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OVERVIEW

Micro-evolutionary change in relation to insecticide resistance in the peach–potato aphid, *Myzus persicae*

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Abstract. 1. Phenotypic diversity is the fuel that powers evolution.

2. Asexual organisms rely on mutation whereas sexual organisms combine mutation with recombination.

3. Few organisms provide examples of species that are both sexual and asexual, but aphids do.

4. To examine evolution on perceptible timescales requires strong evolutionary forces and, as Darwin noted, agricultural practices provide strong selection. In the case of aphids, insecticides provide a considerable force in the elimination of genotypes.

5. Insecticide resistance in *Myzus persicae* (Sulzer) has arisen independently through point mutation and gene amplification on a number of occasions and at different times. Resistance to organophosphates, pyrethroids, and pirimicarb (a dimethyl carbamate) is now widespread.

6. In this paper, we examine these three elements: sexual recombination, clonal expansion, and insecticide selection in the peach–potato aphid *M. persicae* in relation to the evolution of insecticide resistance and survival of the fittest clone.

Key words. Adaptation, aphid, clone, insecticide resistance, *Myzus persicae*, population genetics.

Introduction

Working at a time before DNA was discovered, or genetics was understood, Darwin spent most of his life looking for examples of evolution in action. He proposed a process whereby organisms exhibited natural phenotypic variation and this variation sometimes conferred an advantage, but only to some individuals. Such an advantage ensured the organism's survival to reproduction and as a consequence, an increased chance of contributing genes to the next generation. Darwin had the insight to see how, over extended periods of time, this process would lead to differentiated forms of an organism and eventually to speciation. The organisms that Darwin studied were mainly composed of sexual populations where the effects of selection normally take a long time. Darwin also noted how selective breeding could alter domesticated forms of animals on a perceptible timescale. An example of what Darwin was looking for can be found in aphids which respond rapidly to selection as they reproduce both sexually and asexually (Dixon, 1998; Simon *et al*., 2002). The sexual stages provide the opportunity for recombination to generate novel gene combinations, incorporating any new mutations along the way. In the presence of selection, asexual stages provide a means of rapidly amplifying successful gene combinations. We will use the peach–potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), as an example of this rapid selection. The life cycle of this species involves sexual forms, produced every autumn, which mate and lay overwintering eggs on the primary woody host, peach, *Prunus persica* (L.) (van Emden *et al*., 1969). Asexual forms are produced for the remainder of the year, although in some areas with relatively few peach trees, such as the U.K. (Blackman, 1971, Kasprowicz *et al*., 2008a,b) or central and southern Greece (Margaritopoulos *et al*., 2002; Blackman *et al*., 2007), asexual forms can predominate. In some cases, asexual reproduction predominates on summer crops despite the presence of peach orchards. In Caserta, southern Italy, where tobacco fields are located near peach orchards, the tobacco-adapted populations, *Myzus persicae nicotianae* Blackman (Eastop & Blackman, 2005), consist of asexual genotypes

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while the individuals on peach lack the genetic capability to colonise tobacco and migrate to other crops (Margaritopoulos *et al*., 2003; Blackman *et al*., 2007). In *M. persicae*, individuals with successful genes and gene combinations can increase quickly through asexual clonal expansion. These clones can remain over many seasons as permanent asexual populations, but these will have little (functional parthenogens = facultative forms; see Appendix) or no ability (obligate parthenogens = anholocyclic forms) to contribute their successful genes to the gene pool (Blackman, 1971, 1972; Poupoulidou *et al*., 2006). Alternatively, successful holocyclic clones (i.e. with an autumnal sexual phase) generated during one season will be more numerous at the end of that season and they will generate many sexual stages, resulting in their genes being passed on to more zygotes. For *M. persicae* one of the main contributions to its success has been the rapid evolution and spread of insecticide resistance genes into many genotypes and populations (Devonshire *et al*., 1998; Foster *et al*., 2000). In these papers the authors describe how this species has evolved several different types of insecticide resistance. A metabolic mechanism, known as esterase resistance, confers strong resistance primarily to organophosphates by the overproduction of carboxylesterases that sequester or degrade insecticide esters before they reach their target sites in the insect nervous system. Another, target-site mechanism, known as MACE (modified acetylcholinesterase), confers virtual immunity specifically to the dimethyl-carbamate, pirimicarb. Two more closely-related target-site mechanisms, known as *kdr* and super-*kdr* (*kdr* is a knock-down mechanism conferring resistance to pyrethroids, see the Appendix for more details), confer strong resistance to pyrethroid insecticides. In this paper, we review the latest research on the population structure and adaptive physiology of this major pest species in relation to insecticide resistance.

Materials and methods

Aphid samples

The data set consisted of 215 aphid lineages (microsatellite 'multilocus genotypes' or MLGs) collected from 14 countries in four continents mainly from peach and tobacco but other herbaceous hosts were included (Table 1). To obtain these unique genotypes, thousands of individuals had been sampled and analysed in the various study areas over a 10-year period, mostly from crops, but also from 12.2 m high suction traps. Most lineages were reared parthenogenetically under laboratory conditions and specimens from each lineage were stored at -80°C or in tubes filled with absolute ethanol (room temperature) until DNA extraction. Some of the samples consisted of a single aphid collected directly from the source tree, plant or suction trap and stored as above.

DNA extraction and microsatellite genotyping

Details on DNA extraction, microsatellite loci amplification, analysis and visualisation are presented in a previous paper (Malloch *et al*., 2006). Six microsatellite loci, M35, M40, M49, M63, M86, and myz9 (Sloane *et al*., 2001), were chosen on the basis of their resolution (based on allele numbers of 12, 11, 35, 19, 21, and 18, respectively, giving 2.43×10^{13} possible combinations). Many of the lineages had been analysed in earlier work (e.g. Fenton *et al*., 2003; Margaritopoulos *et al*., 2009). However, for this study, additional samples from Chile, Turkey, and Greece were included.

Hardy–Weinberg equilibrium

Deviation from Hardy–Weinberg equilibrium (HWE) at each locus was examined separately using the U-test (Raymond & Rousset, 1995). A Markov chain (MC) method is used for the unbiased estimation of the exact P-value of this test (Guo & Thompson, 1992). A multi-sample score test (Raymond & Rousset, 1995), which is performed by MC algorithm, was used as a global test across loci. These two tests were performed using GENEPOP version 4.0.

Bayesian clustering analysis

A Bayesian clustering method (Pritchard *et al*., 2000) as implemented in the program STRUCTURE version 2.2 was used to infer the number of K unknown genetic populations in which the sampled multilocus genotypes can be split. This model-based Bayesian method also assigns a probability that an individual belongs to a discrete population or to more than one population when it is admixed. The data set was analysed using admixture (each individual draws some fraction of its genome from each of the K populations) and no admixture (individuals are discretely from one population or another) ancestry models and K values 1–10 without incorporating population information. Also, two models for the allele frequencies were used for each of the two ancestry models. The independent allele frequency model assumes that the allele frequencies in each population are independently drawn from a distribution. The correlated allele frequency model assumes that allele frequencies in the different populations are likely to be similar (probably due to migration or shared ancestry). Ten independent runs for each K value were conducted with 100 000 iterations after a burn-in period of 20 000 iterations in each run.

Growth measurements

From colonies grown for at least 10 asexual generations on oilseed rape (OSR), 20 one-day-old nymphs of each lineage were placed onto 20 individual oilseed rape seedlings (2 weeks old). The seedling and pot were covered with a clear Perspex® tube (8 cm external diameter × 7 cm internal diameter × 16 cm length; Stockline Plastics, Glasgow, U.K.). To allow air-flow, each tube was capped with a thin mesh (mesh size 200 μm, John Lewis, Edinburgh, U.K.) held in place with a strong rubber band. The pots were then placed in a controlled environment room at 18°C ± 2°C in a LD 16:8 h cycle for 15 days. The experiment was terminated by cutting each plant at its base and placing the plant and the aphid colony into

Table 1. *Myzus persicae* lineages used in the study.

Region	Collection year	Locality	Crop	Colour		Total
				Green	Red	
Canada	2005	—	Pepper	1	1	2
Argentina	1993	—	Peach	1	—	1
Chile	2005	El molle	Weed		1	1
Chile	2008	Colina, Santiago	Potato	10	—	10
U.K. (Scotland)	1995, 2001–2004	Various	Bsprt, cabbage, oilseed rape, potato	14	3	17
U.K. (England)	1987, 1991, 1997, 1999, 2002–2003	Lincs, Yorks, Kent Herts, Suffolk, Cambs, Norfolk	Bsprt, cabbage, oilseed rape, sugar beet, potato	7	2	9
North Greece	2006	Meliki	Peach	20	0	20
North Greece	2006	Meliki	Tobacco	8	1	9
Eastern central Greece	2006	Lechonia	Peach	19	—	19
South Greece	2005	Naphplion	Tobacco	1	4	5
Greece	2008	—	—	4	—	4
France	2001, 2003	Bellegarde, Nimes	Peach	53	5	58
France	2001	Nimes	Weeds	4	—	4
Germany	1999	—	Tobacco	—	2	2
Slovenia	2006	Ljubljana, Krosko	Pepper, potato	16	—	16
Spain	1999	Madrid	Tobacco	1	2	3
Spain	1992	—	Pepper	1	—	1
Turkey	1998	—	Pepper	1	0	1
Turkey	2008	—	—	3	—	3
Japan	1993	Kyoto	Peach	1	—	1
Japan	1982 1997	Kyoto, Funehiki	Tobacco	—	2	2
Japan	1995, 2001	Kyoto	Radish, potato	1	1	2
Sri Lanka	2006	Sri Lanka	Weed	2	—	2
Australia	2005	—	Peach	1	—	1
Australia	2005	—	Potato	1	—	1
New Zealand	2005	Lincoln, Christchurch, Dorie, Pukekohe, Rakaia, Ashburton, Pukekohe	Potato	21	—	21
			Total	191	24	215

Samples and their sources used for population genetic analysis. Many of these were described in Margaritopoulos *et al*. (2009), but additional samples from Chile, Greece and Turkey were included in the current work.
Bsprt, Brussels sprout.

screw-topped tubs (6.5 cm × 7.5 cm; VWR, U.K.). These were frozen and stored at −20°C until the colony could be counted by carefully disassembling each plant.

Results and discussion

Sexual populations

Sexual populations of *M. persicae* have been studied from different regions of the world including France (Fenton *et al*., 2003; Guillemaud *et al*., 2003), Australia (Wilson *et al*., 2002; Vorburger *et al*., 2003a) and Greece (Blackman *et al*., 2007; Margaritopoulos *et al*., 2007a). In the current work we present the detailed results of two populations from France and Chile (Tables 2 and 3). The French population was collected from samples in a peach orchard in spring 2002, a time when the population would have completed only one or two asexual generations after emerging from eggs and were chosen as they should represent individuals with the maximum genetic diversity. From an understanding of the life cycle it was hypothesised that this population would be composed of many distinct genotypes but clonal expansion would amplify genotypes on the leaves and trees where they hatched. Where more than one egg was present there would be a mixture of clones. Microsatellite analysis of 103 individuals from 21 trees revealed 37 distinct genotypes with the number of individuals of any one clone ranging from one to eight. Each member of a clone was found on the same tree, but some trees had more than one MLG. Thus the population structure supports the above. The gene frequencies in this population were found to be in HWE apart from M40, which displayed a significant increase in homozygotes (see Table 4 and Margaritopoulos *et al*., 2009). Therefore, it appears that the process of producing independently migrating males and

Table 2. Microsatellite multilocus genotypes (MLGs) of *Myzus persicae* individuals collected on one occasion in spring (2002) from a single peach orchard near Nîme, France.

	Locus											
	M49		M63		M86		M35		M40		myz9	
Clone (tree)	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1E.1 (1)	137	137	170	181	139	139	188	188	**127**	**127**	195	195
1E.A (1)	166	170	174	181	143	143	188	198	**120**	**120**	220	220
1E.D (1)	149	149	166	172	115	126	188	198	120	125	195	207
1 W.2E (2)	141	170	172	174	115	139	188	188	**122**	**122**	208	220
2E.1E (3)	164	166	172	181	143	143	188	188	**120**	**120**	220	220
2E.B (3)	137	141	170	172	141	143	182	188	125	133	220	220
2 W.1 (4)	151	209	170	172	115	145	188	188	127	133	220	220
2 W.1E (4)	201	207	172	174	115	143	190	198	122	133	195	222
2 W.2E (4)	149	166	170	181	97	139	188	188	127	133	207	208
3E.C (5)	137	211	178	181	115	143	188	188	122	127	207	220
3 W.B (6)	149	168	170	170	139	143	188	188	122	127	207	226
4 W.1E (7)	137	149	170	172	115	154	188	188	122	133	207	220
4 W.2E (7)	137	151	172	181	97	141	188	188	127	133	220	220
4 W.F (7)	141	149	172	181	139	143	188	188	122	133	207	214
5 W.1 (8)	149	166	170	172	99	139	188	188	122	133	195	220
5 W.1E (8)	149	166	164	170	139	143	188	198	125	125	195	222
5 W.C (8)	166	166	166	170	99	115	188	198	120	122	220	220
6 W.1E (9)	149	166	172	174	139	139	188	188	122	127	207	207
6 W.B (9)	137	137	172	172	113	139	188	188	122	120	208	214
7 W.1 (10)	149	170	172	181	115	143	188	188	**127**	**127**	195	208
7 W.B (10)	149	170	172	181	115	143	188	188	122	133	207	218
W2*1.1 (11)	151	166	170	170	141	143	188	188	127	133	195	222
W2*1.2E (11)	151	166	170	170	141	143	188	188	127	133	207	207
W2*13.1 (12)	149	149	172	181	139	143	188	188	127	133	208	220
W2*18.1E (13)	149	149	164	181	143	143	188	188	122	127	195	222
W2*4.1 (14)	149	170	166	181	126	143	182	188	120	133	207	207
W2*4.1E (14)	149	149	170	172	97	101	188	198	**122**	**122**	220	220
W2*9.1 (15)	135	149	174	183	139	139	188	198	120	127	220	220
W3*1.1 (16)	151	166	166	170	113	143	188	198	**120**	**120**	218	226
W3*10.1 (17)	166	201	164	172	115	141	188	198	**127**	**127**	195	195
W3*10.2E (17)	166	166	172	172	141	141	188	188	**127**	**127**	220	220
W3*10D (17)	137	166	164	187	101	143	186	188	**127**	**127**	195	208
W3*11.2E (18)	137	166	166	172	99	115	188	188	122	133	195	208
W3*14.1E (19)	149	166	172	181	139	154	184	188	127	133	195	220
W4*1.1E (20)	137	153	172	181	101	139	188	188	**127**	**127**	207	222
W5*1.1 (21)	141	207	166	176	99	139	188	198	122	127	195	222
W5*1A (21)	135	141	172	174	143	143	188	198	**127**	**127**	207	214

MLGs were identified by their unique microsatellite profiles. Each MLG (of clone) has a unique identification code corresponding to the tree it was collected on. This code has been simplified in the parentheses. The numbers in bold indicate homozygotes. Six loci were used and these are shown in columns 2–13 as pairs of alleles. As an example, tree 1 had three MLGs associated with it.

females at the end of a season produces reasonable mixing between clones, but it is perhaps not a surprise that some deviations from HWE are present in such a population as mating within a clone is also likely to occur. By the end of a season some clones will be present as multiple individuals either through selection (e.g. by insecticides) or by chance and abundant sexual clonal lineages will produce males and females which could then mate with each other. The M40 locus may be linked with insecticide resistant traits and therefore it is continuously selected, but further research is needed to confirm this hypothesis. Heterozygote deficiency in microsatellite loci appears to be common among aphid species e.g. *Sitobion avenae* [France (Simon *et al.,* 1999)], *Rhopalosiphum padi* [France (Delmotte *et al*., 2002)], *M. persicae* [France (Fenton *et al*., 2003; Guillemaud *et al*., 2003), Australia (Wilson *et al*., 2002) and Greece (Margaritopoulos *et al*., 2007a)] and it has been recorded in both sexual and asexual populations. However, some studies have also found less deviation from HWE in sexual populations of *M. persicae* when compared to others e.g. Australia (Wilson *et al*., 2002) and Greece (Margaritopoulos *et al*., 2007a).

The result of microsatellite analysis of a Chilean population represents a new analysis and is presented in Table 3. In this case the population was collected in spring 2008 from three

Table 3. Microsatellite multilocus genotypes (MLGs) of *Myzus persicae* individuals collected on one occasion in spring (2008) from a single potato field near Colina, Chile.

	Locus											
	M49		M63		M86		M35		M40		myz9	
Clone	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
3B	137	149	172	172	101	141	188	188	120	122	207	208
3A	137	155	172	201	101	126	188	188	120	127	195	222
2G	144	172	163	170	136	139	184	184	127	135	203	230
2A	168	170	166	183	99	107	184	188	133	133	222	224
2F	137	149	172	187	101	141	188	188	110	122	195	208
1F	137	149	172	187	128	141	184	188	122	127	195	208
1A	137	166	168	181	113	126	FAIL	188	120	120	195	208
1G	137	155	172	201	99	126	186	188	120	127	195	222
1B	137	137	172	181	139	141	188	200	122	133	195	208
1H	137	149	172	187	99	141	186	188	110	122	195	208

MLGs were identified by their unique microsatellite profiles. Six loci were used and these are shown in columns 2–13 as pairs of alleles. There were 10 MLGs in the sample from Chile. A sample from the same plant shares the same number (1, 2, or 3).

potato plants in a single field at the same time. Thirteen individuals were analysed and 10 MLGs were identified. Interestingly, all the loci in this population were found to be in HWE suggesting that these individuals had a recent zygotic origin (Table 4). These results indicate that the sexual cycle is present in Chile and in the case of these populations, that genetic diversity can be maintained when moving to secondary hosts. Sexual cycle in spring populations on peach have been reported in Chile by Zúñiga (1969).

A previous study (Fuentes-Contreras *et al*., 2004) on populations from tobacco from various regions in Chile covering a 300 km latitudinal survey found only one asexual genotype which is likely of European origin (Margaritopoulos *et al*., 2009), whereas our results reveal a number of MLGs on a potato crop which appear to originate from sexual reproduction. It seems likely that the answer to this contradiction is host specialisation. A single tobacco-adapted asexual lineage has spread and predominated in tobacco crops in Chile, whilst populations with a sexual phase on peach do not prefer tobacco and instead colonise crops other than tobacco. This is a similar situation to that observed in southern Italy (Margaritopoulos *et al*., 2003).

Table 4. Population parameters from France and Chile.

Locus	F_{IS}	P_{def}	P_{exe}
France			
M49	0.076	0.171	0.889
M63	—0.050	0.810	0.170
M86	0.153	0.089	0.906
M35	—0.039	0.079	0.925
M40	0.143	0.006	0.994
myz9	0.145	0.195	0.810
Overall	0.081	0.023	0.991
Chile			
M49	—0.124	0.937	0.341
M63	—0.047	0.829	0.522
M86	—0.053	0.819	0.473
M35	0.107	0.340	0.813
M40	0.140	0.259	0.807
myz9	—0.264	1.000	0.079
Overall	—0.044	0.777	0.220

Hardy–Weinberg equilibrium (P_{def}, probability for deficiency; P_{exe}, probability for excess); and F_{IS} values in the samples from Chile and France.

Amplified asexual populations

The study of *M. persicae* sexual populations demonstrates the maintenance of considerable genetic diversity, which approaches the levels expected from an organism without a parthenogenetic asexual stage. Sex in the *M. persicae* population is maintained through selection on the primary host peach, as obligate sexual lineages must complete their life cycle as eggs on this tree. The *M. persicae* populations found in areas thousands of miles from peach growing areas have a dramatically different population structure. One of the best understood populations is that of the U.K. This population is composed of an extremely limited number of genotypes. Some of these MLGs have clonally expanded on a vast scale. For the last decade less than 30 MLGs have been found in the U.K. and of these, only 16 have been found on more than one occasion (Table 5). The recent ancestral U.K. population was most probably composed of two insecticide-sensitive genotypes (I and J) and a clone carrying some insecticide resistance (C). These clones are entirely asexual and can no longer produce sexual forms (Pozarowska, 1987; Kasprowicz *et al*., 2008b). The selective action of new insecticides such as dimethyl carbamates has created a dynamic situation. Clones resistant to the dimethyl carbamate (pirimicarb) carrying the MACE mutation (A, B, H, M, N, O, and P) appear to have entered the U.K., with most expanding rapidly (Kasprowicz *et al*., 2008a). In these populations, the rapid expansion appeared to be followed by subsequent population collapses, presumably due to the fitness cost of resistance mechanisms, actions of

Table 5. Microsatellite multilocus genotypes (MLGs) of thousands of lineages of *Myzus persicae* collected from many crops and locations over a decade in the U.K. (1995–2009).

	Locus											
	M49		M63		M86		M35		M40		myz9	
Clone	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
A	149	155	172	181	113	139	198	198	122	133	195	222
B	155	159	166	201	99	139	198	202	127	135	208	222
C	153	166	166	170	136	141	188	198	122	122	195	203
D	153	153	170	201	126	139	188	198	127	133	203	222
E	155	164	164	170	103	107	200	204	122	133	203	207
F	149	155	181	201	126	139	188	196	122	127	208	222
G	137	180	163	183	107	132	180	194	127	133	218	226
H	137	176	166	166	103	107	182	198	133	135	203	205
I	153	207	166	166	126	141	188	198	120	133	201	222
J	153	166	166	170	115	141	188	188	120	122	203	220
K	137	186	157	181	119	139	188	200	122	133	212	220
L	137	155	166	181	139	141	188	200	133	133	195	208
M	120	170	174	176	103	126	200	200	114	135	201	205
N	137	144	157	170	99	103	180	198	114	125	203	220
O	176	211	164	174	99	101	182	182	133	133	197	222
P	170	201	164	170	103	107	182	204	120	133	222	230

The 16 MLGs (clones) collected in the U.K. Six loci were used and these are shown in columns 2–13 as pairs of alleles. Each clone is labelled with a letter of the alphabet.

hymenopterous parasitoids, or an inability to adapt to winter (see later).

For example, in their first season, resistant genotypes from an obligate sexual population would respond to winter by producing sexual forms. These would be a reproductive dead end if they could not find peach trees and such lines would cease. The appearance and disappearance of MACE genotypes occurred between the years 2001 and 2007 and has been described as clonal turnover (see Fig. 1; Kasprowicz *et al*., 2008a). However in 2007, a new genotype (O), that only contained MACE, appeared in large numbers and instead of reducing in numbers this appeared to increase, so that by 2008 it dominated field and aerial populations. Large numbers of type O were found in spring of 2008 in areas that had never been treated with insecticides, suggesting that it had a phenotype that was much better adapted to local conditions than previous MACE clones. In general, the last decade has seen warmer winters, with the most likely cause being climate change. Therefore, non-endogenous *M. persicae* clones have not been subjected to very cold winters. Mean temperatures over most of the U.K. in January, 2009 were generally below the 1971–2000 normal and around 1.5°C below across much of southern England (http://www.metoffice.gov.uk/climate/). This could have had an impact on the survival of type O in the 2009 season. To test this hypothesis, samples of *M. persicae* caught from 18 May to 7 June 2009 in the U.K. nationwide network of suction traps were subjected to DNA analysis and the results are presented in Table 6. It is clear that type O has survived the most challenging winter in a decade and it seems poised to become the dominant clone. Type O must have some physiological advantages over the previous clones, as discussed in the sections below.

Assessments of *M. persicae* collected from U.K. crops suggest that the esterase- and *kdr*-based mechanisms are linked, with esterase-R_2 and -R_3 aphids tending to carry *kdr* and esterase-S and -R_1 aphids tending not to do so (Foster *et al*., 2002) (R and S indicate lines of *M. persicae* resistant and susceptible to insecticides, respectively; see Appendix). Such an association may reflect the predominance of anholocycly for this species in the U.K. and would result in independently associated mechanisms remaining closely linked through parthenogenesis. However, the situation is not universal, as *M. persicae* commonly undergoes sexual reproduction in mainland Europe (Margaritopoulos *et al*., 2002; Guillemaud *et al*., 2003; Blackman *et al*., 2007), a process that should inevitably uncouple genes that are not closely physically linked in the genome. It would appear therefore that differences in various aspects of behaviour influencing aphid survival may be primarily due to pleiotropic effects of the *kdr* mechanism deleteriously affecting response to important environmental cues, supplemented by additional fitness costs associated with carboxylesterase overproduction. At this stage, the role of the MACE mechanism in contributing to reduced fitness, alone or in combination with other mechanisms, remains unclear.

The 'in between' situation – asexual clones in sexual areas

The previous sections describe two extremes found in *M. persicae* populations. Sexual populations on or near peaches are genetically diverse, whereas distant populations in agricultural areas are asexual, very numerous but genetically restricted. Between these extremes are asexual clones found in areas alongside sexual clones. For example, some of

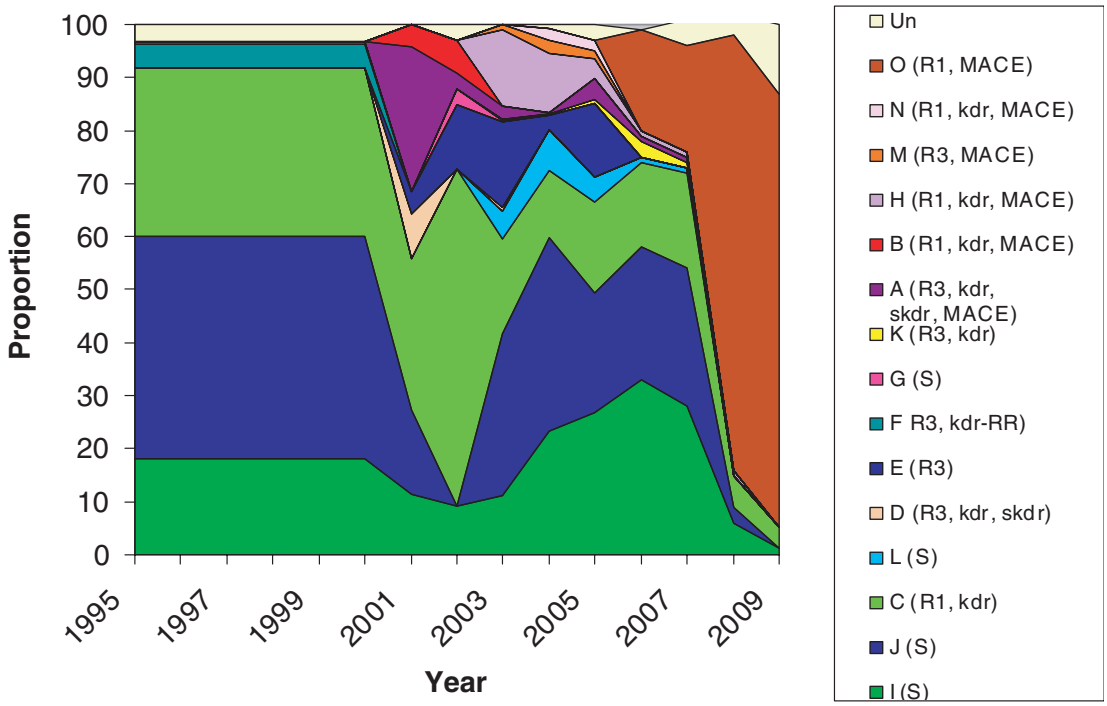

Fig. 1. The yearly proportions of the clones that make up the *Myzus* population in the U.K. For most seasons, between 100 and 300 *M. persicae* individuals were analysed. The early population was stable (from <1995 to 2001). In 2001, MACE-resistant clones arrived and these have been turning over from 2001 to 2007 (see text for detailed explanation and Fig. 3 for full resistance traits). In 2006, clone O was detected for the first time and in recent years it has come to dominate the population.

Table 6. Multilocus genotypes (MLGs) of 2009 early season *Myzus persicae* samples from U.K. suction traps.

Alata no.	Bulletin week	*kdr*	MACE	Clone
K20/5	18 to 24 May	SR	SS	C
Wr23/5	18 to 24 May	SS	SR	P
BB28/5	25 to 31 May	SR	SS	C
BB31/5	25 to 31 May	SS	SR	O
Wr28/5 1	25 to 31 May	SS	SR	Fail
H28/5	25 to 31 May	SS	SR	P
K1/6	1 to 7 June	SR	SR	H
Wr1/6	1 to 7 June	SS	SR	Fail
Wr4/6 1	1 to 7 June	SS	SR	O
Wr4/6 2	1 to 7 June	SS	SR	P
Wr4/6 3	1 to 7 June	SS	SR	O
H2/6	1 to 7 June	SS	SR	P

Myzus persicae alatae collected in four suction traps (K, Kirton; Wr, Writtle; H, Hereford; BB, Brooms Barn) were analysed for insecticide resistance [column 3 knock down resistance (*kdr*), column 4 modified acetylcholinesterase (MACE)] and then genotyped with markers M49, M63, and M86. These markers were sufficient to allocate the individuals into known genotypes (clone column 5).

the same insecticide resistant genotypes found in the U.K. can also be found in countries where sexual reproduction occurs (Fenton *et al*., 2005; van Toor *et al*., 2008). In the peach-growing regions in northern Greece, sexual clones predominate on tobacco crops but obligate or functional parthenogenetic genotypes are also found (Margaritopoulos *et al*., 2002; Blackman *et al.,* 2007). In Japan, most of the lineages from tobacco in regions where peach is grown were functional parthenogens (Takada, 1986; Takada & Tamura, 1987; Shigehara & Takada, 2003). The co-existence of sexual and asexual clones in the same area has serious implications for the evolution of asexuality in aphids and in the genetic structure of their populations. Previous studies on *M. persicae* have suggested gene flow between the two modes of reproduction, through the residual ability of asexual genotypes to produce males and/or mating females, and the creation of new asexual genotypes (Blackman, 1972; Margaritopoulos *et al*., 2002; Guillemaud *et al*., 2003; Vorburger *et al*., 2003a; Blackman *et al*., 2007; Margaritopoulos *et al*., 2007a). The recent creation of asexual genotypes and/or the gene-flow between sexual and asexual aphids are considered the reasons for the absence of heterozygote excess in asexual populations and the slight differences in heterozygosity between these two modes of reproduction in *M. persicae* (Guillemaud *et al*., 2003; Margaritopoulos *et al*., 2007a). Shigehara and Takada (2003) reported that major changes had occurred over a 20-year period in the genotypic composition of tobacco-feeding populations in Japan. They found phenotypes with new combinations of aphid colour and esterase banding pattern, including several resistant to insecticides. These were presumed to have been generated through inbreeding between sexual and facultative asexual genotypes (i.e. there has been some degree of sexual 'leakage' between the lifecycle forms).

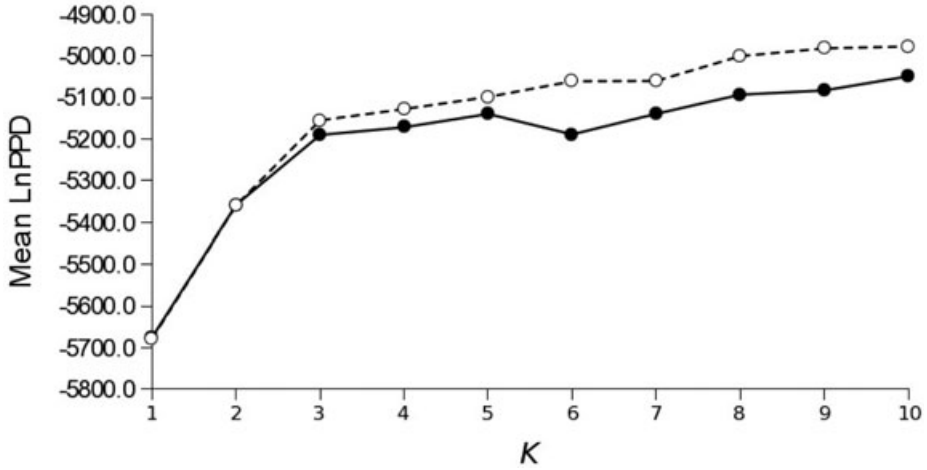

Fig. 2. Mean posterior probability of the data (LnPPD) over 10 simulations against K clusters (see the plateau reached at $K = 3$, produced from the admixture model). Solid circles represent independent allele frequency model. Open circles represent correlated allele frequency model.

Global perspective

The posterior probabilities (PPD) of the data set, which included 197 sexual and asexual parthenogenetic lineages from a previous study (Margaritopoulos *et al*., 2009) with an additional 18 lineages, were calculated with STRUCTURE software (Pritchard *et al.,* 2000) for K values 1–10. Following the pointers for choosing K provided by Pritchard *et al*. (2000), Garnier *et al*. (2004) and Evanno *et al*. (2005), the best solution for K proved to be 3 in 10 independent runs (Fig. 2). Both admixture and non-admixture models gave this solution. There was a sharp increase of PPD values with K moving from 1 to 3. For $K > 3$ the gain of information is rather less and exhibited gradually lower values. A plateau appears to be reached at $K = 3$ and the resolution from the fourth K cluster onwards is less. It seems that splitting samples into three clusters represents the optimal subdivision of the data and avoids unjustified and less informative oversplitting. According to Evanno *et al*. (2005), the modal value of the distribution of ΔLnPPD (absolute value of LnPPD increase divided by standard deviation) is located at the real K. The height of this modal value is used as an indicator of the strength of the signal detected by STRUCTURE. In our case, the highest values were observed at $K = 3$ for both admixture and non-admixture models (results not shown). The three clusters corresponded to genotypes from: 1. Europe, 2. tobacco and 3. Australasia. Clusters 1 and 3 correspond to the generalist *M. persicae persicae* whereas Cluster 2 corresponds to the tobacco-adapted subspecies *M. persicae nicotianae*. Cluster members are spread over all continents and in most of the countries from which populations have been examined. This strongly suggests that the globalisation of agriculture has had an immediate impact on *M. persicae* populations. Studies have provided more direct evidence of this through the spread of obligate parthenogenetic genotypes sampled many miles apart in different countries, e.g. Clones B (U.K. and Turkey) and D (U.K. and New Zealand) (Fenton *et al*., 2005; van Toor *et al*., 2008) and Clone M in U.K. and Slovenia, an MLG found in France and Greece and yet another MLG in U.K., Greece, and Bulgaria (Margaritopoulos *et al*., 2009). These studies reveal that widespread clones appear to occur as a result of selection for insecticide resistance in agriculture (Fenton *et al*., 2005; Zamoum *et al*., 2005; Kasprowicz *et al*., 2008a; van Toor *et al*., 2008). We have examined a relatively small number of individuals in the global *M. persicae* population, yet have detected these dispersed clones. Hence, the number of successful insecticide resistant genotypes is still relatively limited, despite the possibility of resistance genes combining into more genotypes in sexual populations each year. In addition to the spread of resistant clones, it has also been found that asexual tobacco aphid lineages have spread between neighbouring countries such as Greece and Italy (Blackman *et al*., 2007) and a widespread *nicotianae* lineage was found in southern Greece and Slovenia (Margaritopoulos *et al*., 2009). A distinct tobacco lineage has been found in Greece and Chile (Margaritopoulos *et al*., 2009) and due to the lack of genetic variation of *nicotianae* in Chile (a single genotype has been detected; Fuentes-Contreras *et al*., 2004) compared to the European populations, an old world origin of southern American *nicotianae* has been suggested (Blackman *et al*. 2007). Several studies have revealed that the spread of certain genotypes over distant geographical areas is a common phenomenon among aphid species [e.g. *Sitobion* spp. (Wilson *et al*., 1999), *S. avenae* (Sunnucks *et al*., 1996; Simon *et al*., 1999; Haack *et al*., 2000; Llewellyn *et al*., 2003]) including *M. persicae* (Vorburger *et al*., 2003a; Fenton *et al*., 2005; Blackman *et al*., 2007; van Toor *et al*., 2008). The rapid spread of the *M. persicae sensu lato* lineages in different countries and continents should be attributed mostly to human transport and commerce, as suggested earlier for many pest aphid species by Loxdale *et al*. (1993). While winged aphids may be transported very rapidly over great distances by low-level jet streams (Elton, 1925) other studies have found that particular genotypes remain localised (Kasprowicz *et al*., 2008b). The widespread lineages probably represent asexual genotypes reproducing parthenogenetically

all year round. This trait enables them to spread because their reproduction will not be altered by temperature, day length or the requirement for peaches to complete their life cycle. These clones might represent 'general-purpose genotypes' or GPGs (Lynch, 1984) with broad ecological tolerance, which predominate in fluctuating environments through selection, although anthropogenic activities, e.g. insecticide selection pressure, might also be involved (Zamoum *et al*., 2005; Kasprowicz *et al*., 2008a).

In addition to such anthropogenic activity, *M. persicae* populations are influenced by natural mating and biological processes according to geographical region (Australasia vs Southern Europe) and to tobacco adaptation, i.e. *nicotianae* versus *persicae* (tobacco vs. other crops, tobacco or peach in tobacco regions versus peach in non-tobacco regions). Bayesian clustering and genetic distance analyses in previous studies demonstrated the separation of the tobacco aphid populations as well as the regional population structure of *persicae* (Margaritopoulos *et al*., 2007a, 2009).

Physiological adaptation of Myzus persicae *clones*

Clonal growth. The success of different *M. persicae* clones is driven by many physiological factors. As a species, it is apparently capable of colonising over 300 plant species spread over 72 families (Gladders & Peters, 1986). The growth of individual genotypes on different plants could make an important contribution to their relative success. In Germany, Weber (1985) studied over 1,000 lineages of *M. persicae* collected from potato and sugar beet fields. Some clonal lineages reproduced better on the original source plant than on an alternative species and this performance was stable over several generations, showing that some clones were better adapted to particular plant hosts. Other clones performed equally well on both hosts, showing these to be more generalist. Edwards (2001) also found significant variation in the mean relative growth rates between *M. persicae* clonal lineages grown on lupin, but no significant differences were found on chickpea, lentil, pea, or faba bean. None of these studies involved genetic characterisation of the populations concerned. Vorburger *et al*. (2003b) compared the reproductive potential of genetically-characterised sexual and asexual clones. The authors hypothesised that widespread asexual clones would be more generalist than sexual clones, as they have been subjected to greater plant selection on secondary hosts. While differences between clones were found there was no correlation between life cycle and their performance on different host plants and there was little support for the existence of GPGs.

Whatever, there certainly is clear evidence for one *M. persicae* specialist, the subspecies *M. persicae nicotianae* associated with tobacco. This subspecies can be distinguished from clones of *M. persicae* originating from other host plants, both morphologically (Blackman, 1987; Margaritopoulos *et al*., 2000, 2003, 2007b) and genetically (Blackman & Spence, 1992; Margaritopoulos *et al*., 1998; Blackman *et al*., 2007; Margaritopoulos *et al*., 2007a). However, the identical DNA sequence at some loci (Field *et al*., 1994; Clements *et al*., 2000) reveals that some level of interbreeding must occur. The multivariate morphometric studies were performed on clones reared under controlled environmental conditions on the same host plant, indicating that these differences have a genetic basis. These studies have also found that the tobacco-feeding subspecies retains its host-related properties through time, even in regions, such as Greece and Japan, where there is a bisexual generation on the shared primary host and there is a potential for inbreeding. The adaptation to tobacco involves negative trade-offs that reduce the performance of tobacco aphids on other crops (Nikolakakis *et al*., 2003), and there is selection against host migrants (Margaritopoulos *et al*., 2005). These studies also found correlations between acceptance and performance traits, but the genetic basis has not yet been confirmed by direct breeding experiments. The two taxa experience multifarious divergent selection (selection against cross-host migrants and their subsequent generations), which is crucial for the maintenance of host specialisation. Outdoor choice experiments with winged females from Greece show that the two taxa have evolved an improved host recognition mechanism which is based on chemical cues perceived prior to the initiation of feeding (Margaritopoulos *et al*., 2005). This has also been demonstrated in experiments with Chilean aphids (Troncoso *et al*., 2005). Gene flow between the two taxa is reduced due to differences in the mode of reproduction (asexual *versus* sexual) or to the existence of prezygotic reproductive isolation mechanisms in sexual populations (Margaritopoulos *et al*., 2007a). The physiological studies described above reveal that different *M. persicae* genotypes display subtle differences in reproduction which are driven by the host plants they encounter, i.e. differences in host performance between clones have a genetic basis. These differences, except between the two subspecies, are not as pronounced as in other aphids such as the pea aphid, *Acyrthosiphon pisum* (Harris) (Via, 1991).

The U.K. *M. persicae* population is well characterised and immigrant genotypes, such as clone O, can be recognised. Our own work has examined the growth rate of U.K. *M. persicae* on a range of host plants. The interaction between plant and genotype will be considered elsewhere (Fenton *et al*., 2009). This discussion will examine their insecticide resistance status in relation to their growth rates, as it had been earlier found that reproductive performance was seemingly affected by resistance to chemical insecticides (Eggers-Schumacher, 1983; Foster *et al*., 2000, 2003a). The results of clone growth with oilseed rape as a host are shown in Fig. 3. Clone F had the smallest colony size. This clone's growth rate has previously been characterised using different methods (Foster *et al*., 2000), and both studies found a low growth rate. This is the only genotype that is homozygous for the *kdr* mutation which confers resistance to pyrethroids (see Appendix). One suggestion is that the low reproductive rate is a result of the double mutation. In agreement with this view is the finding that *kdr* homozygotes are very rare in the field, even in the zygotes of sexual populations where the *kdr* allele is frequently found (Anstead *et al*., 2007). However, it is more likely that this is caused by the maladaptive behaviour of genotypes bearing the double *kdr* allele (see below) whilst the association between resistant phenotype and low reproductive rates is coincidental.

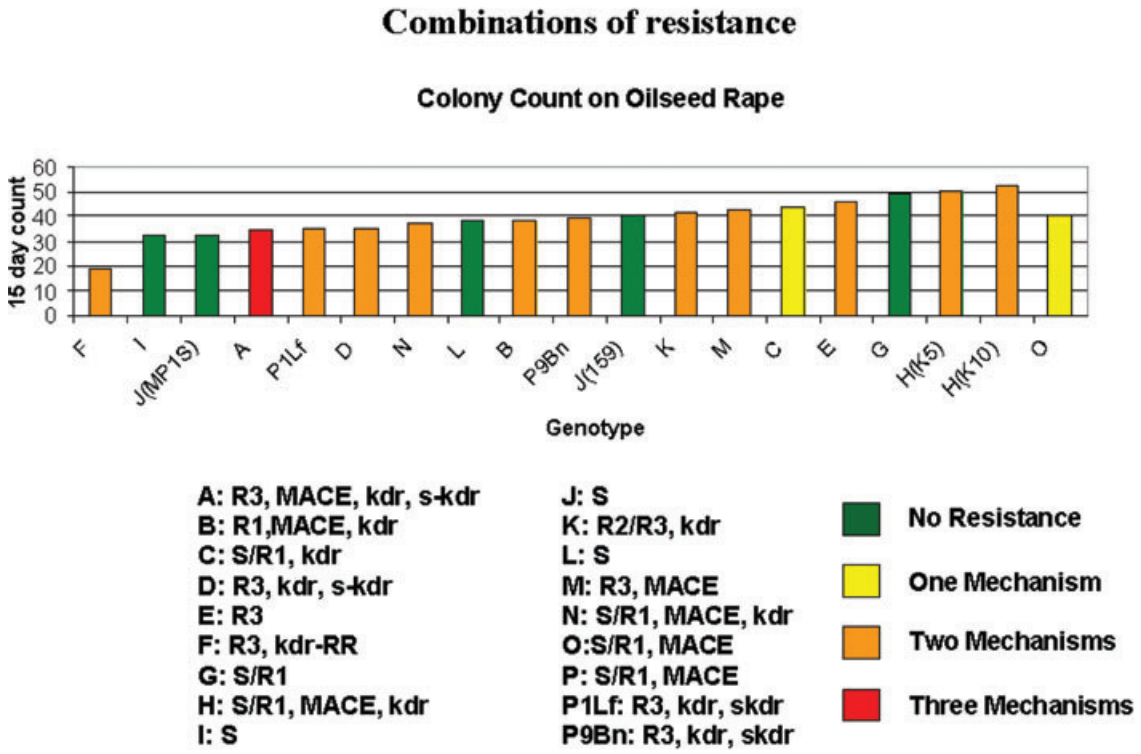

Fig. 3. The colony count of 16 U.K. clonal lineages of *Myzus persicae* in number of individuals after 15 days growth at 18°C on oilseed rape. The experiment was started with 1-day-old nymphs. The lineages with an identical multilocus microsatellite genotype are believed to derive clonally from the same stem mother. These are further differentiated by their unique lineage name in parentheses.

Examination of the impact of other insecticide resistance mechanisms on growth rates is difficult because there is often only one example in each class and there are no obvious patterns (Fig. 3). The performance of clones carrying MACE can be compared where some mechanisms can be matched. To some extent the clones that appear later have better growth rates than their predecessors (Fig. 4) and are found in the field in greater numbers and over a number of years. Clone H, despite carrying both MACE and *kdr*, was the most successful clone until recently and it had the best growth rate of all the clones. While clone N arrived later, it is very rare in the field and has a lower growth rate. Clones A and M are similar, as both have R_3 levels of esterase resistance and carry MACE. Clone M is the most recent to arrive in the U.K. and this has better growth characteristics than A. However, as clone A is *kdr*/*kds* (*kds*, knock-down susceptible) and super *kdr*/*kds* then this could have an impact on its growth rate. Nonetheless, it could be suggested that this supports a hypothesis that over time, resistance mechanisms recombine into better adapted genetic backgrounds. If resistance mechanisms incur a fitness penalty, then one initial mechanism to counter this would be to increase reproductive rates, if possible. The current insecticide selection regime in the U.K. appears to have favoured clone O which carries only MACE, but it does not reproduce particularly rapidly on OSR. Many of the earlier genotypes carried high levels of esterase and/or the *kdr* mechanism and these may have suffered a fitness cost or costs (see section below). Unlike all the previous MACE resistant clones in the U.K., type O appears to be fitter under UK field conditions compared to other resistant clones and that would help increase its frequency. Indeed, its lack of both *kdr* and high/extreme (R_2 and R_3) esterase resistance would suggest that it is not very vulnerable to parasitoids (see later), although this aspect of its fitness remains to be tested. Type O has all the properties of an aphid 'super clone' i.e. a genotype that can build up to very large numbers over many seasons and occur over a large geographical area. This is similar to what may have occurred with clone C, which appears to have become well adapted to being a *kdr* heterozygote (Kasprowicz *et al*., 2008a).

Pleiotropic effects of insecticide resistance

Significant selection pressure from the application of synthetic insecticides dates back just over 50 years, but the intensity of usage in some areas of agriculture and horticulture has imposed extremely strong selection pressure. Nevertheless, susceptible forms often persist and increase in frequency when insecticide selection pressures are relaxed – for example, over winter months when many field crops are either unavailable or untreated.

Growth rates as a measure of potential fitness costs associated with insecticide resistance suggest that there may in fact be no direct link (see above). However, field and laboratory

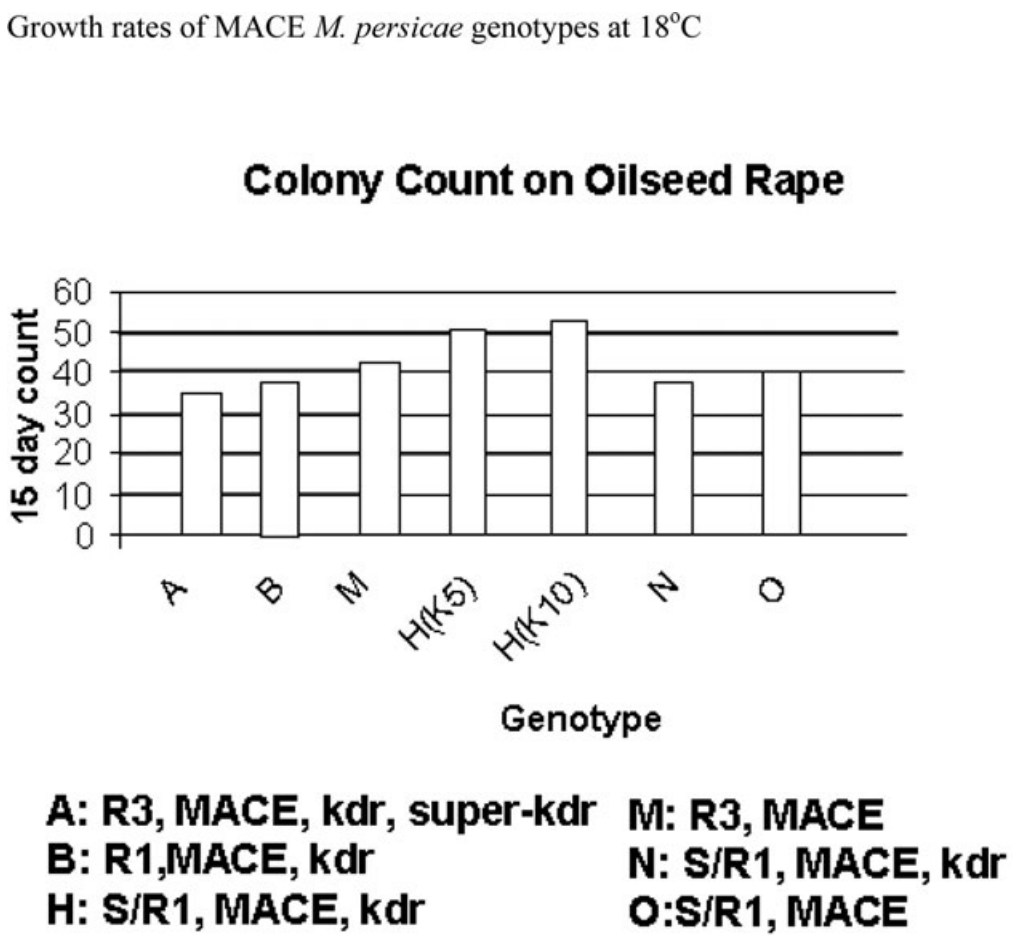

Fig. 4. Direct comparison of colony counts between genotypes with MACE insecticide resistance. The counts represent number of individuals. The clones arriving more recently are shown on the right. Clones H and M were detected in the same year.

studies using *M. persicae* that have been placed under various kinds of stress provide some of the best evidence of negative pleiotropic effects of resistance genes on fitness. In this species, these take the form of poor over-winter survival and increased vulnerability to attack by parasitoids, both apparently caused by maladaptive aphid behaviour.

Reduced overwintering ability

Monitoring of U.K. *M. persicae* populations in the late 1980s showed an apparent fall in the frequencies of aphids carrying high and extreme (R_2 and R_3) esterase-based resistance during the winter months, i.e. R_2 and R_3 phenotypes (Furk *et al.*, 1990), possibly due to counteracting selection in the absence of insecticides. This view was supported by a lower frequency of winged aphids carrying these levels of resistance that were caught in 12.2m suction traps during the spring/summer migrations compared with aphids caught in the previous autumns. Subsequent winter field experiments using populations augmented with *M. persicae* asexual clones initially reared in the laboratory showed that aphids carrying higher levels of esterase resistance suffered greater mortality compared with their lower esterase counterparts during colder, wetter and windier weather conditions (Foster *et al.*, 1996).

Movement from leaves

Movement of aphids from senescing host plant leaves to younger leaves is an important aspect of aphid fitness as individuals remaining at the time of leaf abscission risk starvation during the period of locating another host plant (Harrington & Taylor, 1990). This danger increases dramatically under cold and wet conditions when movement can be severely restricted. Aphids are therefore under strong selective pressure to recognise cues associated with leaf senescence and to respond quickly. Studies of this important aspect of behaviour using U.K. *M. persicae* clones, performed at low temperatures (around 5°C) in the laboratory and in the field, reveal that the rate of aphid movement is inversely associated with esterase-based resistance level (Foster *et al.*, 1996, 1997), i.e. aphids carrying greater esterase resistance tended to move at slower rates from deteriorating leaves. As a

result, they run greater mortality risks after becoming separated from their host plants after leaf fall.

Vulnerability to parasitoids

Another very important component of aphid fitness is the aphid response to the alarm pheromone, (*E*)-β-farnesene. Any reduction in this is highly maladaptive as the dispersal of aphids through this stimulus is an important behavioural adaptation for avoiding attack by natural enemies (Pickett *et al*., 1992). Studies on *M. persicae* show that strong heritable variability in this defence behaviour is consistently associated with the possession of two insecticide resistance mechanisms, esterase and *kdr*. The different alarm responses of insecticide-susceptible and -resistant aphids provided the necessary material to test the hypothesis that interactions with the third trophic level (hymenopterous parasitoids) can play a significant inhibitory role in the evolution of adaptive traits at the second trophic level (aphids), based on a fitness trade-off between insecticide resistance and avoidance of parasitism through defence behaviour. *Myzus persicae* clones carrying the extremes in esterase resistance and *kdr* were exposed to synthetic alarm pheromone to confirm their level of response (Foster *et al*., 2005). Observations of these clones during periods of exposure to adult female parasitoids, *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae), were then made in Petri dishes. Clones showing a consistently high alarm response (insecticide-susceptible forms) displayed a range of behaviours during and after parasitoid attack that were significantly associated with greater survival (avoidance of parasitisation) compared with aphids showing a low alarm response (insecticide-resistant forms). Furthermore, the differential behaviour culminated in the latter suffering significantly higher levels of mummification (Foster *et al*. 2007). This phenomenon may well contribute to the recent fluctuations in resistance frequencies observed in wild *M. persicae* populations in the U.K. (Foster *et al*., 2002).

The finding of increased vulnerability of insecticide-resistant aphids to parasitoids demonstrated for the first time that fitness trade-offs involving interactions between trophic levels can (apparently) be strong enough to affect the evolution and dynamics of insecticide resistance in an insect population. It also suggested an important role for parasitism in the maintenance of genetic diversity within host populations.

Why might insecticide resistance impose fitness costs?

Theoretically, the probability of insecticide resistance having a fitness trade-off is dependent on the type of mechanism involved. Roush and McKenzie (1987) proposed that metabolic mechanisms that increase production of detoxifying enzymes more often impose serious fitness costs compared with target-site mechanisms that are based on simple point mutations affecting the binding of insecticide molecules. In the case of *M. persicae*, both types of resistance mechanism appear to impose handicaps. For the esterase-based, metabolic mechanism, which confers resistance to organophosphates, one potential explanation for this phenomenon is the significant investment of resources in the over-production of a carboxylesterase enzyme, which in R_3 forms represents a non-trivial amount of total body protein, i.e. $\sim$1% (Devonshire & Moores, 1982). Such large amounts of carboxylesterase could cause some form of biochemical disruption to biological pathways, a view supported by studies of another metabolic resistance mechanism in the Australian sheep blowfly, *Lucilia cuprina* (L). In this pest, overproduction of esterases conferring resistance to an organophosphate have been shown to be associated with greater body asymmetry (McKenzie & O'Farrell 1993; Freebairn *et al*., 1996), which is used as a surrogate measure of overall fitness. These enzymes may also function by mediating interactions between cells in the differentiation of terminal structures used in the perception of important stimuli. Indeed, carboxylesterases share amino acid sequence identity with cell adhesion molecules of the nervous system (Hortsch & Goodman, 1991). As a result, overproduction of metabolic enzymes could affect aphid behaviour through adverse effects on their peripheral perception. It is also possible that the mutations conferring metabolic resistance create some form of physical disruption to the aphid genome as they are associated with large sections of DNA and in some cases a large chromosome translocation (Field *et al*., 1988).

Monitoring studies on the esterase mechanism in *M. persicae*, carried out in the U.K. since 1996, have allowed a rare measure of the changes in insecticide resistance frequencies occuring when insecticidal pressure, in this case by organophosphates, is reduced. These compounds have been steadily phased out in the last decade in this country, with a recent sharp decline over several years, to the point where very few such insecticides now remain available to growers. In parallel, since 2003 there has been a sharp fall in the frequency of *M. persicae* carrying high (R_2 or R_3) levels of esterase resistance (Harrington *et al*, 2009), which supports the theory that a lowering of favourable selection by insecticides leads to counter-acting selection imposed by fitness costs. Despite this, it is still possible to find small numbers of R_3 resistant genotypes remaining at low levels in the population, as has been found for type A (Fig. 1 and Kasprowicz *et al*., 2008a).

Turning to the *kdr* target-site mechanism, resistance to pyrethroids is conferred by an alteration in the voltage-gated sodium channel of nerve axon membranes (Martinez-Torres *et al*., 1999), which may also reduce the sensitivity of the aphid nervous system to stimuli used by insecticide-susceptible forms for survival. This view is supported by neurophysical studies incorporating *kdr* mutations into the insect *para* gene, which codes for the sodium channel protein responsible for nerve axon polarisation and depolarisation. When these mutations are expressed *in vitro*, using *Xenopus* oocytes, the single amino acid substitution conferred by the *kdr* mutation shifts the steady-state activation curve for the sodium current by 15 mV in the depolarising direction (Vais *et al*., 1997). Such a change results in an abnormal elevation in action potential thresholds (Smith *et al*., 1997; Vais *et al*., 1997, 2000), thereby making

the nerves in resistant insects less responsive and potentially disrupting the perception and behavioural response to various stimuli important for survival.

Evolution may not always lead to insecticide resistance imposing a handicap. This is supported by examples of the development of modifier genes that ameliorate fitness costs to some extent (McKenzie, 1994). However, these mutations may not be feasible for target site insecticide resistance when it is based in highly conserved nerve proteins.

Conclusions and future perspectives

Aphid pests, like all other organisms, evolve through heritable genetic variation, selection and adaptation. However, evolution of adaptive traits within agricultural environments often occurs rapidly through strong selection pressures imposed by intense man-made activity, exemplified by exposure to insecticides. Therefore, in this situation, insecticide-resistant individuals should suffer fitness handicaps in the absence of insecticide pressure compared with 'wild-type', fully insecticide-susceptible individuals, because if they didn't, resistance alleles would be more common in the population before selection occurs (Crow, 1957; Baker, 1977). In support of this contention, there is growing evidence that fitness handicaps are caused by disruptive side-effects on aspects of fitness, including behavioural traits in houseflies (Foster *et al*., 2003b), and *M. persicae* currently provides one of the clearest demonstrations of this phenomenon.

Metabolic resistance mechanisms, based on increased activity of enzymes that detoxify or intercept insecticide molecules, may interfere with normal biological function. In addition, target site mutations conferring resistance to insecticides aimed at the insect nervous system often occur in highly conserved nerve proteins and as a result, will probably have sub-lethal effects on nerve function through deleterious changes in perception or behaviour. Aphids bearing insecticide resistance mechanisms may therefore be poorly adapted for survival in the absence of insecticide selection pressure, particularly under other types of ecological stress (McKenzie, 1996). Thus, adaptive changes driven by exposure to insecticides could be constrained by deleterious pleiotropic costs associated with genes conferring resistance.

The documented examples of maladaptive effects of insecticide resistance on aphid behaviour and ultimately fitness allow a rare insight into the fundamental processes driving adaptation and evolution (Foster *et al*., 1996, 1997, 2003a, 2005). This is because insecticide resistance often occurs through rapid responses to unambiguous, intense selective agents over relatively short timescales in human terms. [An aphid asexual generation time has been estimated as 7–10 days at ~21°C and there may be 14 or so per growing season in temperate regions of the globe. For general information about pest aphids, see Blackman and Eastop (2000).] The clonal nature of aphids and their alternation of sexual and asexual reproduction also makes them a highly tractable model system for studying evolution in action.

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Appendix: glossary

Anholocyclic: obligatory asexual forms reproducing by continuous apomictic (mitotic) parthenogenesis.

Carbamates: insecticides based on esters of carbonic acid that target acetylcholinesterase in the nervous system.

Clone: a lineage of aphids descended from a single stem mother (i.e. the fundatrix, the first asexual generation after egg hatch in the spring or from another asexual progenitor) and usually tracked in the field using high resolution molecular markers such as microsatellites (Goldstein & Schlötterer, 1999). However, it is likely that clone mates have some differences due to mutational changes in the huge genome of aphids (Vorwerk & Forneck, 2007).

F_{IS}: the inbreeding coefficient of an individual (I) relative to the subpopulation(s)

Functional or facultative sexual forms: life cycle types which are basically holocyclic, but maintain all year round parthenogenesis when (warm) weather conditions allow (Simon *et al*., 2002; Bale *et al*., 2007).

Genetic turnover: changes in gene frequency over generations.

Holocyclic: an aphid lifecycle form which includes an autumn sexual phase during which winged pre-sexual females (gynopare) and winged males return from a secondary herbaceous host/s to the primary woody host, peach. Here, the gynoparae produce wingless sexual females which, after mating, lay cold hardy overwintering eggs between the scales of the leaf buds.

kdr: knock-down mechanism conferring resistance to pyrethroids. Genetically, *kdr* can be homozygous (*kdr*/*kdr*) or heterozygous (*kdr*/*kds*). Most individuals are heterozygous and denoted *kdr*. The only exception in this study is type F which is *kdr*/*kdr*.

kds: knock-down susceptible.

MACE: modified acetylcholinesterase.

Microsatellite: polymorphic DNA loci, consisting of repeating units of 1–6 bp in length, that are used as genetic molecular markers.

MLG: multilocus genotype produced using high resolution molecular markers such as microsatellites, ribosomal DNA markers (i.e. rDNA intergenic spacers or IGS) or amplified polymorphic DNA (AFLPs) (Loxdale & Lushai, 2007). The profiles of such are assumed to be stable over the course of one or a few growing seasons (Haack *et al*., 2000), but are also liable to mutational changes over longer periods (Wilson *et al*., 1999).

Organophosphates: insecticides based on esters of phosphoric acid that target acetylcholinesterase in the nervous system.

Pleiotropy: the genetic effect of a single gene on multiple phenotypic traits.

Pyrethroids: synthetic insecticides, similar to the natural chemical pyrethrins produced by the flowers of pyrethrums, that target sodium channels in the nervous system.

R: lines of *M. persicae* resistant to insecticides and conferred by elevated carboxylestrase levels (Field & Blackman, 2003).

S: lines of *M. persicae* susceptible to insecticides.

Super-kdr: super knock-down mechanism conferring resistance to pyrethroids.