but produce males that migrate to the primary host, where they mate with the cyclical parthenogens (androcyclic); and (3) morphs with an intermediate kind of life cycle, with females reproducing continuously by parthenogenesis, but occasionally producing females that migrate to the primary host, where they originate females and males which mate to produce the overwintering eggs (Blackman, 1971, 1972). The abundance of morphs with different kinds of reproduction in a population depends greatly on the availability of the primary host and the severity of the climate (Margaritopoulos *et al.*, 2003; Vorburger, 2004; Blackman *et al.*, 2007). This versatility in reproduction allows the species to display an abacus of strategies, profiting from the advantages of both sexual and asexual reproduction (Vorburger *et al.*, 2003b). Interaction among genetic, environmental, climate and stochastic factors gives rise to a mosaic of possibilities that ultimately determine the structure of *M. persicae* populations in agroecosystems. A population of *M. persicae* in secondary hosts in summer generally consists of a mixture of clones, some of them migrating from the primary host, and others obligate parthenogenetic clones from the previous winter (Blackman *et al.*, 2007).

A geographic genetic structure may result from restricted gene flow among local populations due to regional variations in environmental conditions (Vorburger *et al.*, 2003a). However, other factors, such as demographic events associated with the plasticity of reproductive modes (Delmotte *et al.*, 2002; Guillemaud *et al.*, 2003; Blackman *et al.*, 2007; Margaritopoulos *et al.*, 2007a), host specialization (De Barro *et al.*, 1995; Sunnucks *et al.*, 1997) and the alternation between primary and secondary hosts (Sunnucks *et al.*, 1997), may also influence differentiation in aphid populations. Host plant-related variations in the performance of different genotypes have been observed in *M. persicae* (Weber, 1985, 1986; Edwards, 2001; Vorburger *et al.*, 2003b). Molecular and morphological studies have revealed that populations of *M. persicae* on *Nicotiana tabacum* L. (Solanaceae) are genetically and morphologically different from those on other host plants, and support the existence of a host race associated with tobacco (Blackman, 1987; Blackman & Spence, 1992; Margaritopoulos *et al.*, 1998, 2000, 2003, 2007a, 2007b; Zitoudi *et al.*, 2001; Blackman *et al.*, 2007). Microsatellite or DNA short tandem repeats (STR) are codominant markers that have been successfully used to provide information about the population structure in aphids in relation to their life cycle, host, geographical distribution and dynamics (Sunnucks *et al.*, 1997; Simon *et al.*, 1999; Delmotte *et al.*, 2002; Guillemaud *et al.*, 2003; Vorburger *et al.*, 2003a; Vorburger, 2006; Blackman *et al.*, 2007; Margaritopoulos *et al.*, 2007a).

In agricultural areas of south-eastern Spain, agroecosystems are very diverse, with a great variety of crops intermingling with uncultivated land harbouring many wild plant species that are potential hosts for *M. persicae* (Sanchez *et al.*, 2011). In the southern part of that region, the mild climate and the abundance of both the primary and secondary hosts enable *M. persicae* to reproduce continuously through parthenogenesis on herbaceous plants, or to engage in cyclical switching between the secondary and primary hosts. These favourable conditions allow *M. persicae* to overwinter on wild herbaceous plants, which serve as a source of aphids for temporal crops (e.g. pepper grown in greenhouses). In the northern part of the working area, winter temperatures frequently fall below 0°C and the survival of aphids with anholocylic life cycles is expected to be lower than in the southern part (Blackman, 1974). The aim of this work was to determine the spatial and temporal genetic population structure of *M. persicae* in the diverse agroecosystems of Murcia province (south-eastern Spain) in relation to host plants and the climatic conditions of the area. We were also interested in comparing the population structure in temporal elements of the landscape, like pepper crops, *versus* permanently available resources, such as *Prunus* spp., and the herbaceous plants used as secondary hosts. For that purpose, we scored eight microsatellite loci in *M. persicae* collected from different host plant species in the province over a period of four years. These data provided information about genetic diversity, the population structure at spatial and temporal scales, and the colonization pattern of pepper greenhouses by *M. persicae*.

Materials and methods

Sampling of M. persicae

M. persicae was collected from 2005 to 2008 from several areas of the province of Murcia in south-eastern Spain (table 1, fig. 1). Samples were collected from 130 sampling sites in the Campo de Cartagena area, 20 sites from Aguilas, ten sites around Murcia city and 14 sites in the northern part of Murcia Province. The isocline maps for the average and minimum temperatures in January were produced by inverse distance weighting interpolation of the average temperature records from 2000 to 2010 obtained from 40 meteorological stations

Table 1. Localities, area, year and month of sampling, hosts and number of individuals genotyped (*N*) in *M. persicae* samples.

Locality	Area	Year	Month	Host	Sample code	*N*
Campo de Cartagena	South	2005	March, April and May	Pepper	CAPEP05	122
			March, April and May	Herbaceous plants	CAHER05	222
			April	*Prunus dulcis*	CAPRU05	29
		2006	March, April and May	Pepper	CAPEP06	26
			April and October	Herbaceous plants	CAHER06	121
		2007	February, April and May	Herbaceous plants	CAHER07	79
		2008	April and June	Pepper	CAPEP08	61
			February	Herbaceous plants	CAHER08	70
			April	*P. dulcis*	CAPRU08	43
Aguilas, Murcia	South	2005	April	Herbaceous plants	AGHER05	27
Jumilla, Murcia	North	2008	April	*Prunus persica*	JUPRU08	26
Murcia, Murcia	South	2008	April	*P. persica*	MUPRU08	15
Noroeste, Mucia	North	2005	April	Herbaceous plants	NOHER05	19
			April and May	*P. dulcis*	NOPRU05	25
	North	2008	April	Herbaceous plants	NOHER08	16
			April	*P. dulcis*	NOPRU08	22

of SIAM, IMIDA (http://siam.imida.es) (fig. 1). The southern part of the province is within the warm temperate zone as described by Blackman (1974), corresponding to the 10°C isotherm, with average temperatures in January between 10 and 20°C. The northern part of the province is within the medium temperate zone corresponding to the 0°C isotherm, with average temperatures in January between 0 and 10°C (fig. 1).

The distance between sampling sites within the same area ranged from 0.5 to 10 km. *M. persicae* was collected from different secondary host plant species of Chenopodiaceae [*Chenopodium album* L., *Chenopodium murale* L., *Beta vulgaris* L. and *Beta maritima* L.], Convolvulaceae [*Convolvulus arvensis* L. and *Convolvulus althaeoides* L.], Brassicaceae [*Sisymbrium irio* L., *Brassica oleracea* L., *Diplotaxis erucoides* (L.), *Eruca vesicaria* (L.) Cav., *Moricandia arvensis* (L.) DC., *Raphanus raphanistrum* L. and *Rapistrum rugosum* (L.) Al], Malvaceae [*Malva parviflora* L.], Rosaceae [*Prunus dulcis* (Mill.) and *Prunus persica* (L.) Batsch] and Solanaceae [*Capsicum annuum* L. and *Solanum tuberosum* L.]. The primary host samples were collected from *P. dulcis* in the southern part of the province and from *P. dulcis* and *P. persica* in the northern part of the province. The aphids were collected separately from individual plants and introduced into single translucent plastic containers. To reduce the chances of individuals descending from the same progenitor, individual plants at the sampling sites were more than 5 m apart. The *M. persicae* samples were taken to the laboratory in refrigerated boxes, placed in glass vials with absolute ethanol and stored at 4°C.

Amplification of microsatellites loci

Two individuals for each plant species, collected from different individual plants at each sampling site, were analysed. DNA was extracted from apterous adult females of *M. persicae* using the Quelex 100 chelating ion exchange resin method (Malloch *et al.*, 2006). The loci used in this study were two X-linked (M86 and Myz25) and six autosomal (Myz9, M35, M37, M40, M49 and M63) loci (Sloane *et al.*, 2001; Wilson *et al.*, 2004). The X-linked loci, M37-M49 and M35-M63 belonged to three different linkage groups (Sloane *et al.*, 2001). Myz9 and M40 belonged to another two different linkage groups (Sloane *et al.*, 2001). Amplification reactions were prepared using 2 µl of approximately 0.5 ng µl^{-1} of genomic DNA, 0.5 µM of each primer, 0.2 mM of dNTPs, 10 mM Tris/HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% of Tween and 0.5 unit of DNA polymerase (DFS-Taq DNA polymerase, Bioron GmbH, Ludwigshafen, Germany), in a total volume of 20 µl. Forward primers were 5′ labelled with 6-FAM, VIC, NED or PET dyes (Applied Biosystems, Foster City, California). Polymerase chain reaction (PCR) amplifications were carried out in an Eppendorf Mastercycler EPgradient (Eppendorf AG, Hamburg, Germany) under the following conditions: 2 min at 94°C followed by 36 cycles of 15 s at 94°C, 30 s at 61°C and 30 s at 72°C and then a 10 min incubation at 72°C. The PCR products were run at 1:50 dilution on an AB3730 DNA Analyzer (Applied Biosystems) with the LIZ500-labelled size standard (Applied Biosystems). Fragments were detected with Peak ScannerTM v1.0 (Applied Biosystems) and verified manually.

Genetical statistical analyses

A total of 923 *M. persicae* were successfully genotyped for the eight microsatellite loci (table 1). To prevent unreliable results due to the inclusion of multiple clonal copies (Sunnucks *et al.*, 1997), genotypes with the same score for the eight microsatellites on samples from the same plant species and sampling sites were excluded from the analyses. We assumed that two individuals were in the same genotype or clone whenever they had the same score for the eight microsatellites. The number of alleles per locus and population, heterozygosity, deviation from Hardy–Weinberg equilibrium (HWE), the fixation index (F_{IS}) and genotypic linkage disequilibrium (LD) were calculated using the FSTAT 2.9.3.2 software (Goudet, 2002). All probabilities were Bonferroni corrected (Rice, 1989). The frequency of null alleles was estimated using Genepop 4.0 (Rousset, 2008). ANOVA was used to test whether the proportion of a linked pair of loci in samples (number of linked loci/total number of possible pairwise comparisons) was significantly influenced by linkage group (same or different chromosome), host (primary/secondary host) and geographical area (north/south). The

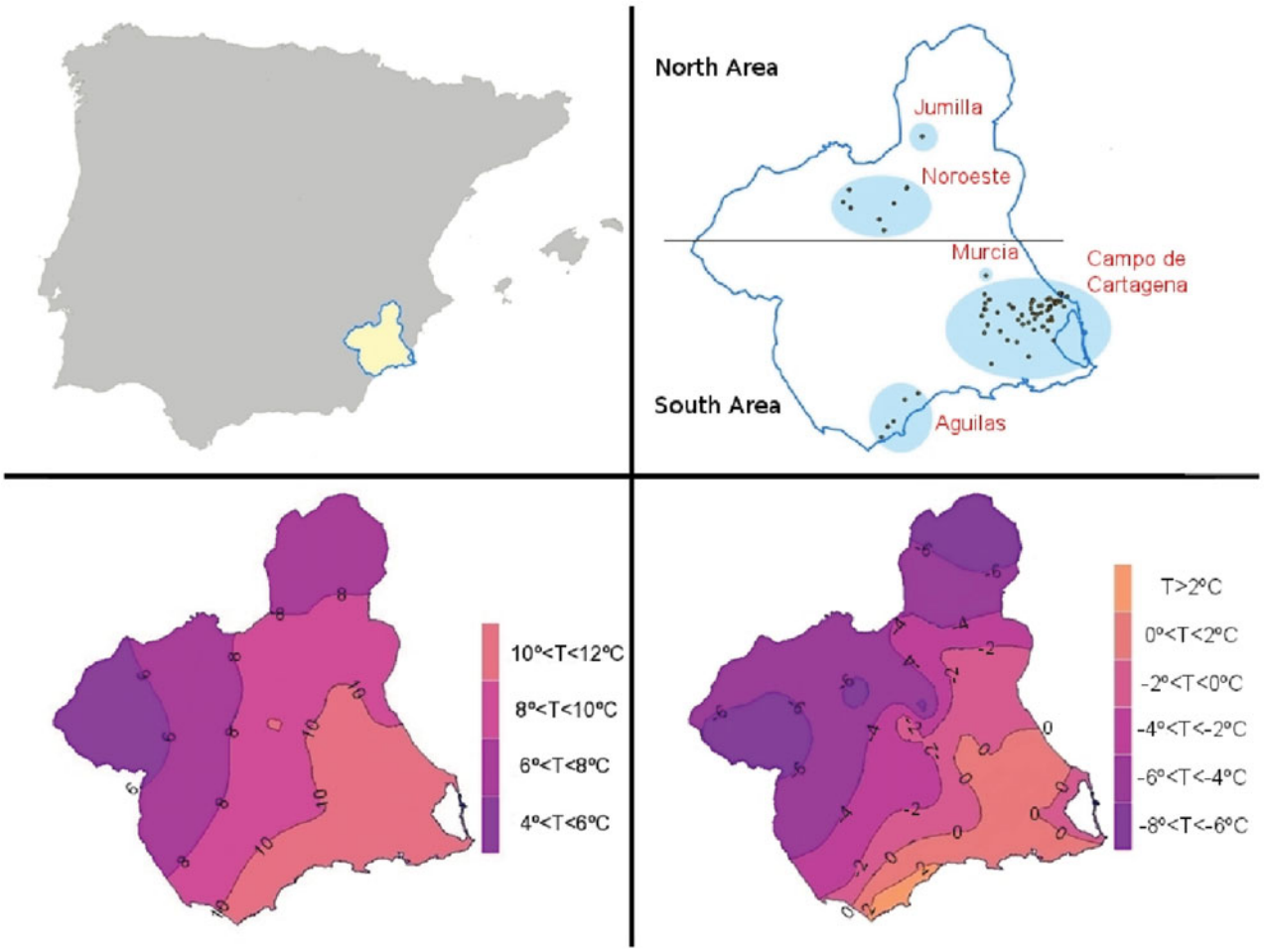

Fig. 1. Location of the sampling areas (top) and maps with the isoclines of the average (bottom left) and minimum temperatures in January (bottom right).

number of alleles per individual, the proportion of genotypes (number of different genotypes/number of individuals genotyped) and the number of loci not under HWE in the samples were tested by ANOVA as a function of the host and the geographical area. Data were transformed by the natural logarithm of $(x+1)$ when needed to account for heteroscedasticity. All the above statistical analyses were performed using the R software (R Development Core Team, 2005).

Pairwise genetic distances for populations were calculated using the Cavalli-Sforza and Edwards chord distance (D_C) (Cavalli-Sforza & Edwards, 1967) using FSTAT. D_C is considered the most efficient distance for obtaining the correct tree topology under different conditions for microsatellite markers (Takezaki & Nei 1996). A neighbour-joining tree for all populations (table 1) was constructed using Population 1.2.32 (Langella, 1999). The dendrogram was displayed using Treeview (Page, 1996). Genotypic differentiation between populations was tested by F_{ST} according to Weir & Cockerham (1984), and *P*-values obtained after 36,000 permutations using FSTAT; probabilities were Bonferroni corrected (Rice, 1989). Population differentiation in *M. persicae* in relation to host plant families was tested using the samples from Campo de Cartagena collected in 2005. To test whether samples of different plant families represented different *M. persicae* subpopulations, aphids collected from different plant species were pooled by family to calculate pairwise F_{ST}-values. For analysis of the genetic population structure at geographical and temporal scale, F_{ST}-values were calculated by grouping the samples from each year by crop (pepper), secondary host (herbaceous plants other than pepper) and primary host (*Prunus* spp.). Two AMOVA analyses were performed using ARLEQUIN 2.0 (Excoffier *et al.*, 2005), grouping the samples according to host category (pepper; secondary and primary hosts) and year, respectively. The AMOVA in which the samples were grouped by year was performed using only the samples from 2005 and 2008 because some of the host categories were not available for 2006 and 2007. The population structure was inferred using STRUCTURE v2.3 following the method described by Pritchard *et al.* (2000), assuming a model with 1 to 10 populations (*K* clusters). Each *K* was replicated 20 times for 100,000 iterations after a burn-in of 100,000 without any prior information on the population of origin. The *ad hoc* statistic ΔK, based on the rate of change in the log probability between successive *K* values, was used to estimate the uppermost hierarchical level of structure (optimal *K*) (Evanno *et al.*, 2005).

Table 2. Population code: 1 = AGHER05, 2 = CAPEP05, 3 = CAPEP06, 4 = CAPEP08, 5 = CAHER05, 6 = CAHER06, 7 = CAHER07, 8 = CAHER08, 9 = CAPRU05, 10 = CAPRU08, 11 = JUPRU08, 12 = MUPRU08, 13 = NOHER05, 14 = NOHER08, 15 = NOPRU05, and 16 = NOPRU08.

		Samples															
Loci		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
M86	n	11	6	7	8	13	11	8	11	8	14	8	7	7	10	7	8
	H_o	0.917	1	0.933	1	0.974	0.973	1	0.974	0.765	0.871	1	0.778	0.929	0.727	0.385	0.765
	H_e	0.854	0.734	0.818	0.783	0.795	0.845	0.763	0.831	0.875	0.828	0.875	0.778	0.743	0.939	0.865	0.807
	F_{IS}	−0.075	−0.367*	−0.146	−0.282*	−0.225*	−0.154*	−0.318*	−0.176	0.121	−0.053	−0.148	0.000	−0.261	0.204	0.542*	0.055
Myz25	n	5	4	4	4	6	5	6	5	5	7	5	5	4	3	3	4
	H_o	0.792	1	0.867	0.737	0.984	0.947*	1	0.974	0.588	0.677	0.667	0.556	0.786	0.818	1	0.647
	H_e	0.671	0.556	0.729	0.544	0.655	0.713	0.805	0.685	0.529	0.661	0.712	0.680	0.669	0.658	0.649	0.709
	F_{IS}	−0.184	−0.810*	−0.221	−0.36	−0.505*	−0.331	−0.248	−0.429*	−0.115	−0.025	0.065	0.101	−0.182	−0.259	−0.576	0.051
Myz9	n	14	7	7	7	18	11	9	9	5	12	8	3	10	9	7	7
	H_o	0.833	0.959	0.933	0.921	0.948	0.853	0.926	0.974	0.824	0.839	0.81	0.778	0.929	0.818	0.769	0.882
	H_e	0.777	0.721	0.818	0.755	0.750	0.795	0.763	0.793	0.62	0.82	0.826	0.621	0.815	0.913	0.649	0.799
	F_{IS}	−0.075	−0.332*	−0.146	−0.223	−0.264*	−0.074	−0.218	−0.232	−0.341	−0.032	−0.01	−0.273	−0.146	0.100	−0.194	−0.109
M49	n	16	6	6	10	17	9	8	12	12	15	16	6	10	11	8	6
	H_o	0.917	0.699	0.933	0.868	0.921	0.84	0.63	0.947	1	0.774	0.667	0.444	0.929	0.818	0.846	0.824
	H_e	0.926	0.718	0.834	0.816	0.744	0.843	0.752	0.836	0.913	0.906	0.895	0.830	0.860	0.948	0.852	0.865
	F_{IS}	0.010	0.021	−0.123	−0.079	−0.239*	0.003	0.148	−0.136	−0.099	0.148	0.259	0.439	−0.083	0.130	0.008	0.049
M63	n	9	7	7	8	10	11	9	10	10	10	8	4	8	9	7	7
	H_o	0.958	1	0.933	0.974	0.911	0.907	0.852	0.816	1	0.742	0.905	0.667	1.000	1.000	0.923	0.941
	H_e	0.842	0.757	0.844	0.802	0.813	0.87	0.788	0.766	0.838	0.747	0.754	0.660	0.825	0.909	0.806	0.754
	F_{IS}	−0.141	−0.325*	−0.11	−0.217	−0.120*	−0.042	−0.086	−0.066	−0.201	0.007	−0.206	−0.055	−0.221	−0.106	−0.152	−0.258
M37	n	6	3	4	4	10	6	4	7	7	7	6	1	4	4	5	3
	H_o	0.458	0.274	0.6	0.342	0.482	0.573	0.296	0.395	0.529	0.452	0.429	0.000	0.357	0.636	0.538	0.588
	H_e	0.726	0.252	0.543	0.446	0.474	0.504	0.384	0.431	0.754	0.528	0.512	0.000	0.675	0.688	0.751	0.456
	F_{IS}	0.345	−0.143	−0.212	0.235	−0.024	−0.139	0.168	0.039	0.282	0.109	0.165	NA	0.454	0.079	0.273	−0.301
M40	n	7	4	5	7	9	7	6	6	5	7	5	5	6	6	4	4
	H_o	0.917	0.945	0.867	0.974	0.812	0.853	0.926	0.816	0.941	0.903	0.714	1.000	0.929	0.818	0.923	0.471
	H_e	0.787	0.629	0.770	0.742	0.62	0.77	0.712	0.773	0.766	0.797	0.625	0.771	0.762	0.853	0.717	0.613
	F_{IS}	−0.169	−0.507*	−0.13	−0.317*	−0.311*	−0.108	−0.308	−0.064	−0.252	−0.136	−0.2	−0.321	−0.229	0.027	−0.303	0.229
M35	n	6	4	4	5	8	7	6	6	6	6	6	3	4	8	5	4
	H_o	0.667	0.986	0.533	0.921	0.906	0.653	0.556	0.947	0.647	0.71	0.714	0.667	1.000	0.727	1	0.529
	H_e	0.784	0.645	0.708	0.652	0.706	0.748	0.588	0.592	0.699	0.777	0.698	0.667	0.659	0.823	0.797	0.756
	F_{IS}	0.142	−0.535*	0.22	−0.425*	−0.283*	0.122	0.011	−0.613*	0.036	0.074	−0.062	−0.185	−0.549	0.121	−0.268	0.306

Number of alleles (n), observed (H_o) and expected (H_e) heterozygosity, F_{IS}-values (*significantly different from expected in HW equilibrium after Bonferroni correction). NA, not available.

Table 3. Pairwise comparison for genetic differentiation based on F_{ST}-values for *M. persicae* samples on different plant families. URT, urticaceae; SOL, solanaceae; PLA, plantaginaceae; MAL, malvaceae; BRA, brassicaeae; CON, convolvulaceae; CHE, chenopodiaceae; ROS, Rosaceae (*Prunus* spp.). NS = non-significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Significance after the correction of P by Bonferroni. Number of individuals genotyped (excluding duplicates) in brackets.

	Samples								
	Pepper (73)	URT (5)	SOL (13)	PLA (7)	MAL (56)	BRA (57)	CON (16)	CHE (37)	ROS (17)
Pepper		NS	***	NS	***	***	NS	***	***
URT	0.015		NS	NS	NS	NS	NS	NS	NS
SOL	0.053	0.043		NS	NS	NS	NS	NS	**
PLA	0.021	0.007	0.029		NS	NS	NS	NS	NS
MAL	0.026	0.000	0.018	0.014		NS	NS	NS	***
BRA	0.041	0.019	0.011	0.002	0.007		NS	NS	***
CON	0.022	0.004	0.033	0.022	0.001	0.008		NS	**
CHE	0.052	0.045	0.037	0.014	0.017	0.013	0.001		***
ROS	0.130	0.111	0.080	0.089	0.090	0.072	0.080	0.087	

Results

Microsatellite markers, linkage disequilibrium and genetic diversity

All eight microsatellite loci amplified successfully for the *M. persicae* samples. A total of 289 different genotypes were found in the 630 individuals scored for the eight microsatellites, excluding duplicates from the same sampling site and plant species. Polymorphism was observed for the eight loci in all the samples with the exception of MUPRU08, which was monomorphic for M37 (table 2). The average allelic richness across loci was higher for the herbaceous (mean, 5.75: range, 3–10.53) and primary host (5.56: 1–10) than for pepper (4.52: 2.06–6.53) (table 2) samples. The maximum number of alleles was observed in Myz9 (18), followed by M49 (17) (table 2). The maximum number of alleles across samples was found in the samples from herbaceous plants in 2005 (91) followed by the samples in the primary host in 2008 (78) (table 2). There were no significant differences in the number of alleles between primary and secondary hosts ($F = 0.368$, df = 1, 12, $P = 0.556$), nor between samples from the southern and the northern part of the province ($F = 0.723$, df = 1, 12, $P = 0.412$). However, the number of alleles on pepper samples in Campo de Cartagena was significantly lower than on samples from herbaceous plants ($F = 9.07$, df = 1, 5, $P = 0.030$) and *Prunus* ($F = 6.78$, df = 1, 3, $P = 0.080$). The proportion of unique genotypes in the primary host was similar in the northern (mean ± SE, 0.961 ± 0.036) and the southern (0.987 ± 0.013) part of the province. In the secondary host, the proportion of unique genotypes was higher in the north (0.801 ± 0.159) than in the south (0.318 ± 0.063). The ANOVA denoted significant differences in the proportion of unique genotypes between areas ($F = 18.50$, df = 1, 12, $P < 0.001$) and host ($F = 58.58$, df = 1, 12, $P < 0.001$), with a significant area–host interaction ($F = 11.10$, df = 1, 12, $P < 0.01$). The proportion of unique genotypes on pepper and herbaceous plants in the surroundings of greenhouses in Campo de Cartagena was not significantly different ($F = 1.13$, df = 1, 5, $P = 0.336$).

The average proportion of null alleles across samples ranged from 0.04 to 5.27%. In samples from the southern part of the province, significant disequilibrium was detected between loci belonging to the same and different linkage groups, both in herbaceous plants and *Prunus* spp. The average linkage proportions for pairs of loci located in the same chromosome in the southern part of the province were 0.333 and 0.222 in herbaceous and *Prunus*, respectively. For the loci in different chromosomes, the linkage proportion was higher in herbaceous plants (0.395) than in *Prunus* spp. (0.133). In the northern part of the province, no linkage disequilibrium was observed between loci located in different groups, and low linkage was observed for loci located in the same group in samples from herbaceous plants (0.02). The proportion of linked loci did not differ significantly in relation to linkage group ($F = 0.021$, df = 1, 28, $P = 0.887$) or host (herbaceous plants/*Prunus*) ($F = 0.962$, df = 1, 28, $P = 0.335$), but significant differences were found between the southern and the northern part of the province ($F = 6.10$, df = 1, 28, $P = 0.020$).

Heterozygosity was high in most of the samples and loci (table 2). The average H_o ranged from 0.452 to 0.900, and the average H_e from 0.513 to 0.844 (table 2). Heterozygosity excess was observed in most of the samples, with a significant departure from HWE in all samples on pepper and herbaceous plants in Campo de Cartagena (table 2); the average number of loci not in HWE was the same for pepper and herbaceous plant samples (three loci). Significantly lower heterozygosity than expected was found only for M86 in NOPRU08 (table 2). The number of loci not in HWE was significantly higher in the southern than in the northern part of the province ($F = 5.45$, df = 1, 12, $P = 0.034$), and in the secondary host than in the primary host ($F = 4.22$, df = 1, 12, $P = 0.062$).

Genetic population structure in M. persicae *in relation to host plant*

The F_{ST}-values pointed to a degree of differentiation in *M. persicae* samples according to the primary and secondary hosts. The overall F_{ST}-values among the samples from different families of herbaceous plants in the environment of pepper greenhouses were low (0.001–0.053) and not significant in any case (table 3). These low F_{ST}-values indicate null or little genetic differentiation among samples in relation to plant family. However, significant F_{ST}-values were found between pepper and most of the herbaceous plant samples, and between herbaceous plants (including pepper) and the primary host (table 3). The highest F_{ST}-values (0.072–0.130) were found between samples on the primary host and the rest of the samples (table 3). Given the lack of population differentiation in relation to plant families, *M. persicae* samples

Table 4. Pairwise comparison for genetic differentiation based on F_{ST}-values for *M. persicae* samples (see Table 1 for sample codes). *P*-values obtained after 136,000 permutations. NS = non-significant, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; *P* significance after Bonferroni correction.

		Samples															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	AGHER05		***	NS	NS	***	NS	NS	*	NS	NS	*	NS	NS	NS	NS	**
2	CAPEP05	0.071		***	***	***	***	***	***	***	***	***	***	***	***	***	***
3	CAPEP06	0.019	0.090		NS	**	NS	NS	NS	***	NS	**	*	NS	NS	***	**
4	CAPEP08	0.027	0.037	0.045		***	***	**	NS	***	**	***	*	*	NS	***	***
5	CAHER05	0.034	0.030	0.058	0.024		***	**	**	***	***	***	***	***	**	***	***
6	CAHER06	0.019	0.086	0.010	0.044	0.050		***	***	***	***	***	**	**	NS	***	***
7	CAHER07	0.052	0.089	0.078	0.064	0.041	0.068		*	***	**	***	**	*	NS	***	***
8	CAHER08	0.032	0.043	0.058	0.017	0.022	0.057	0.050		***	NS	**	*	NS	NS	***	***
9	CAPRU05	0.037	0.130	0.116	0.099	0.082	0.101	0.096	0.071		**	**	*	*	*	NS	***
10	CAPRU08	0.020	0.084	0.060	0.034	0.045	0.047	0.061	0.033	0.053		NS	NS	**	NS	**	NS
11	JUPRU08	0.036	0.111	0.081	0.060	0.055	0.065	0.050	0.050	0.044	0.010		NS	***	**	***	NS
12	MUPRU08	0.068	0.144	0.135	0.088	0.104	0.106	0.109	0.093	0.101	0.022	0.042		**	*	*	NS
13	NOHER05	0.013	0.061	0.074	0.041	0.034	0.069	0.060	0.039	0.051	0.046	0.066	0.115		NS	NS	***
14	NOHER08	0.009	0.096	0.001	0.058	0.060	0.006	0.075	0.051	0.062	0.040	0.051	0.114	0.056		**	NS
15	NOPRU05	0.038	0.156	0.129	0.113	0.109	0.112	0.132	0.093	0.017	0.063	0.082	0.127	0.060	0.081		***
16	NOPRU08	0.049	0.129	0.085	0.067	0.080	0.069	0.092	0.075	0.071	0.013	0.010	0.044	0.089	0.053	0.102	

on herbaceous plants in the same year and locality were grouped together for the following analyses.

Genetic population structure at geographical and temporal scale

F_{ST}-values ranged from 0.001 to 0.156 and denoted a variable degree of differentiation among samples in the three groups of hosts (pepper, herbaceous plants and *Prunus*) in the different geographical areas and years (table 4). F_{ST}-values in the four years of the study ranged from 0.037 to 0.090 among pepper samples, from 0.022 to 0.068 among samples of herbaceous plants and from 0.010 to 0.127 among samples of *Prunus* (table 4). F_{ST}-values among pepper and herbaceous plant samples in Campo de Cartagena ranged from 0.010 to 0.017, with lower F_{ST}-values among pepper and herbaceous plant samples in the same year than among those in different years (table 4). Significant differences were found among pepper samples from Campo de Cartagena and samples on herbaceous plants from other localities in approximately half of the cases (table 4). F_{ST}-values always showed significant differences in pairwise comparisons among pepper and *Prunus* samples, with the exception of CAPEP06 and CAPRU08 (table 4). Samples on herbaceous plants in the north and the south of the province differed significantly in six out of ten pairwise comparisons (table 4). No significant differences were found among any *Prunus* samples in the same year, which was also sometimes the case with pairwise comparison among samples from different years (table 4).

AMOVA pointed to a significant population structure when samples were grouped by host category (pepper, secondary and primary hosts). Differences between individuals within samples accounted for most of the genetic variation (94.14%), followed by the variation among samples within groups (4.15%) and differences among groups of hosts (1.71%) (table 5). When samples were grouped by year, most of the genetic variation was also due to differences between individuals within samples (94.80%), followed by variations among samples within groups (4.54%) and between groups (0.66%) (table 6). Two main clusters may be observed in the dendrogram showing the relationship among *M. persicae* samples: one of the clusters includes all the samples from *Prunus* and the other all the samples from pepper and the rest of herbaceous plants (fig. 2). There was no clear structure associated with the year of sampling, although, samples from the same year were frequently located in the same branch. A structural analysis was run for *K* values ranging from 1 to 10, with the *ad hoc* statistic (ΔK) reaching its maximum at $K=3$ (55.4 ± 3.9, average ± SE) followed by $K=2$ (52.4 ± 4.2). For $K=2$, most of the individuals from the primary host were included in the first cluster, while the samples from pepper and the rest of the secondary hosts were a mixture of individuals assigned to both clusters (fig. 3). The percentage of individuals assigned to the first cluster in the primary host ranged from 88.2 to 99.3%. In the pepper samples, most of the individuals were generally included in the second cluster (47.4–70.9%), while for the other secondary hosts the highest numbers of individuals were assigned to the first cluster (52.7–81.8%) (table 7). For $K=3$, most of the individuals from the primary host had the highest probability of ancestry in the first cluster (79.3–96.5%) (fig. 3, table 7). In pepper, a high percentage of individuals were assigned to the second cluster (40.9–64.2%), but the results varied with the years (table 7). In the samples from herbaceous plants, there was no clear pattern in the assignment of individuals to clusters (table 7).

Table 5. Hierarchical analysis of molecular variance (AMOVA) for samples of *M. persicae* collected from 2005 to 2008 grouped by host category (crop, secondary and primary hosts). Degrees of freedom (df), sums of squared deviations (SSD), the percentage of the total variance because of each level, and the probability test calculated after 1023 permutations.

Source of variation	df	SSD	Variance component	Percentage	*P*-value
Among groups	2	66.7	0.052	1.71	<0.001
Among samples within groups	13	154.6	0.125	4.15	<0.001
Within samples	1212	3436.0	2.835	94.14	<0.001

Table 6. Hierarchical AMOVA for samples of *M. persicae* collected from 2005 and 2008 on different host categories grouped by year. Degrees of freedom (df), sums of squared deviations (SSD), the percentage of the total variance because of each level, and the probability test calculated after 1023 permutations.

Source of variation	df	SSD	Variance component	Percentage	*P*-value
Among groups	1	24.5	0.019	0.66	<0.001
Among populations within groups	10	116.2	0.134	4.54	<0.001
Within populations	960	2680.9	2.793	94.80	<0.001

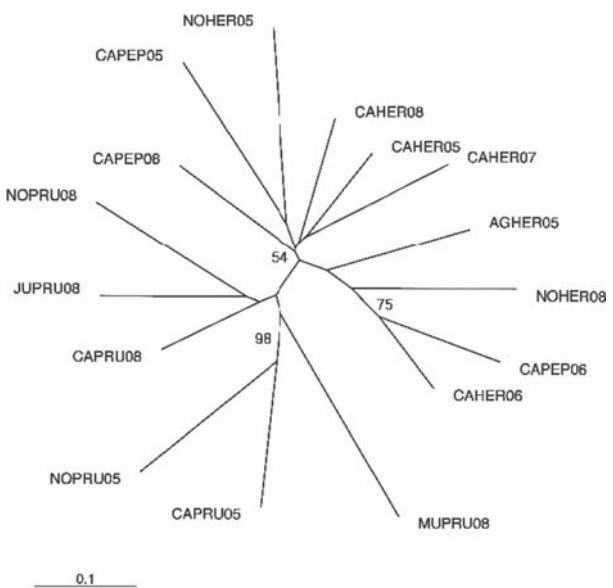

Fig. 2. Population dendrogram for samples of *M. persicae* collected from 2005 to 2008 on different host plants (see table 1 for sample codes). Numbers on nodes show bootstrap support (10,000 pseudo replications).

Discussion

Genetic diversity

In this work, we investigated the population structure of *M. Persicae* in diverse agroecosystems in areas with different climatic conditions and in relation to the primary and secondary hosts. We also looked at the change in the population structure in temporal hosts (pepper crops) *versus* permanent hosts (*Prunus* spp. and herbaceous plants). The high genetic diversity found in Murcia province agrees with the high haplotype diversity observed using other markers such as RAPD (Random amplified polymorphic DNA) (Martinez-Torres *et al.*, 1997) or rDNA fingerprinting (Fenton *et al.*, 1998). The differences in the proportion of unique genotypes, linkage disequilibrium, number of alleles and the number of loci under HWE indicated divergences in the population structure of *M. persicae* according to the host (primary or secondary) and the geographical area of distribution. The proportion of unique genotypes was higher in *Prunus* than in the secondary host plants but, while in *Prunus* the proportion was similar in the northern and southern part of the province, on the secondary host the proportion of unique genotypes was lower in the southern area. No significant differences were found in unique genotypes between pepper and herbaceous plant samples in the vicinity of greenhouses in the southern area, although, a lower number of alleles were observed on pepper than on herbaceous plants and *Prunus*, which might be due to a founder effect. The higher proportion of unique genotypes in herbaceous plants in the northern part of the province might be explained by the lower survivorship of obligate parthenogens in severe winter conditions, and consequently, by a lower representation of anholocyclic forms in populations. Lower genotypic variability is to be expected in obligate parthenogenetic aphids as a result of the absence of recombination in asexual reproduction (Delmotte *et al.*, 2001, 2002; Guillemaud *et al.*, 2003). These findings agree with the distribution and abundance of holocyclic and anholocyclic *M. persicae* in relation to climatic conditions predicted by Blackman (1974). The different climatic conditions of our two geographical areas of study may have a significant effect on the *M. persicae* population structure. In the southern part of the province anholocyclics may easily survive mild winter temperatures, while in the northern part their survivorship will depend greatly on the severity of winter temperatures. Vorburger *et al.* (2003a) found that both the abundance of peach trees and temperature influenced the distribution of genotypes with different reproduction strategies, and the genetic diversity in populations of *M. persicae* in Victoria, Australia. Cold winters may eliminate anholocyclic *M. persicae*, reducing genetic variability and imposing population 'bottlenecks' (Fenton *et al.*, 1998). The results of the present work also agree with those of Guillemaud *et al.* (2003), who found a higher proportion of multilocus genotypes in mixed samples of obligate and cyclical parthenogens than on peach samples of cyclical parthenogens.

Table 7. Results of Bayesian analyses using STRUCTURE. Percentage of individuals in the *M. persicae* samples assigned to each cluster according to their respective probabilities of ancestry for $K=2$ and $K=3$ in samples.

	$K=2$		$K=3$		
Sample codes	Cluster 1	Cluster 2	Cluster 1	Cluster 2	Cluster 3
CAPEP05	29.1	70.9	21.3	64.4	14.3
CAPEP06	52.6	47.4	13.3	40.0	46.7
CAPEP08	43.1	56.9	36.6	55.3	8.2
AGHER05	66.7	33.3	50.8	27.1	21.9
CAHER05	52.7	47.3	39.1	44.6	15.9
CAHER06	62.2	37.8	25.6	30.7	43.7
CAHER07	63.2	36.8	53.8	34.6	11.5
CAHER08	53.4	46.6	47.5	46.2	6.3
NOHER05	59.5	40.5	46.8	37.1	16.1
NOHER08	81.8	18.2	40.9	18.2	40.9
CAPRU05	88.2	11.8	88.2	11.8	0.0
CAPRU08	89.2	10.8	79.3	8.8	11.9
MUPRU08	98.1	1.9	93.0	6.4	0.0
JUPRU08	99.2	0.8	96.5	0.0	3.0
NOPRU05	88.2	11.8	88.2	11.8	0.0
NOPRU08	99.3	0.7	95.0	0.0	5.0

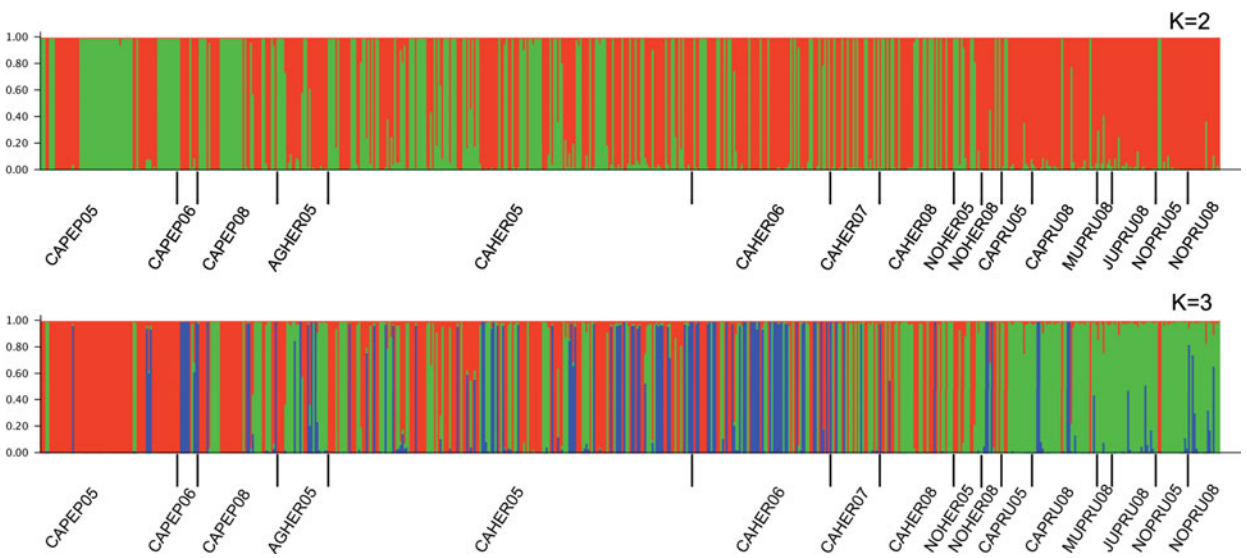

Fig. 3. STRUCTURE analyses for $K=2$ and 3 clusters. *M. persicae* population structure based on microsatellite scores. Each line represents a single individual and is fragmented in different colours according to the coefficient of ancestry of each individual.

Linkage disequilibrium was frequent in samples from the southern part of the province, both in the primary and secondary hosts, but rare in the northern area. A similar degree of association found at both, loci belonging to the same and different linkage groups, might be explained by the generation of new genotypes resulting from the mutation of obligate parthenogens. The significant linkage found on peach samples in the southern part of the province might be explained by the contribution of androcyclic and facultative parthenogenetic clones to the population pool reproducing sexually in the primary host. Gillemaud *et al.* (2003), found no significant linkage among loci in samples of *M. persicae* populations collected on peach, with frequent linkage disequilibria in samples from aerial traps, supposedly integrated by a mix of obligate and cyclical parthenogens. Linkage disequilibrium at loci belonging to different linkage groups was also observed in *M. persicae* by Wilson *et al.* (2002).

The significant heterozygosity excess found on pepper and herbaceous plants in the southern part of the province may be attributed to the high representation of obligate parthenogens in *M. persicae* populations in the secondary hosts. The same diversity pattern associated with the over representation of obligate parthenogens in populations has been reported for other aphids such as *Sitobion* spp. (Sunnucks *et al.*, 1996; Wilson *et al.*, 1999), *Aphis gossyii* Glover (Fuller *et al.*, 1999) and *Myzus antirrhinii* Macchiati (Hales *et al.*, 2002). This higher heterozygosity in obligate parthenogenetical lineages may be explained by the accumulation of mutations at loci during extended cycles of asexual multiplication, among other factors

(Birky 1996; Fenton *et al.*, 1998; Guillemaud *et al.*, 2003; Vorburger *et al.*, 2003a). The hybridization between highly differentiated populations is another factor leading to heterozygosity excess in aphid populations (Delmotte *et al.*, 2001). This argument favours our hypothesis of interbreeding between androcyclic and facultative obligate parthenogens with cyclical parthenogens in the primary host in Murcia province. Complementary studies will help determine the contribution of hybridization to the genetic variability of *M. persicae* populations.

Population genetic structure in relation to host plants

Host specialization is common in aphids and the formation of host races has been reported in several polyphagous species (Blackman & Eastop 2000; Frantz *et al.*, 2006). Divergences in the populations of *A. pisum* associated with host specialization in alfalfa [*Medicago sativa* L. (Fabaceae)] and red clover [*Trifolium pratense* L. (Fabaceae)] have been reported both in the ancestral geographic range of distribution and in the New World (Via, 1999; Hawthorne & Via 2001; Simon *et al.*, 2003; Frantz *et al.*, 2006). The association of lineages with specific host plant species was also reported in *Sitobion avenae* (F.) (Hemiptera: Aphididae) in France (Sunnucks *et al.*, 1997). The specialization of *M. persicae* seems to be mostly related to tobacco and little evidence of population differentiation has been reported in other host plants. Molecular, morphological and behavioural studies pointed to significant differences between *M. persicae* clones on tobacco and other host plants in tobacco-growing regions in northern and central Greece (Margaritopoulos *et al.*, 2000, 2003, 2007a; Nikolakakis *et al.*, 2003). The overrepresentation of one *M. persicae* genotype on *Solanum physalifolium* Rusby (Solanaceae) is the only evidence provided as a possible case of specialization of *M. persicae* in a host other than tobacco. Fenton *et al.* (1998) found no differences in the distribution of genotypes between *Brassica* and potato crops. Similarly, Zitoudi *et al.* (2001) found no specific band pattern in clones collected from different host plants using RAPD. In the present work, we found no evidence of *M. persicae* population divergence in any of the families of herbaceous plants commonly present in the vicinity of pepper greenhouses in Murcia province.

M. persicae *population structure at geographical and temporal scale*

Analysis of the population structure using microsatellite markers revealed a marked microgeographic structure associated with primary and secondary hosts, and variable with time. F_{ST}-values ranging from 0.034 to 0.130 indicated moderate differentiations between *M. persicae* populations in the primary and secondary host plants; samples from the herbaceous plants and *Prunus* also grouped into two distinct clusters in the NJ dendrogram. As stated above, these differences in structure might be due to populations in herbaceous plants being made up of both obligate and cyclical parthenogenetic genotypes, while cyclical parthenogens would be the predominant forms in the primary host. Bayesian analyses showed that the population of *M. persicae* in Murcia province could be grouped into two or three clusters. In both cases ($K = 2$ and 3), the samples from the primary host were mainly composed of individuals belonging to the first cluster, while the samples from the secondary host were made up of genotypes belonging to the first, second and third (in the case of $K = 3$) clusters. The existence of two clusters could be explained according to reproductive modes: *Prunus* would mainly host holocyclic genotypes, while herbaceous plants would host both holocyclic and anholocyclic forms. The high level of organization ($K = 3$) predicted by STRUCTURE is difficult to explain because no obvious pattern is observed in the secondary host. Genetic differentiation between populations with a high percentage of cyclical parthenogens and those consisting mostly of anholocyclic lineages were reported in tobacco-growing regions in Greece (Margaritopoulos *et al.*, 2007a). Martinez-Torres *et al.* (1996) predicted that a different distribution of anholocyclic and holocyclic aphids in populations would result in significant structuring of populations as long as gene flow between the two lineages and migration levels were limited. The little differentiation between samples of *M. persicae* on pepper and herbaceous plants in the same year seems to indicate that herbaceous plants in the vicinity of greenhouses are the most likely source of aphids for pepper crops. F_{ST}-values among pepper and herbaceous plant samples from Campo de Cartagena ranged from 0.001 to 0.017, with lower F_{ST}-values between samples on pepper and herbaceous plants from the same year than between those in different years. The differences between herbaceous plants and pepper found in some years could be due to a founder effect, as populations in greenhouses may be composed of the few lineages that spread from herbaceous plants and *Prunus*.

The significant change observed in the structure of *M. persicae* populations on the secondary hosts from one year to another might be due to a high turn-over of obligate parthenogenetic genotypes surviving the winter, combined with a variation in the contribution of genotypes generated by cyclical parthenogens reproducing sexually in the primary host. Moreover, the changes in the structure of the population found on *Prunus* between years might also be connected with the selection of genotypes in secondary hosts that migrate later to the primary host. Impoverishment and increased differentiation between summer and autumn populations of *M. persicae* were reported in central and northern France (Guillemaud *et al.*, 2003). Treatment with insecticides may also have a strong impact on the shaping of *M. persicae* population in agroecosystems, selecting genotypes resistant to insecticides (Devonshire *et al.*, 1998).

Overall F_{ST}-values (0.022 ± 0.009) among *Prunus* samples from distant localities in the same year indicate a low degree of population differentiation, possibly due to the homogenization of populations by the displacement of *M. persicae* over a greater distance than considered in this study (<100 km). In contrast, samples on herbaceous plants showed higher geographical differentiation on the same spatial scale (overall $F_{ST} = 0.040 \pm 0.006$). A higher degree of population homogenization and, therefore, lower population differentiation is expected in cyclical than in obligate parthenogenetic aphid populations due to the migrations of cyclical parthenogens between the primary and secondary hosts.

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