DOI: 10.1111/j.1570-7458.2009.00904.x

Response of the melon aphid, *Aphis gossypii*, to host-plant resistance: evidence for high adaptive potential despite low genetic variability

Eric Lombaert[1]*, Jérôme Carletto[1], Christine Piotte[1], Xavier Fauvergue[1], Hervé Lecoq[2], Flavie Vanlerberghe-Masutti[1] & Laurent Lapchin[1]

[1]*UMR IBSV, INRA-UNSA-CNRS, 06903 Sophia-Antipolis, France, and* [2]*Unité de Pathologie Végétale, INRA, 84000 Avignon, France*

Accepted: 15 July 2009

Key words: cotton aphid, gene-for-gene interaction, *Vat* gene, genetic polymorphism, microsatellites, overcoming of host-plant resistance, phenotypic plasticity, pest control, Hemiptera, Aphididae

Abstract

In agrosystems, pests are submitted to strong human-imposed selective pressures to which they sometimes adapt rapidly, either through selection of genotypes resulting from mutation and/or recombination events, or through phenotypic plasticity. Understanding how insects respond to such selective pressures is of great importance for sustainable pest management strategies, such as the use of resistant plants. In this study, we investigated the genetic and phenotypic variability of anholocyclic *Aphis gossypii* Glover (Hemiptera: Aphididae) strains, in response to the resistance gene *Vat* that is present in melon crops. Forty-nine aphid colonies were sampled on several melon crops in southern France, genotyped using 15 microsatellite loci, and tested in phenotypic experiments using *Vat* or non-*Vat* melons. The level of genetic polymorphism between these colonies was low, as only seven multilocus genotypes were detected. In contrast, the phenotypic variability for life-history and behavioral traits between colonies, including those sharing the same genotype, was unexpectedly high, with a continuum of response to the *Vat* gene from complete susceptibility to strong virulence. The low genetic polymorphism associated with a strong phenotypic variability highlights the high adaptive potential of *A. gossypii* and the major role of environmental cues in shaping phenotypic responses of this aphid to pest management strategies.

Introduction

The cotton or melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a cosmopolitan species colonizing more than 600 host plants. It is the vector of more than 50 plant viruses and a major pest of many crops, including melon and other cultivated members of the Cucurbitaceae (Blackman & Eastop, 1984; Ebert & Cartwright, 1997). This aphid species is considered to be anholocyclic, that is, it reproduces continuously by apomictic parthenogenesis.

The adaptive potential of an insect is strongly linked to its mode of reproduction. Sexual reproduction provides ample opportunity to generate diversity through mutation and recombination, enabling populations to respond to environmental modifications. By contrast, asexual reproduction is considered an evolutionary dead end (Crow, 1994; Griffiths & Butlin, 1995), due to the loss of genetic variation and the accumulation of deleterious mutations (Muller, 1932; Halkett et al., 2005). Asexuality is likely to be particularly disadvantageous in highly unstable and stochastic anthropogenic environments, such as agroecosystems. However, some major agricultural pests, including *A. gossypii*, display obligate parthenogenesis. This type of reproduction, combined with strong selective pressure, should lead to strong reduction in levels of genetic polymorphism (Fuller et al., 1999), and coping with environmental heterogeneity may partly rely on adaptive phenotypic plasticity (reviewed in Roff, 2002).

Many insects, and especially aphids, are known to have adapted rapidly to recent strong selective pressures such as

*Correspondence: Eric Lombaert, INRA – Centre de Recherches de Sophia-Antipolis, 400 Route des Chappes, BP 167, 06 903 Sophia-Antipolis Cedex, France. E-mail: lombaert@sophia.inra.fr

insecticides (e.g., Denholm et al., 2002). Nevertheless, there have been few studies on the ability of aphids to overcome host-plant resistance, which is also a commonly used method in pest management (see Painter, 1951). Selection of biotypes has been described in the case of the aphid *Schizaphis graminum* (Rondani) that overcome sorghum resistance through increased pectin methylesterase activity in saliva (Dreyer & Campbell, 1984). The existence of pre-adapted biotypes has been suggested to be crucial for a rapid response of aphids to host-plant resistance (Porter et al., 1997; Goggin et al., 2001).

Aphis gossypii is a highly polyphagous species but several host races have been described, including a race on Cucurbitaceae (Carletto et al., 2009). This host race consists of *A. gossypii* genotypes that have specialized on cucurbits while remaining generalist within the plant family. For control of this pest in melon crops in southern France, a specific host-plant resistance conferred by a gene called *Vat* ['virus aphid transmission' (Pitrat & Lecoq, 1982)] has been used commercially for nearly 15 years and is now present in about 80% of French melon cultivars (Sauvion et al., 2005). *Vat* has three specific effects on *A. gossypii*, viz.: (1) antibiosis, modifying the life-history traits of the insects (decreasing their longevity, growth, and fecundity), (2) antixenosis, modifying insect behavior (having a negative effect on their settling behavior), and (3) complete and specific resistance of the plant to the transmission of non-persistent viruses by the aphids (Pitrat & Lecoq, 1980; Lecoq et al., 1981). The *Vat* gene is of high economic value and it is therefore important to evaluate the capacity of *A. gossypii* to overcome this resistance, and to assess the aphid's adaptive potential in the face of this recent selective pressure. *Vat* is a member of the NBS-LRR superfamily of plant resistance genes (Pauquet et al., 2004). Genes of this type are thought to be involved in gene-for-gene interactions, as has been shown in some pathosystems (Bogdanove, 2002; Belkhadir et al., 2004; McHale et al., 2006). The gene-for-gene concept, in which the resistance gene of the plant corresponds to an avirulence gene in the pathogen, may also be applied to plant-insect interactions (Kaloshian, 2004; Smith & Boyko, 2007). In this case, the response of the herbivore should be qualitative and binary, with either a failure (death or population with very low rate of increase) or a success (population with high rate of increase) of the settlement (Stahl & Bishop, 2000). Therefore, if *A. gossypii* populations were shown to overcome *Vat*, the most parsimonious hypothesis would be that a low level of genetic variability of this aphid should be associated with a low level of phenotypic variability.

In this study, we investigated the intraspecific genetic and phenotypic variability of *A. gossypii*, by comparing the response to *Vat* of 49 clonal lineages sampled on *Vat* and non-*Vat* melon plants in southern France. Genetic polymorphism was analyzed using 15 microsatellite loci, and we tested each of the three known effects of *Vat* (antibiosis, antixenosis, and resistance to transmission of non-persistent virus). The implications of our results in relation to the adaptation of insects to strong and recent selective pressures and to the development of future pest management methods are discussed.

Materials and methods

Aphid sampling and rearing

During the spring and summer of 2003 and 2004, we sampled 49 established aphid colonies from commercial melon crops, *Vat* and non-*Vat* cultivars, in southern France. The distance between two samples ranged from 0.1 to 115 km, the mean distance being 32 km. For each colony, 10–20 apterous individuals were collected from 1 to 3 leaves of a single plant; three were genotyped with microsatellite markers as described below and 5–10 others were kept alive on small cucumber seedlings [*Cucumis sativus* cv. Serit (Cucurbitaceae)] for phenotypic experiments. Once infested, the seedlings were maintained individually in small rectangular boxes ($6 \times 3 \times 10$ cm) to prevent cross-contamination and they were removed and replaced by new seedlings every 10 days. The rearing was done in duplicate, under controlled environmental conditions (20 °C, 70% r.h., and L16:D8). The genotype of each aphid colony was checked a second time just before the phenotypic experiments. The duration of this rearing (from sampling to experiments) varied considerably between colonies (27–369 days), depending on sampling date. Two laboratory clones were also used for the experiments: Lab 1 and Lab 9, collected in 1988 on cultivated Cucurbitaceae at two localities from southern France.

Measurement of genetic polymorphism

Genetic variability between the 51 aphid colonies (49 field colonies + 2 laboratory strains) was assessed using 15 microsatellite loci. DNA was extracted from individual aphids using 50 µl of a 5% (wt/vol) Chelex solution (Bio-Rad, Hercules, CA, USA). Amplifications were conducted in PCR reactions carried out under multiplex conditions. The forward primer for each microsatellite locus was labeled with a fluorescent dye (FAM, NED, PET, VIC) chosen to allow the simultaneous analysis of several microsatellite loci, which were distributed in two sets. The first set contained eight microsatellite loci specific to the *A. gossypii* genome (Vanlerberghe-Masutti et al., 1999), viz., Ago24-FAM, Ago53-VIC, Ago59-NED, Ago66-VIC, Ago69-NED, Ago84-PET, Ago89-PET, and Ago126-FAM. PCR reactions and identification of alleles were carried out

as described in Brévault et al. (2008). The second set contained five microsatellite loci originally designed for *Aphis fabae* Scopoli (Gauffre & D'Acier, 2006), viz., AF48-VIC, AF63-PET, AF82-PET, AF86-FAM, and AF153-NED, one locus originally designed for *Sitobion miscanthi* (Takahashi) (Wilson et al., 2004), S17b-FAM, and one locus originally designed for *Rhopalosiphum padi* (L.) (Simon et al., 2001), R5.10-NED. PCR reactions were performed under the same multiplex conditions as the first set of loci, except that 0.5 µl of Q-solution (Qiagen, Venlo, The Netherlands) was added to the PCR mix and the annealing temperature was 58 °C. The PCR products for each set of loci were separated and detected by capillary electrophoresis and automatic sequencing (3100 Genetic Analyzer, ABI, Foster City, CA, USA). Results were interpreted with STR and 2.2.241 software (Acid Nucleic Analysis Software, http://www.vgl.ucdavis.edu/informatics/strand.php), which determines the size of the allele at each microsatellite locus by comparison with the size standard. Each individual was assigned a multilocus genotype representing the combination of alleles for the 15 microsatellite loci.

Measurements of phenotypic variability

Effect of plant resistance on life-history and behavioral traits. A single experiment was conducted to assess the impact of *Vat* on *A. gossypii* colonies, evaluating both antibiosis (impact on life-history traits) and antixenosis (impact on behavioral traits) and determining the value of a global fitness indicator combining both mechanisms. The experiment had a factorial design, including two treatments: the melon cultivar (with two levels, corresponding to susceptible and resistant cultivars) and the aphid colony (with 51 levels, corresponding to the 51 colonies). Plants and aphids were prepared and all tests were carried out under controlled conditions (25 °C, 65% r.h., and L16:D8).

Before the experiment, 3–5 individuals of each aphid colony were taken from the cucumber seedlings and reared on cucumber leaf disks for three generations. This made it possible to maintain low densities, avoiding the production of winged aphids. For the experiment, two cultivars of *Cucumis melo* L. (Cucurbitaceae) were used at the five-leaf stage: 'Védrantais', which is susceptible to *A. gossypii* (+/+), and 'Margot', which is resistant (*Vat/Vat*). Each melon was planted and attached to a stake in a 0.4-1 plant pot. The soil used was Agrior No. 2; no fertilizer was added. For each of the 102 combinations of treatment levels, 20 replicates were used, giving a total of 2,040 tests. The experimental unit was the melon leaf: 4 leaves were used for each plant replica.

On the 1st day of the experiment, a single 2- to 3-day-old adult aphid was placed on the lower side of a leaf. We prevented the aphid from walking off the leaf by applying a ring of glue to the leaf petiole. Each leaf was orientated in different directions to prevent aphids from falling on the lower leaf. Residence time (a behavioral trait) was measured by determining the position of the adult aphid at 24, 48, and 72 h. The aphid was considered to be 'absent', if it was stuck in the glue or had dropped off the leaf. The 'residence-time' variable was the number of days that the adult stayed on the leaf. Fitness was roughly estimated by calculating the '3-day fecundity' variable, i.e., the number of larvae per adult counted 72 h after the initial infestation.

Effect of plant resistance on virus transmission. We evaluated the capacity of each aphid colony to transmit non-persistent viruses to *Vat* and non-*Vat* plants, using the two cultivars described above. It was possible to test only 50 colonies (48 field colonies +2 laboratory clones). Each aphid colony was multiplied on a young cucumber plant (2–3 leaf stage) for 1 month in an acrylic glass cage under controlled conditions (23 °C, 65% r.h., and L16:D8). During this month, a new cucumber plant was added in the cage on two occasions, to provide the aphids with favorable growth conditions. The day before the experiment, approximately 60 apterous adults of each colony were isolated on cucumber leaf disks.

There were two treatments in this experiment: melon cultivar (Védrantais or Margot) and aphid colony (50 colonies). A clone of the aphid *Myzus persicae* (Sulzer), which is known to transmit non-persistent virus on *Vat* plants (Lecoq et al., 1981), was used as a control for transmission on *Vat* melon. The non-persistent virus used was the 'Zucchini yellow mosaic virus' strain, ZYMV-E15 (Lecoq & Purcifull, 1992). The experimental unit was the melon seedling, grown to the 1st-leaf stage in a greenhouse before the experiment. Five replicates were performed for each set of conditions.

On the day of the experiment, aphids were starved for 1 h. They were then loaded with virus particles by allowing them to probe infected zucchini (*Cucurbita pepo* L.) leaves for 1.5 min. For each colony, three aphids were then immediately placed on the leaf of a non-infected melon seedling. Aphids were allowed to probe the melon leaf for 1–2 h. Then, aphids were eliminated by spraying every seedling with the insecticide Confidor® (0.5 ml l^{-1}; imidacloprid). Spraying was repeated about 12 h later. We checked for symptoms by visual examination 2–3 weeks after the experiment.

Data analysis

Due to the unbalanced nature of the data, statistical analyzes were performed in two steps. We first assessed the impact of the resistance gene *Vat* on the behavioral and

life-history traits of each colony. This made it possible to observe the variability of reaction norms (i.e., the set of phenotypes produced by one aphid colony in a range of environmental parameters; Schlichting & Pigliucci, 1998; Debat & David, 2001), and therefore, potentially, to detect colonies able to overcome *Vat*. We then explored the genetic and environmental factors with a potential effect on the variability of reaction norms for our fitness indicator, '3-day fecundity', which might account for the adaptive potential of the aphid.

Variability of the impact of resistance gene on residence-time and daily fecundity. In our analysis of behavioral traits (i.e., residence time), we evaluated the effects of melon cultivar (i.e., the test plant), colony, and their interaction on the 'residence-time' variable of each aphid, by fitting a generalized linear model to the data with a multinomial probability distribution and a cumlogit link function. The variable 'plant replica' was also added to the model.

For the analysis of life-history traits (i.e., daily fecundity), a similar approach was used, fitting a generalized linear model to the data, with a Poisson probability distribution and a log link function. The response variable was the '3-day fecundity' variable, but we excluded the effect of antixenosis by adding the 'residence-time' variable as an offset to the statistical model (an offset is a regression variable with a constant coefficient for each observation). This procedure is equivalent to that used for testing daily fecundity, but it avoids the need to analyze a ratio with a distribution less clear than that for counts. Finally, for both traits, we tested (1) for each aphid colony the null hypothesis that the two cultivars were the same, and (2) for the *Vat* cultivar the null hypothesis that the aphid colonies were the same.

Genetic and environmental factors responsible for the variability of 3-day fecundity. We used a generalized linear model with a Poisson probability distribution and a log link function to evaluate the factors best accounting for the variability of the fitness indicator (i.e., 3-day fecundity). The response variable was the '3-day fecundity' variable, and four main effects were tested: melon cultivar (Védrantais or Margot), aphid genotype (15-locus microsatellite genotype), the plant on which the colony was sampled in the field (i.e., plant of origin, either *Vat* or non-*Vat* melon), and the number of days for which the colony was reared on cucumber seedlings in the laboratory before being tested (i.e., rearing duration). The interactions between cultivar and aphid genotype, plant of origin, and rearing duration were introduced into the model to explore the factors responsible for variation in response to *Vat*.

A complementary analysis was carried out to determine whether colony variability within multilocus genotypes could be detected. A similar model was used, but with aphid genotype and colony nested within 'aphid genotype' as explanatory variables.

In each generalized linear model, we carried out type 3 analyzes. If the data were found to be slightly overdispersed, the covariance matrix was rescaled using a dispersion parameter estimated by dividing the deviance by the number of degrees of freedom. All statistical analyzes were performed with SAS software, version 9.1.3 (SAS Institute, 1999).

Results

Genetic variability

Genotyping revealed that aphids from the colony sampled on a particular plant in the field were characterized by the same multilocus genotype, and thus very likely corresponded to a single clone (i.e., each colony descended from a single individual). Seven multilocus genotypes were identified among the 49 colonies (Table 1). We found no evidence for genetic structuring of aphid colonies with respect to *Vat*: with the exception of two rare genotypes (C10 and Z2), all genotypes were found on both susceptible and resistant (*Vat*) plants. Three multilocus genotypes (C9, C11, and NM1) characterized three quarters of the sampled aphid colonies. The two laboratory clones, Lab 9 and Lab 1, originally collected from the field 20 years ago, had the C9 and NM1 multilocus genotypes, respectively.

Phenotypic variability

Phenotypic variability and reaction norms. High-phenotypic variability was observed for both the 'daily-fecundity' and 'residence-time' traits (Table 2). For both variables, we observed high levels of variability between aphid colonies, and a strong impact of plant cultivar (and thus of *Vat*). In addition, the significant aphid colony*plant cultivar interaction indicates that aphid colonies did not respond similarly to *Vat*, suggesting that the phenotypic plasticity of the response to the plant was genetically variable. The 'plant replica' factor was not significant. Concerning the 'virus transmission' trait, 85.2% of the aphids tested transmitted the ZYMV virus on non-*Vat* melon, as opposed to 1.2% on *Vat* melon. *Myzus persicae* efficiently transmitted virus particles on both non-*Vat* and *Vat* melon.

Overcoming the Vat *gene.* Of the 51 aphid colonies tested, 28 showed no significant difference in daily fecundity between *Vat* (resistant) and non-*Vat* (susceptible) melon plants (Table 3). For the other 23 aphid colonies, daily

Table 1 Multilocus genotypes of 49 aphid colonies and resistance profile of the plants on which colonies were sampled

			Microsatellite locus												
MLG	N	nV/V	Ago24	Ago53	Ago59	Ago66	Ago69	Ago84	Ago89	Ago126	s17b	AF86	AF48	AF153	AF63	R5.10	AF82
C9	23	12/11	153–157	116–116	182–182	152–152	109–114	112–118	150–150	176–176	138–148	218–218	302–302	281–300	300–300	243–243	210–221
C10	1	0/1	157–157	116–116	182–182	152–152	108–109	118–118	150–150	176–176	138–148	210–218	302–302	281–300	300–300	237–243	216–221
C11	7	2/5	153–157	116–116	182–200	152–152	109–109	108–112	150–150	176–176	138–148	210–218	302–302	281–300	300–300	232–243	204–210
NM1	7	6/1	153–153	113–116	184–217	152–156	109–115	116–116	150–158	166–177	137–139	210–218	302–302	281–281	300–310	239–239	210–214
Z2	4	4/0	153–153	110–116	147–182	147–152	114–116	118–118	150–164	176–176	143–148	218–235	302–303	281–290	295–300	243–247	195–210
Z6	5	4/1	153–157	110–116	147–182	147–152	109–116	118–118	150–152	176–176	144–148	218–239	302–302	281–290	292–300	243–248	193–221
Z7	2	1/1	113–157	110–116	147–182	147–152	109–116	118–118	150–152	176–176	144–148	218–239	302–302	281–290	292–300	243–248	193–221
Total	49	29/20															

Multilocus genotypes were based on the combinations of genotypes at 15 microsatellite loci. The size of each allele is indicated in base pairs.
MLG, multilocus genotype; N, no. colonies; nV, no. colonies sampled on non-*Vat* melon; *V*, no. colonies sampled on *Vat* melon.

Table 2 Generalized linear model testing the effect of *Vat* on two traits of 51 *Aphis gossypii* colonies

	Trait: daily fecundity			Trait: residence time		
	Test: Poisson regression			Test: multinomial regression		
Effect	χ^2	d.f.	P-value	χ^2	d.f.	P-value
Colony	890.78	50	<0.0001	209.42	50	<0.0001
Cultivar	281.84	1	<0.0001	522.25	1	<0.0001
Colony* cultivar	241.93	50	<0.0001	89.51	50	0.0005
Plant	12.51	8	0.1298	11.88	8	0.1565

fecundity was always higher on susceptible than on resistant melon. Only eight aphid colonies displayed no significant difference in residence time on the two cultivars. All the other aphid colonies stayed significantly longer on non-*Vat* than on *Vat* melon. Mean daily fecundity and mean residence time were positively correlated when paired over all aphid colonies (Spearman's rank correlation: r = 0.51, P<0.0001). Eight aphid colonies were not significantly affected by antixenosis and antibiosis. However, these aphid colonies performed differently on melon plants. Overall, strong differences in colony performance on the *Vat* cultivar were confirmed by statistical analyzes for both antibiosis (χ^2 = 460.1, d.f. = 50, P<0.0001) and antixenosis (χ^2 = 218.5, d.f. = 50, P<0.0001). In contrast, the blocking of non-persistent virus transmission by *Vat* was not overcome by any of the aphid colonies (Table 3).

Factors determining the response to the Vat *gene.* Statistical analyzes of the effects of other explanatory variables on the fitness indicator '3-day fecundity' are summarized in Table 4, model I. *Vat* (factor 'cultivar') was found to have a significant effect, confirming the results described above. In addition, genotype and rearing duration appeared to have a significant effect. Both interactions of these factors with cultivar were significant, indicating that the response to *Vat* was also linked to the genotype of the aphid and some of the aphid's previous experience (i.e., the duration of rearing on cucumber seedlings between the sampling in the field and the date of the experiment). With increasing time (which is correlated to the number of generations) spent on cucumber, the fitness indicator '3-day fecundity' decreased on susceptible melon (cv. Védrantais) and increased on *Vat* melon (cv. Margot) plants. The plant on which the colony aphid was sampled (*Vat* or non-*Vat*) had no significant effect on the variable '3-day fecundity'.

Genotype had a strong effect but, as can be seen in Figure 1, high variability was also observed between

Table 3 Daily fecundity (antibiosis), residence time (antixenosis), and ZYMV virus transmission rates for each of the 51 aphid colonies on non-*Vat* and *Vat* melon

Colony	Plant of origin	Genotype	Mean daily fecundity non-*Vat*/*Vat* melon	Antibiosis (P-value Poisson)	Mean residence time (day) non-*Vat*/*Vat* melon	Antixenosis (P-value multinomial)	% virus transmission rates non-*Vat*/*Vat* melon
3 81	*Vat*	C9	1.63/1.53	0.7842	2.10/1.40	0.1026	100/0
4 104	*Vat*	C9	6.07/6.80	0.5211	2.45/1.92	0.2363	100/0
3 90	*Vat*	C9	7.13/6.37	0.3808	3.05/2.45	0.136	100/0
3 99	*Vat*	C9	7.41/6.58	0.3287	2.80/2.10	0.1062	100/0
4 A15	Non-*Vat*	C9	6.91/5.90	0.3295	3.30/1.45	**<0.0001**	100/0
4 76	*Vat*	C9	7.61/6.52	0.3247	3.03/1.24	**<0.0001**	80/40
3 42	Non-*Vat*	C9	6.85/6.24	0.3075	3.20/2.25	**0.0134**	60/0
4 A1	Non-*Vat*	C9	8.21/7.26	0.2438	3.25/2.15	**0.0038**	100/0
3 91	*Vat*	C9	6.32/5.00	0.2072	3.05/1.50	**<0.0001**	100/0
4 X6	Non-*Vat*	C9	7.32/6.10	0.1297	3.15/2.05	**0.0114**	60/0
3 94	*Vat*	C9	4.52/3.07	0.1205	2.76/1.55	**0.003**	60/0
4 114	Non-*Vat*	C9	3.99/2.95	0.0828	3.40/2.55	**0.0127**	100/0
3 80	Non-*Vat*	C9	4.83/3.40	0.0718	2.65/1.65	**0.0256**	60/0
4 56	*Vat*	C9	9.25/6.86	**0.0276**	2.90/1.94	**0.003**	80/0
3 92	*Vat*	C9	7.00/5.38	**0.027**	3.35/2.00	**0.0003**	100/0
4 X8	Non-*Vat*	C9	6.65/4.43	**0.0256**	3.25/1.25	**<0.0001**	100/0
4 12	*Vat*	C9	6.97/5.31	**0.0141**	3.10/2.08	**0.0066**	80/0
4 111	Non-*Vat*	C9	6.49/4.25	**0.0044**	3.35/1.90	**0.0001**	60/0
4 A14	Non-*Vat*	C9	7.23/4.32	**0.001**	3.20/1.35	**<0.0001**	80/0
4 118	Non-*Vat*	C9	5.97/4.37	**0.0009**	3.35/2.65	**0.0315**	80/0
3 83	*Vat*	C9	7.97/5.32	**0.0003**	3.25/2.13	**0.0002**	60/0
4 105	Non-*Vat*	C9	5.71/2.93	**0.0003**	3.15/1.83	**0.0001**	100/0
4 X9	Non-*Vat*	C9	8.10/3.50	**0.0001**	2.71/0.70	**<0.0001**	100/0
Lab9	Lab strain	C9	7.53/4.74	**<0.0001**	3.18/1.64	**<0.0001**	100/0
4 A3	*Vat*	C10	4.39/3.67	0.421	2.80/1.44	**0.0026**	100/0
4 107	*Vat*	C11	3.36/2.65	0.2771	1.75/1.05	0.1168	100/0
3 98	*Vat*	C11	1.70/1.21	0.1363	2.30/2.10	0.7135	60/0
4 83	*Vat*	C11	3.67/3.65	0.9717	3.25/1.90	**0.0005**	60/0
3 61	*Vat*	C11	2.99/2.33	0.14	3.03/1.00	**<0.0001**	100/0
4 99	Non-*Vat*	C11	5.92/4.47	0.1248	3.05/1.50	**0.0003**	80/0
4 X10	Non-*Vat*	C11	3.56/2.62	0.1133	3.25/2.00	**0.0027**	80/0
4 109	*Vat*	C11	3.91/3.04	0.1036	3.25/2.24	**0.0017**	80/0
4 31	Non-*Vat*	NM1	3.52/4.06	0.3194	3.15/2.15	**0.0032**	80/0
4 X12	Non-*Vat*	NM1	6.76/4.76	**0.0028**	3.45/1.55	**<0.0001**	100/0
4 22	*Vat*	NM1	6.23/2.59	**0.0002**	3.40/0.66	**<0.0001**	80/0
4 106	Non-*Vat*	NM1	7.69/3.14	**<0.0001**	2.61/1.03	**0.0001**	100/20
3 88	Non-*Vat*	NM1	7.38/2.88	**<0.0001**	2.55/1.15	**0.0019**	80/0
Lab1	Lab strain	NM1	6.20/1.81	**<0.0001**	2.86/0.91	**<0.0001**	100/0
4 E1	Non-*Vat*	NM1	6.05/2.40	**<0.0001**	3.45/2.15	**<0.0001**	100/0
4 X18	Non-*Vat*	NM1	5.60/3.07	**<0.0001**	2.65/1.55	**0.0159**	80/0
4 A9	Non-*Vat*	Z2	3.31/3.36	0.9162	3.50/2.40	**0.0003**	100/0
4 103	Non-*Vat*	Z2	6.03/4.71	0.1196	2.95/2.03	**0.0133**	80/0
4 A11	Non-*Vat*	Z2	4.3/1.54	**0.0001**	2.65/0.70	**<0.0001**	80/0
4 A8	Non-*Vat*	Z2	3.94/1.08	**<0.0001**	2.95/0.75	**<0.0001**	-/-
4 X1	Non-*Vat*	Z6	4.05/3.78	0.6537	3.35/2.95	0.5065	80/0
3 62	*Vat*	Z6	3.45/2.53	0.1087	3.35/2.40	**0.0018**	60/0
4 X3	Non-*Vat*	Z6	7.49/1.88	**<0.0001**	3.15/0.87	**<0.0001**	100/0
4 112	Non-*Vat*	Z6	6.11/0.20	**<0.0001**	2.94/0.50	**<0.0001**	100/0

Table 3 (Continued)

Colony	Plant of origin	Genotype	Mean daily fecundity non-*Vat*/*Vat* melon	Antibiosis (P-value Poisson)	Mean residence time (day) non-*Vat*/*Vat* melon	Antixenosis (P-value multinomial)	% virus transmission rates non-*Vat*/*Vat* melon
4 11	Non-*Vat*	Z6	5.55/2.80	**<0.0001**	3.50/2.50	**<0.0001**	80/0
4 A2	*Vat*	Z7	3.35/4.07	0.2318	3.05/2.45	0.0674	100/0
4 113	Non-*Vat*	Z7	5.00/3.41	0.0533	3.13/1.18	**<0.0001**	80/0

Bold P-values indicate significant difference between the values of a given trait on the two cultivars.

colonies sharing the same multilocus genotype. This was confirmed by the statistical analyzes summarized in Table 4, model II, in which the variable 'aphid colony' nested within genotype was highly significant. Thus, different genotypes had different responses, but the same genotype did not necessarily display the same phenotype.

Discussion

Our results highlight the high adaptive potential of *A. gossypii* facing strong recent selective pressures. The high level of phenotypic variability observed is far from the binary response expected under the hypothesis of a gene-for-gene interaction underlying virulence/resistance. Our results show that genetic polymorphism alone cannot entirely account for phenotypic variability. The mismatch between genetic and phenotypic data illustrates the major role of environmental cues in shaping adaptive phenotypes.

Table 4 Generalized linear models testing (model I) the effect of various genetic and environmental factors, and (model II) the effect of nesting colony within genotype, on the 3-day fecundity of aphids

	3-day fecundity – Poisson regression		
Effect	χ^2	d.f.	P-value
Model I			
Var	113.61	1	<0.0001
Gen	169.61	6	<0.0001
Po	0.16	1	0.6915
Dur	6.18	1	0.0129
Var*gen	22.38	6	0.0010
Var*po	1.3	1	0.2541
Var*dur	5.97	1	0.0146
Model II			
Gen	122.36	6	<0.0001
Colony (gen)	211.37	44	<0.0001

Var, cultivar; gen, multilocus genotype; po, plant of origin; dur, rearing duration.

Seven multilocus genotypes were identified by microsatellite analyzes over 49 field-collected aphid colonies, and no genetic structure of aphid populations based on host-plant resistance conferred by *Vat* was observed. One multilocus genotype predominated, accounting for almost 50% of the samples. This very low level of genetic polymorphism was consistent with previous studies showing genetic specialization of *A. gossypii* on cucurbit plants (Fuller et al., 1999; Carletto et al., 2009). Other selective pressures, such as insecticide use, biological control, and/or the spatial structure of the environment (e.g., Zamoum et al., 2005; Lombaert et al., 2006; Brévault et al., 2008) may account for the low level of genetic polymorphism observed in this clonal species.

This study reveals the existence of field aphid colonies capable of overcoming *Vat*. In a similar system, some

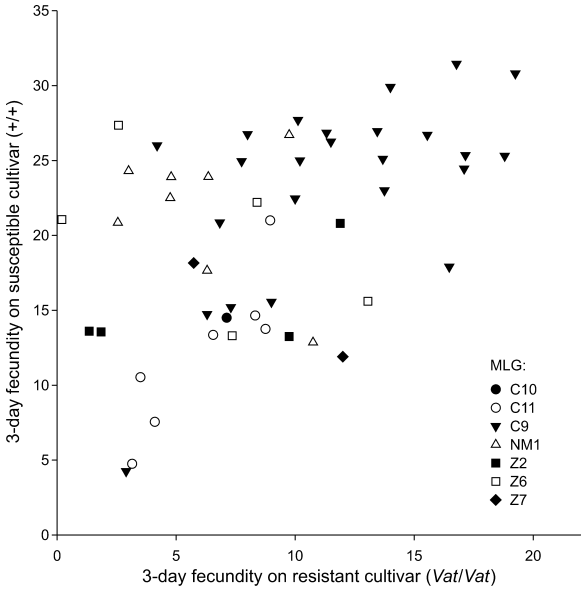

Figure 1 Phenotypic variability within multilocus genotypes. Each point is the mean 3-day fecundity of one colony on cultivars Margot (*Vat*/*Vat*) and Védrantais (+/+); MLG, multilocus genotype (see Table 1).

lineages of the potato aphid, *Macrosiphum euphorbiae* (Thomas), were found to overcome the *Mi* gene on tomato (Goggin et al., 2001; Hebert et al., 2007). In our system, antibiosis was overcome much more frequently than antixenosis, but the correlation between these two traits suggests that they may be governed by common mechanisms, although co-selection may also have occurred. *Vat* had a highly variable impact, depending on the aphid colony considered, with a continuum of responses observed, from extreme susceptibility to strong virulence (host-plant resistance completely overcome). High-phenotypic variability has previously been reported in aphids faced with various selective pressures, such as parasitoids (Ferrari et al., 2001) or host-plant species (Webster et al., 1992; Caillaud et al., 1995; Gorur et al., 2005). In this study, the variability observed for antibiosis and antixenosis traits was greater than expected for an NBS-LRR gene involved in interactions of the gene-for-gene type (Bogdanove, 2002). However, no variability and no overcoming of *Vat* resistance were observed for the trait 'virus transmission'. This suggests that antibiosis/antixenosis and resistance to the transmission of non-persistent viruses are driven by separate mechanisms. This confirms that the blockage of virus transmission is probably not linked to an impact of *Vat* on aphid behavior (Chen et al., 1997; Martin et al., 2003). It also suggests that *A. gossypii* is always effectively recognized by *Vat* melon plants, even if the antibiosis/antixenosis phenomenon is overcome.

We showed that genetic polymorphism could account partly for some of the observed continuum of phenotypes. The statistically significant interaction between aphid genotype and plant cultivar highlights the genetic variability of reaction norms and, thus, of the response to *Vat*. The genetic basis of the response of aphids to host-plant resistance has been described in other systems (e.g., Bournoville et al., 2000), in which the observed phenotypic variability closely matched genetic variability. In contrast, genetics does not provide a complete explanation in our study, as we observed highly significant trait variability within multilocus genotypes. We may have used too few microsatellites in this study to assess overall genetic polymorphism accurately. We tested this hypothesis, by plotting genotypic diversity against the number of loci, using MULTILOCUS software (that randomly samples from 1 to 14 loci from the dataset and calculates the number of different genotypes and the genotypic diversity; Agapow & Burt, 2001). Our analyzes were highly discriminating, as four loci were needed, on average, for the detection of six genotypes, and 15 loci detected only one additional genotype (data not shown). However, *Vat* has exerted a selective pressure for only 15 years and a virulence gene may have recently emerged and been selected for, while no mutation has yet occurred at the 15 microsatellite loci, which are considered to be selectively neutral. Therefore, it might not be possible to discriminate susceptible and virulent colonies on the basis of their multilocus genotypes. However, this is not the case for another recent selective pressure viz., that from insecticides. Indeed, Brévault et al. (2008) revealed that insecticide-susceptible and -resistant *A. gossypii* individuals displayed different multilocus genotypes.

Plant of origin had no effect on phenotypic variability, in contrast to the duration of laboratory rearing on cucumber. Longer durations of rearing on cucumber were associated with better performance of the aphid colony on *Vat* plants and poorer performance on non-*Vat* plants. Polyphagous aphids must modify their strategies for resource acquisition when they encounter a new plant species/cultivar (Francis et al., 2006; Lombaert et al., 2006). We can therefore assume that the use of cucumber plants in our rearing method has some similarity with the use of *Vat* melon.

Several hypotheses can be proposed to explain the mismatch between genetic and phenotypic data. Firstly, aphids may be subject to genetic selection on cucumber plants. Some colonies were reared in the laboratory for almost a year before the experiment, corresponding to about 40 generations. Clonal species, such as aphids, may evolve much more rapidly than was previously thought (Lushai et al., 2003; Wilson et al., 2003). However, the rearing procedure involved a very small population of aphids, greatly reducing the likelihood of mutant aphid development, and particularly that of mutant aphids developing independently in several colonies. Secondly, there may have been a gradual change in the microbial symbiont flora. Aphids harbor several secondary (or facultative) symbiotic bacteria affecting aphid fitness, including adaptation to the host plant (Adams & Douglas, 1997; Wilkinson et al., 2001; Tsuchida et al., 2004). Thirdly, there may be epigenetic control of phenotypic plasticity, possibly extending over several generations, through maternal effects (Bernardo, 1996; Mousseau & Fox, 1998). Phenotypic plasticity is known to be involved in the evolution of trophic interactions (Nylin & Gotthard, 1998; Agrawal, 2001) and is well documented for various aphid traits (Kawada, 1988; Field & Blackman, 2003; Halkett et al., 2004; Mondor et al., 2005). Plasticity associated with host selection has also been described in other phytophagous insects (De Souza et al., 2001; Agrawal et al., 2002; Lazarevic et al., 2002; Spitzer, 2004). Studies on this topic are still rare for aphids (but see Via, 1991), but phenotypic plasticity may be the cornerstone of host-plant adaptation in *A. gossypii*, which may be considered a generalist pest on plants of

the cucurbit family (e.g., Fuller et al., 1999). The variability of reaction norms found in this study implies that plasticity has a genetic basis, and therefore could theoretically be influenced by natural or artificial selection (Pigliucci, 2005). More generally, phenotypic plasticity may have first played a major role in the pre-adaptation of *A. gossypii* to various plant families. However, the cost of maintaining a high level of phenotypic plasticity (DeWitt et al., 1998) for insects to attack plants from different families may have been too great, leading to genetic specialization through genetic assimilation (Pal & Miklos, 1999; Pigliucci et al., 2006).

Our findings show that *A. gossypii* has high adaptive potential, despite its low genetic polymorphism, highlighting the potential importance of extended phenotypic plasticity. We showed that patterns associated with gene-for-gene interactions are less obvious when phenotypic plasticity is involved. Overall, the arms race between human-imposed selective pressures and the short-term adaptation of crop pests may be far more complex than previously thought. This opens up new perspectives in the management of insect resistance, which is usually based on adaptive genetic polymorphism in the target species (Roush, 1993; Gould, 1995, 1998).

Acknowledgements

We thank Michèle Salles, Aurélie Blin, and Catherine Rys for technical assistance with the laboratory experiments, Roger Boll and François-Xavier Moratin for help with aphid sampling, and Nathalie Boissot and Catherine Dogimont, who kindly provided the melon seeds. This work was supported by the 'Santé des Plantes et Environnement' Department of the 'Institut National de la Recherche Agronomique' and by the Agriculture Council of the French region Provence Alpes Côte d'Azur.

References

Adams D & Douglas AE (1997) How symbiotic bacteria influence plant utilisation by the polyphagous aphid, *Aphis fabae*. Oecologia 110: 528–532.

Agapow P-M & Burt A (2001) Indices of multilocus linkage disequilibrium. Molecular Ecology Notes 1: 101–102.

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294: 321–326.

Agrawal AA, Vala F & Sabelis MW (2002) Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: adaptive plasticity? The American Naturalist 159: 553–565.

Belkhadir Y, Subramaniam R & Dangl JL (2004) Plant disease resistance protein signaling: NBS-LRR proteins and their partners. Current Opinion in Plant Biology 7: 391–399.

Bernardo J (1996) Maternal effects in animal ecology. American Zoologist 36: 83–105.

Blackman RL & Eastop VF (1984) Aphids on the World's Crops: An Identification and Information Guide. Wiley, Chichester, UK.

Bogdanove AJ (2002) Protein–protein interactions in pathogen recognition by plants. Plant Molecular Biology 50: 981–989.

Bournoville R, Simon JC, Badenhausser I, Girousse C, Guilloux T & Andre S (2000) Clones of pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae) distinguished using genetic markers, differ in their damaging effect on a resistant alfalfa cultivar. Bulletin of Entomological Research 90: 33–39.

Brévault T, Carletto J, Linderme D & Vanlerberghe-Masutti F (2008) Genetic diversity of the cotton aphid *Aphis gossypii* in the unstable environment of a cotton growing area. Agricultural and Forest Entomology 10: 215–223.

Caillaud CM, Dedryver CA, Dipietro JP, Simon JC, Fima F & Chaubet B (1995) Clonal variability in the response of *Sitobion avenae* (Homoptera, Aphididae) to resistant and susceptible wheat. Bulletin of Entomological Research 85: 189–195.

Carletto J, Lombaert E, Chavigny P, Brévault T, Lapchin L & Vanlerberghe-Masutti F (2009) Ecological specialization of the aphid *Aphis gossypii* Glover on cultivated host plants. Molecular Ecology 18: 2198–2212.

Chen J-Q, Martin B, Rahbe Y & Fereres A (1997) Early intracellular punctures by two aphid species on near-isogenic melon lines with and without the virus aphid transmission (Vat) resistance gene. European Journal of Plant Pathology 103: 521–536.

Crow JF (1994) Advantages of sexual reproduction. Developmental Genetics 15: 205–213.

De Souza ALT, Tanaka MO, Fernandes GW & Figueira JEC (2001) Host plant response and phenotypic plasticity of a galling weevil (*Collabismus clitellae*: Curculionidae). Austral Ecology 26: 173–178.

Debat V & David P (2001) Mapping phenotypes: canalization, plasticity and developmental stability. Trends in Ecology and Evolution 16: 555–561.

Denholm I, Devine GJ & Williamson MS (2002) Evolutionary genetics – Insecticide resistance on the move. Science 297: 2222–2223.

DeWitt TJ, Sih A & Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution 13: 77–81.

Dreyer DL & Campbell BC (1984) Association of the degree of methylation of intercellular pectin with plant resistance to aphids and with induction of aphid biotypes. Cellular and Molecular Life Sciences 40: 224–226.

Ebert TA & Cartwright B (1997) Biology and ecology of *Aphis gossypii* Glover (Homoptera: Aphididae). Southwestern Entomologist 22: 116–153.

Ferrari J, Müller CB, Kraaijeveld AR & Godfray HCJ (2001) Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. Evolution 55: 1805–1814.

Field LM & Blackman RL (2003) Insecticide resistance in the aphid *Myzus persicae* (Sulzer): chromosome location and

epigenetic effects on esterase gene expression in clonal lineages. Biological Journal of the Linnean Society 79: 107–113.

Francis F, Gerkens P, Harmel N, Mazzucchelli G, De Pauw E & Haubruge E (2006) Proteomics in *Myzus persicae*: effect of aphid host plant switch. Insect Biochemistry and Molecular Biology 36: 219–227.

Fuller SJ, Chavigny P, Lapchin L & Vanlerberghe-Masutti F (1999) Variation in clonal diversity in glasshouse infestations of the aphid, *Aphis gossypii* Glover in southern France. Molecular Ecology 8: 1867–1877.

Gauffre B & D'Acier AC (2006) New polymorphic microsatellite loci, cross-species amplification and PCR multiplexing in the black aphid, *Aphis fabae* Scopoli. Molecular Ecology Notes 6: 440–442.

Goggin FL, Williamson VM & Ullman DE (2001) Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the tomato resistance gene *Mi*. Environmental Entomology 30: 101–106.

Gorur G, Lomonaco C & Mackenzie A (2005) Phenotypic plasticity in host-plant specialisation in *Aphis fabae*. Ecological Entomology 30: 657–664.

Gould F (1995) Comparisons between resistance management strategies for insects and weeds. Weed Technology 9: 830–839.

Gould F (1998) Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annual Review of Entomology 43: 701–726.

Griffiths HI & Butlin RK (1995) A timescale for sex versus parthenogenesis – evidence from subfossil ostracods. Proceedings of the Royal Society of London, Series B 260: 65–71.

Halkett F, Harrington R, Hulle M, Kindlmann P, Menu F et al. (2004) Dynamics of production of sexual forms in aphids: theoretical and experimental evidence for adaptive 'coin-flipping' plasticity. The American Naturalist 163: E112–E125.

Halkett F, Simon JC & Balloux F (2005) Tackling the population genetics of clonal and partially clonal organisms. Trends in Ecology and Evolution 20: 194–201.

Hebert SL, Jia LL & Goggin FL (2007) Quantitative differences in aphid virulence and foliar symptom development on tomato plants carrying the *Mi* resistance gene. Environmental Entomology 36: 458–467.

Kaloshian I (2004) Gene-for-gene disease resistance: bridging insect pest and pathogen defense. Journal of Chemical Ecology 30: 2419–2438.

Kawada K (1988) Polymorphism and morph determination. Aphids: Their Biology, Natural Enemies and Control (ed. by AK Minks & P Harrewijn), pp. 255–268. Elsevier, Amsterdam, The Netherlands.

Lazarevic J, Peric-Mataruga V, Stojkovic B & Tucic N (2002) Adaptation of the gypsy moth to an unsuitable host plant. Entomologia Experimentalis et Applicata 102: 75–86.

Lecoq H & Purcifull DE (1992) Biological variability of potyviruses, an example – zucchini yellow mosaic virus. Archives of Virology 5(Suppl.): 229–234.

Lecoq H, Pitrat M & Pansart M-J (1981) La résistance au puceron du melon et son interaction avec le virus chez le melon. Journées d'études et d'informations – Les pucerons des cultures (ed. by B Hurpin), pp. 313–317. Association de Coordination Technique Agricole, Paris, France.

Lombaert E, Boll R & Lapchin L (2006) Dispersal strategies of phytophagous insects at a local scale: adaptive potential of aphids in an agricultural environment. BMC Evolutionary Biology 6: 1–13.

Lushai G, Loxdale HD & Allen JA (2003) The dynamic clonal genome and its adaptive potential. Biological Journal of the Linnean Society 79: 193–208.

Martin B, Rahbe Y & Fereres A (2003) Blockage of stylet tips as the mechanism of resistance to virus transmission by *Aphis gossypii* in melon lines bearing the *Vat* gene. Annals of Applied Biology 142: 245–250.

McHale L, Tan XP, Koehl P & Michelmore RW (2006) Plant NBS-LRR proteins: adaptable guards. Genome Biology 7: 212; doi: 10.1186/gb-2006-7-4-212.

Mondor EB, Rosenheim JA & Addicott JF (2005) Predator-induced transgenerational phenotypic plasticity in the cotton aphid. Oecologia 142: 104–108.

Mousseau TA & Fox CW (1998) The adaptive significance of maternal effects. Trends in Ecology and Evolution 13: 403–407.

Muller HJ (1932) Some genetic aspects of sex. The American Naturalist 66: 118–138.

Nylin S & Gotthard K (1998) Plasticity in life-history traits. Annual Review of Entomology 43: 63–83.

Painter RH (1951) Insect Resistance in Crop Plants. The University Press of Kansas, Lawrence, KS, USA.

Pal C & Miklos I (1999) Epigenetic inheritance, genetic assimilation and speciation. Journal of Theoretical Biology 200: 19–37.

Pauquet J, Burget E, Hagen L, Chovelon V, Le Menn A et al. (2004) Map-based cloning of the *Vat* gene from melon conferring resistance to both aphid colonization and aphid transmission of several viruses. Progress in Cucurbit Genetics and Breeding Research – Proceedings of the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding (ed. by A Lebeda & HS Paris), pp. 325–329. Olomouc, Czech Republic.

Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? Trends in Ecology and Evolution 20: 481–486.

Pigliucci M, Murren CJ & Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. Journal of Experimental Biology 209: 2362–2367.

Pitrat M & Lecoq H (1980) Inheritance of resistance to cucumber mosaic virus transmission by *Aphis gossypii* in *Cucumis melo*. Phytopathology 70: 958–961.

Pitrat M & Lecoq H (1982) Relations génétiques entre les résistances par non-acceptation et antibiose du melon *Aphis gossypii*. Agronomie 2: 503–508.

Porter DR, Burd JD, Shufran KA, Webster JA & Teetes GL (1997) Greenbug (Homoptera: Aphididae) biotypes: selected by resistant cultivars or preadapted opportunists? Journal of Economic Entomology 90: 1055–1065.

Roff DA (2002) Life History Evolution. Sinauer Associates, Sunderland, MA, USA.

Roush RT (1993) Occurrence, genetics and management of insecticide resistance. Parasitology Today 9: 174–179.

SAS Institute (1999) SAS/Stat User's Guide, Version 9.1.3. SAS Institute, Cary, NC, USA.

Sauvion N, Mauriello V, Renard B & Boissot N (2005) Impact of melon accessions resistant to aphids on the demographic potential of silverleaf whitefly. Journal of Economic Entomology 98: 557–567.

Schlichting CD & Pigliucci M (1998) Phenotypic Evolution, A Reaction Norm Perspective. Sinauer Associates, Sunderland, MA, USA.

Simon J-C, Leterme N, Delmotte F, Martin O & Estoup A (2001) Isolation and characterization of microsatellite loci in the aphid species, *Rhopalosiphum padi*. Molecular Ecology Notes 1: 4–5.

Smith CM & Boyko EV (2007) The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomologia Experimentalis et Applicata 122: 1–16.

Spitzer BW (2004) Maternal effects in the soft scale insect *Saissetia coffeae* (Hemiptera: Coccidae). Evolution 58: 2452–2461.

Stahl EA & Bishop JG (2000) Plant-pathogen arms races at the molecular level. Current Opinion in Plant Biology 3: 299–304.

Tsuchida T, Koga R & Fukatsu T (2004) Host plant specialization governed by facultative symbiont. Science 303: 1989.

Vanlerberghe-Masutti F, Chavigny P & Fuller SJ (1999) Characterization of microsatellite loci in the aphid species *Aphis gossypii* Glover. Molecular Ecology 8: 693–695.

Via S (1991) Specialized host plant performance of pea aphid clones is not altered by experience. Ecology 72: 1420–1427.

Webster JA, Inayatullah C & Fargo WS (1992) Variation in fecundity of greenbug (Homoptera, Aphididae) biotypes on resistant and susceptible barley. Journal of Economic Entomology 85: 2023–2026.

Wilkinson TL, Adams D, Minto LB & Douglas AE (2001) The impact of host plant on the abundance and function of symbiotic bacteria in an aphid. Journal of Experimental Biology 204: 3027–3038.

Wilson ACC, Sunnucks P & Hales DF (2003) Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). Biological Journal of the Linnean Society 79: 115–135.

Wilson ACC, Massonnet B, Simon JC, Prunier-Leterme N, Dolatti L et al. (2004) Cross-species amplification of microsatellite loci in aphids: assessment and application. Molecular Ecology Notes 4: 104–109.

Zamoum T, Simon JC, Crochard D, Ballanger Y, Lapchin L et al. (2005) Does insecticide resistance alone account for the low genetic variability of asexually reproducing populations of the peach-potato aphid *Myzus persicae*? Heredity 94: 630–639.