Genetic diversity of the melon aphid Aphis gossypii Glover in different melon growing areas of France

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REDIA, XCII, 2009: 47-50

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GENETIC DIVERSITY OF THE COTTON-MELON APHID, *APHIS GOSSYPII* GLOVER IN DIFFERENT MELON GROWING AREAS OF FRANCE

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Thomas S., Mistral P., Chareyron V., Barral B., Boissot N., Vanlerberghe-Masutti F. – Genetic diversity of the cotton-melon aphid, *Aphis gossypii* glover in different melon growing areas of France.

In melon, the *Vat* gene confers resistance to colonization by the Cotton-Melon aphid species, *Aphis gossypii* Glover. The *Vat* gene has been present in several melon varieties produced in south-eastern France for the past fifteen years, although as time goes by, the risk of the aphids overcoming such resistance is increasing. The study of the genetic structure of Cotton-Melon aphid populations is important in order to understand the efficacy and durability of methods to control pests through the use of resistant plants. Therefore, as outlined in the present paper, we set up field trials in geographically distant melon-producing areas in France and the French West Indies (FWI) using melon plants with the resistant allele or with the susceptible allele at the *Vat* locus. Samples of *A. gossypii* were collected from these crops in the different regions and their genetic diversity analyzed at eight microsatellite loci. We identified 33 multilocus genotypes (MLGs) that were present in multiple copies, of which five were observed in several regions of France whilst two were restricted to the FWI. The genetic diversity was high in south-eastern France, moderate in south-western and western France, and low in Guadeloupe (FWI). Differentiation between pairs of geographical populations in mainland France estimated by multilocus F_{ST} was not significant. Some MLGs (NM1 and C9) were significantly less frequent on melon with the resistant allele at the *Vat* locus than on melon with the susceptible allele at the *Vat* locus, while the frequencies of others (C6, CUC1, CUC13 and CUC25) increased significantly on melon plants with the resistant allele at the *Vat* locus.

KEY WORDS: *Aphis gossypi*, genetic diversity, *Vat*, resistance gene, *Cucumis melo*

INTRODUCTION

The Cotton-melon aphid, *Aphis gossypii* Glover is a cosmopolitan pest of crops, causing direct damage both via the sucking of plant sap and indirectly through the transmission of phytopathogenic viruses. This polyphagous species, which reproduces mainly by apomictic parthenogenesis, is structured into host races that are distributed worldwide (CARLETTO *et al.,* 2009). The genetic diversity of the aphids from the race on cucurbits is split into two divergent phylogenetic lineages. The first is made of only one genotype – defined on the basis of eight microsatellite loci – called NM1, whilst the second occurs as a cluster of genotypes of which C9 is very frequently observed throughout the species' range. Infestation of melon crops by aphids of the cucurbit race may be under the control of the *Vat* gene that confers resistance both to colonization by *A. gossypii* (PITRAT & LECOQ, 1982) and to virus transmission by this aphid (PAUQUET *et al.,* 2004). Laboratory and field observations revealed that melon cultivars with the resistant allele at *Vat* locus are resistant to aphids of genotype NM1 and partially resistant to aphids of genotype C9 (BOISSOT *et al.,* 2008).

The aim of the present study was to compare the genetic structure of *A. gossypii* populations on susceptible (non *Vat*) and resistant (*Vat*) melon crops in different melon growing areas of France, in order to evaluate the intensity of the *Vat* selection pressure. Apterous *A. gossypii* were sampled on both non *Vat* and *Vat* melon plants in five localities corresponding to four geographical regions. The genetic variability of the *A. gossypii* samples was assessed using eight microsatellite markers, first at the locality level and second, by taking the *Vat* selection pressure into account.

MATERIALS AND METHODS

Aphids were sampled in 2007 from one locality in south-eastern France (Aramon) and in 2008 from four localities in south-eastern (Saint-Andiol and Aramon), south-western (Montauban), western France (Angers) and from Guadeloupe, FWI (Petit-canal). We collected only apterous morphs, either as singletons or individuals within a colony *i.e.* and thus presumed to descend from the same asexual founding mother (foundress).

DNA from each of 1,871 individual aphids was extracted using a 5% (w/v) Chelex resin solution as described in FULLER *et al.*, (1999). DNA amplifications at the eight microsatellite loci specific of the *A. gossypii* genome (VANLERBERGHE-MASUTTI *et al.,*1999) were performed in two PCR reactions as described in CARLETTO *et al.*, (2009). The allele size at each locus was identified by comparison with molecular size standard using the software GeneMapper v3.7 and a multilocus genotype (MLG) was subsequently assigned to each aphid.

The clonal diversity of each sample was calculated by using the Shannon-Wiener index: $H = -\Sigma_i p_i \ln p_i$, where p_i represents the relative frequency of the i^{th} multilocus genotype. It was expressed by e^H as proposed by VANO-

– Received 30 July 2009 Accepted 2 September 2009 Published 1 December 2009

VERBEKE & DE MEESTER (1997) to take into account the number of individuals in the sample and the relative abundance of the different MLGs. The values of e^H range from 1 (all individuals have the same MLG) and N (all individuals have a different MLG).

The following analyses were performed with only one representative per MLG (SUNNUCKS *et al.,* 1997): departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium between pairs of loci were calculated using exact tests whilst genetic differentiation between pairs of populations was testing by calculating F_{ST} across loci (Arlequin v. 311). A genetic distance matrix between each pair of MLG was calculated by using the allele shared distance (JIN & CHARKRABORTY, 1994). A phylogenetic network was built according to the Neighbor-Joining (NJ) method (SAITOU & NEI, 1987) using DARwin5 v. 5.0.156.

RESULTS AND DISCUSSION

Twenty-five to >> 200 apterous aphids were collected on both resistant and susceptible melon plants in each field for the total of 1,871 individuals tested (Tab. 1). Allelic diversity at the eight microsatellite loci ranged from three to eight alleles per locus with 48 alleles identified across all loci.

We discriminated 33 frequent MLGs, whilst 71 MLGs were observed only once and related to single, isolated apterae. Most of MLGs corresponded to rearrangements of identical alleles, rather than possession of private alleles. The highest clonal diversity was observed within *A. gossypii* samples from south-eastern France (e^H >7), intermediate diversity in south-western and western France (e^H ~ 4) and low diversity in Guadeloupe (e^H < 2). Five MLGs were present in several regions of France: NM1, C11, C9, CUC2 and MTB. In Guadeloupe, two MLGs were identified (C6 present at 16% and GWD present at 84%) that have never been observed in metropolitan France.

When site-associated populations from mainland France were considered, HWE was observed for the great majority of the loci (no significantly different from expectations at P=0.05). However, differences between observed and expected heterozygosity were highly significant for the majority of the loci when we considered a single population made of one individual per MLG. This could result from a Wahlund effect. In the same way, significant linkage disequilibria were observed for 24 pairs of loci out of 28 when we considered a single population but the number of significant linked loci per locus was lower when site-associated populations were analysed (P<0.05). These results suggest that infrequent sexual reproduction (holocycly) events most probably do occur in French populations, which, if true, challenges the commonly held view that *A. gossypii* has an exclusively clonal reproduction in temperate regions (BLACKMAN & EASTOP, 2000). In contrast, the tropical climate characterizing Guadeloupe where asexual (anholocyclic) propagation is likely to occur all year round and the relative isolation of this small island in the Caribbean basin, could account for the very low clonal diversity found in the population sample from this locality

The F_{ST} analysis conducted on the *A. gossypii* populations collected in France did not reveal any structuring effect as the differentiation between pairs of geographical populations estimated by multilocus F_{ST} was not significant (Tab. 2).

A phylogenetic network was built with the 33 frequent MLGs sampled in this study and the other MLGs described by CARLETTO *et al.*, (2009) (Fig. I). The two MLGs observed in Guadeloupe and 26 of those observed in France belong to the cluster described by CARLETTO *et al.*, (2009). Interestingly, four MLGs detected in south-eastern France were close to the MLG NM1 previously described as the other phylogenetic lineage of *A.gossypii* colonizing Cucurbits.

We compared the frequencies of the nine MLGs detected both on melon plants with the resistant allele at the *Vat* locus and on melon plant with the susceptible allele at the *Vat* locus (Tab. 3). NM1 was significantly less frequent on melon with the resistant allele at the *Vat* locus than on melon with the susceptible allele at the *Vat* locus over all localities except Montauban. There was no significant variation in the frequency of C9 according to the allele of *Vat* except in one locality, Aramon 2008, where it decreased on *Vat* melons. This variation is consistent with the known effects of the *Vat* gene on NM1

Table 1 – number of aphids and clonal diversity (e^H) of *A. gossypii* samples collected from different localities in three areas in France and in the French West Indies.

Localities	Southeast France			Southwest France	Western France	French West Indies	Total
	Aramon	Aramon	Saint Andiol	Montauban	Angers	Guadeloupe Petit-canal	
Year	2007	2008	2008	2008	2008	2008	
n	359	325	305	315	105	462	1871
e^H	9.09	7.34	7.58	3.58	2.93	1.55	12.90

Table 2 – Genetic differentiation according to pairwise F_{ST} pairs of five *A. gossypii* populations corresponding to samples pooled by geographical origin.

	Aramon 2007	Aramon 2008	Saint Andiol 2008	Montauban 2008
Aramon 2008	0.0198^{NS}	0		
Saint Andiol 2008	0.0149^{NS}	-0.0405^{NS}	0	
Montauban 2008	0.0324^{NS}	-0.0296^{NS}	-0.0221^{NS}	0
Angers 2008	-0.0134^{NS}	-0.0487^{NS}	-0.0448^{NS}	-0.0931^{NS}

NS, non-significant; P<0.05.

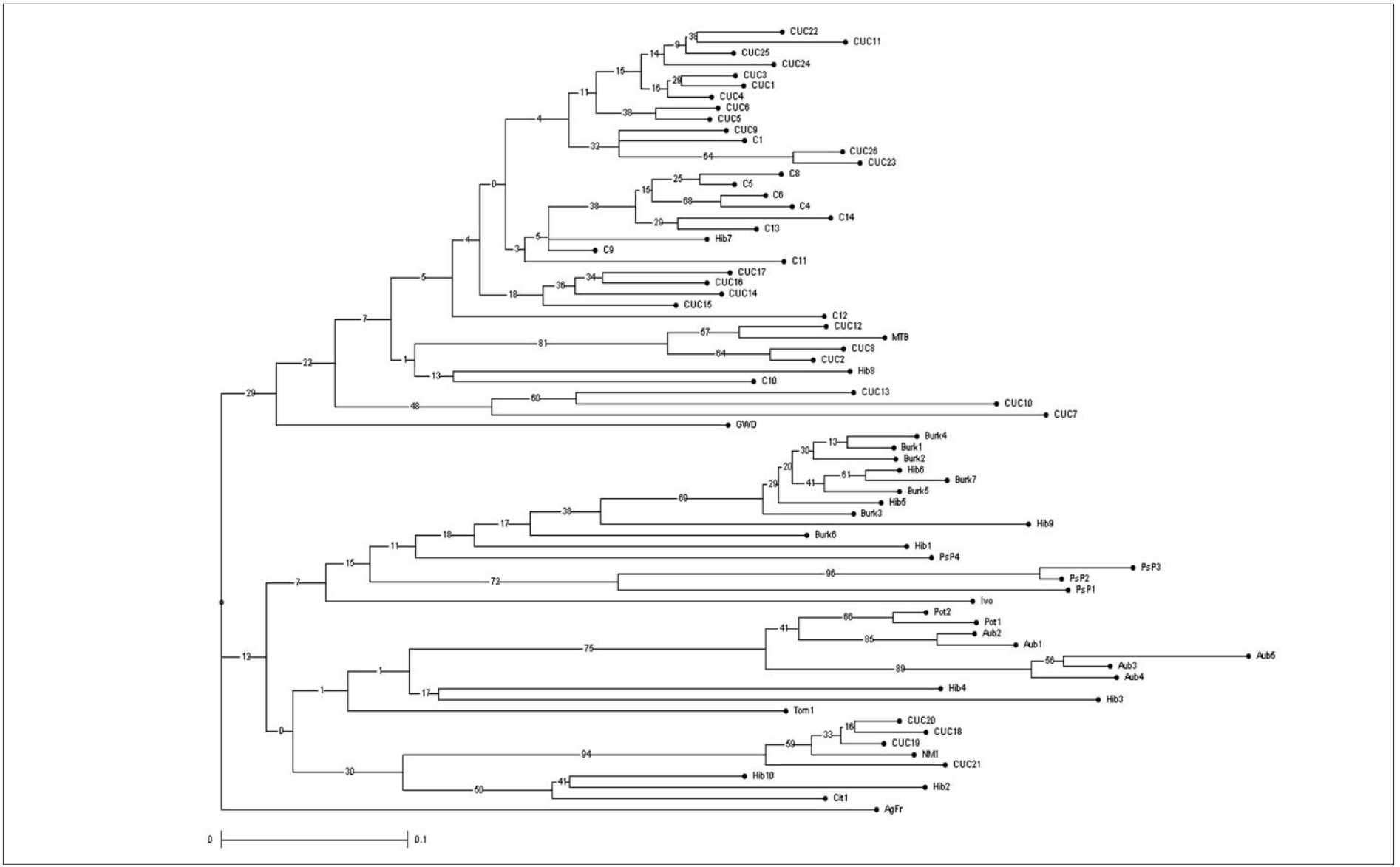

Fig. I – Neighbour-joining tree based on shared allele distances (DAS) calculated with eight microsatellite loci for 72 multilocus genotypes of *A. gossypii* (28 new MLGs from melon crops in France and 44 MLGs from various crops all over the world described by CARLETTO *et al.*, 2009). Bootstrap values are given in percentage over 1000 replications.

Table 3 – Frequencies of MLGs found on both non *Vat* and *Vat* melons in the different localities. Significant differences according to the presence of *Vat* are indicated in bold.

	Aramon 2007		Aramon 2008		Saint Andiol		Montauban		Angers		Guadeloupe	
MLG	Non Vat	Vat	Non Vat	Vat	Non Vat	Vat	Non Vat	Vat	Non Vat	Vat	Non Vat	Vat
NM1	**0.38**	**0.07****	**0.22**	**0.10***	**0.17**	**0.06***	0.21	0.21	**0.52**	**0.20****		
C9	0.04	0.01	**0.32**	**0.16***	0.09	0.05	0.02	0.04				
CUC1	**0.07**	**0.19***	0.10	0.15	**0.15**	**0.41***						
CUC25	**0.05**	**0.25****										
MTB					0.09	0.05	0.56	0.56	0.27	0.40		
CUC13							0.04	0.08	**0.15**	**0.28***		
C11			0.02	0.07	0,.03	0.03			0.01	0.00		
C6											**0.06**	**0.26****
GWD											0.94	0.74

* p value < 5%; ** p value < 1%

and C9 aphid strains observed in laboratory experiments. On the contrary, frequencies of the genotypes CUC1, CUC25, CUC13 and C6 were significantly higher in some localities on melon with the resistant allele at the *Vat* locus *vs.* on melon with the susceptible allele at the *Vat* locus. These four MLGs belong to the second cluster described by CARLETTO *et al.,* (2009) that contains most of the MLGs feeding on Cucurbits. The only cases of heavy aphid colonies developing on plants with the resistant allele at the *Vat* locus were attributed to MLGs C6 and GWD in Guadeloupe and to C11 and MTB in mainland France. Although these genotypes appear to be restricted to two geographical areas, three of them might represent a threat for plant resistance durability because they develop as well on *Vat* as on non *Vat* melons (C11, GWD and MTB, Table 3). This strongly suggests that the ability of these particular genotypes in overcoming *Vat* effects is not associated with a fitness cost. The life history traits (fecundity, host plant range, ability to disperse) of these three genotypes needs to be assessed whilst population genetic surveying should be continued over several years to discern whether the frequency and distribution of these genotypes expands …or not, as the case may be.

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