The Great Lakes Entomologist

Volume 28
Number 2 - Summer 1995 *Number 2 - Summer 1995*
Article 2

June 1995

RAPD PCR Confirms Absence of Genetic Variation Between Insecticide Resistant Variants of the Green Peach Aphid, *Myzus Persicae* (Homoptera: Aphididae)

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ABSTRACT

Previous allozyme analysis has revealed an apparent absence of enzyme variability in the green peach aphid, *Myzus persicae* (Sulzer). We are interested in determining the genetic relatedness of individual *M. persicae* clones carrying different numbers of esterase 4 (E4) gene copies conferring resistance to insecticides, in order to determine how many times and in what geographic locations resistance via gene duplication may have evolved. We have therefore extended the analysis of genetic variability in *M. persicae* to the DNA level using random amplification of polymorphic DNA (RAPD) with single 10 mer oligonucleotide primers. Here we report a lack of variability between resistant clones in Wisconsin populations even at the DNA level. Further, 'fast' E4 (FE4) variants appear to be absent from Wisconsin populations, despite FE4 variants of moderate resistance (R_1) being the most common clones in the United Kingdom. These results suggest that resistance in *M. persicae* may have evolved a very few times and that North American populations may differ from those in Europe by founder effects.

Previous reports on the levels of allozyme variation in the green peach aphid, *Myzus persicae* (Sulzer), have revealed a striking absence of enzyme variability. Thus in surveys conducted in the United Kingdom (Wool et al. 1978; Brookes and Loxdale 1987), Germany (Tomiuk and Wohrmann 1983) and North America (May and Holbrook 1978) all enzymes were found to be monomorphic except for two esterase loci (E1/2 and E4). This means that the predominant reported genetic variability for this aphid is associated with gene duplication of the esterase 4 (E4) gene (Field et al. 1988), which can sequester (Devonshire and Moores 1982) and hydrolyze (Devonshire 1977) a wide range of insecticidal esters.

E4 variants can be classified as E4 or FE4 depending on their mobility on polyacrylamide gels (FE4 or 'fast'E4 shows higher mobility) and S, R_1, R_2 or R_3 in relation to the apparent level of activity observed using artificial napthyl ester substrates (Devonshire 1989). Clones of R_1 activity appear to carry the FE4 enzyme, whilst R_2 and R_3 clones carry E4 (although there is some overlap of E4 and FE4 activities within the resistance classification). Interestingly, recently reported sequence data from these two genes has shown that the DNA flanking this gene is the same *within* E4 and FE4 clones and differs only between E4/FE4 types (Field et al. 1993). However, the precise *number of amplified copies* of each of the E4/FE4 genes and the *number of locations* at

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which they have been amplified in the genome of individual clones remains unpublished. Therefore it is not currently possible to *exactly* correlate the number of amplified E4/FE4 genes with the levels of resistance (S, R_1, R_2 or R_3).

Temperate populations of *M. persicae*, away from their woody host (peach), appear to be largely anholocyclic (asexual reproduction) overwintering as nymphs or adults instead of eggs (Blackman 1971). These observations therefore raise the fascinating possibility that resistance via duplication of E4 (or FE4) may have arisen a limited number of times and has been driven through asexually reproducing populations within a few clonal variants. We are therefore interested in documenting the number of individual clones carrying E4/FE4 mediated resistance. Despite the high level of controversy generated over the reproducibility of random amplified DNA (RAPD) markers (Hederick 1992; Riedy et al. 1992; Ellsworth et al. 1993), previous studies have shown that if care is taken over DNA to primer ratios (Ellsworth *et al.* 1993), that these arbitrary DNA markers *can* be reproducible. RAPD markers have thus been used to address questions in population ecology in a range of insects (Hadrys et al. 1992) and to detect polymorphisms within (Black et al. 1992), and distinguish between (Cenis et al. 1993), aphid species. RAPD markers have also been successfully used to trace clonal lineages within various protozoa (Tibayrenc et al. 1993). We were therefore interested in using RAPD markers to understand the genetic relationships between resistant clonal variants of *M. persicae* in temperate North American populations, in order to address the two related questions. 1) How many times and in what locations has resistance arisen? 2) Do North American and European populations show genetic differences? RAPD PCR markers were chosen in order to represent a random sampling of genetic variation in the *Myzus* genome.

Here we report, following analysis of 35 clones of differing E4 activity with 27 random 10 mer oligonucleotides, that *no reproducible variability* in RAPD banding patterns was observed between *any* of the clones. Further, electrophoretic analysis of the E4 variants in Wisconsin shows an apparent absence of FE4 variants, despite the fact that R_1 clones of FE4 mobility are the most common clones found in crop populations in the United Kingdom. This suggests that resistance caused by gene duplication of E4 has arisen in a very limited number of genetically similar clones.

MATERIALS AND METHODS

Aphid clones. Aphids were collected from crop plants, predominantly potatoes, in July and August of 1992 and 1993 in Wisconsin USA. The numbers of clones collected from different counties (Dane, Oneida, Langdale, Waushara, Columbia, Oconto, Juneau and Adams) are shown in Table 1. Clones were established from individual females on excised potato leaflets kept in small plastic cages at room temperature in the laboratory. Three reference R_1 FE4 clones were collected in September 1992 in the United Kingdom from autumn sown rape near Cambridge, England.

Electrophoresis and RAPD PCR. Polyacrylamide gel electrophoresis was performed as described elsewhere (Brookes and Loxdale 1987). Five individuals from the same aphid clones were also used to make genomic DNA for RAPD PCR. DNA preparation was as for individual *Drosophila* and has been described elsewhere (ffrench-Constant et al. 1993). DNA was resuspended in sterile distilled water to a concentration of 1 ng/μl. 1 ng of DNA was added to a PCR reaction containing: 0.4 mM magnesium chloride, 0.1 mM dNTPs, 0.2 μM of a single random 10 mer primer (purchased from J. Carlson,

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DOI: 10.22543/0090-0222.1876

Table 1. Number and location of *Myzus persicae* clones collected in counties within Wisconsin, USA and East Anglia, UK. Their E4 activity as scored by polyacrylamide gel electrophoresis is shown.

	E4 activity			
County	S	R_1	R_2	R_3
Adams	0	1	0	0
Columbia	1	0	0	0
Dane	0	2	0	0
Juneau	0	0	1	0
Langdale	5	5	0	2
Oconto	0	0	2	0
Oneida	1	4	5	0
Waushara	2	0	1	0
Cambridge, UK	0	1	1	0

University of British Columbia, Canada) and 0.8 units of Taq polymerase. 27 primers (Table 1) were tested on 47 individual clones of differing resistance status. After 3 min. denaturation at 94°C, amplification was performed by 45 cycles of: 1 min denaturation at 94°C, 1 min annealing at 35°C and 2 min extension at 72°C. Products were loaded onto a 4% agarose gel and visualized using ethidium bromide fluorescence. Product sizes were estimated by reference to a 1 kb reference ladder (Bethesda Research Laboratories). Each primer clone combination was repeated at least three times or until a reproducible result was achieved.

RESULTS

E4 Electrophoresis. Clones were classified into susceptible (S, with no discernible E4 activity in individual aphids on a polyacrylamide gel), moderately resistant (R_1), highly resistant (R_2) and extremely resistant (R_3) based on the levels of esterase activity observed at this locus in relation to the R_1 standards from the United Kingdom (Fig. 1). Within Wisconsin clones were predominantly R_1 (38%, n = 32), with similar frequencies of S and R_2 (both 28%), and a lower frequency of R_3 (6%). The collection location and E4 activity are shown in Table 1. There was no apparent geographic structure to the observed levels of resistance within the state. However, none of these variants had the higher mobility FE4 enzyme associated with the English R_1 standards, which are the commonest variants in UK field populations (Fig. 1, lane with asterisk).

RAPD PCR. Following the testing of 27 random primers against 34 (32 from Wisconsin and 2 from the UK) clones of differing resistance status no reproducible variations in DNA banding patterns could be documented. A representative set of reaction products for six primers is shown in Fig. 2. The number and size of the products formed from each random primer, alongside the sequence of the primer is listed in Table 2.

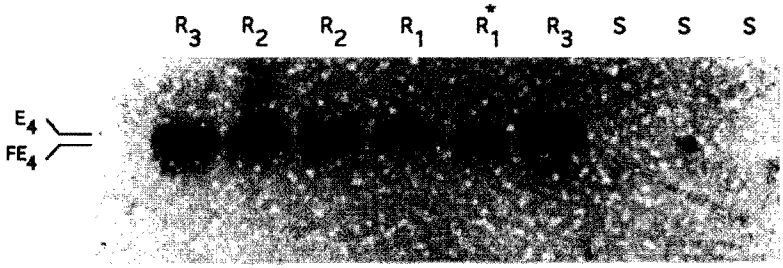

Fig. 1. Polyacrylamide electrophoresis gel of nine *Myzus persicae* clones of differing E4 status: S, susceptible; R_1, moderately resistant; R_2, highly resistant and R_3, extremely resistant. E4's of differing mobility are also indicated, thus the European R_1 clone (asterix) has 'fast' E4 or FE4.

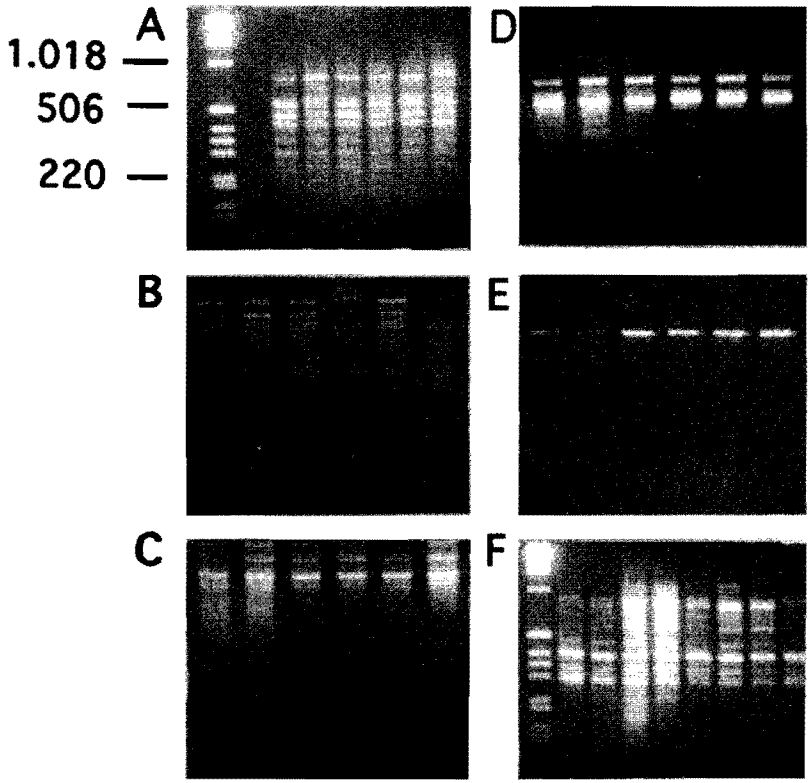

Fig. 2. Amplification products from six different RAPD primers (A, 178; B, 169; C, 177; D, 182; E, 170 and F, 192) used on DNA from six (eight in F) *Myzus persicae* clones of differing E4 resistance status. Sizes of DNA markers are given in base pairs.

https://scholar.valpo.edu/tgle/vol28/iss2/2
DOI: 10.22543/0090-0222.1876

Table 2. Sequence of RAPD primers used in this study and size of amplification products observed in base pairs (bp). Primer numbers refer to those designated by J. Carlson, University of British Columbia.

Primer number	sequence	Approximate size of products (bp)
144	AGA GGG TTC T	800, 1000
148	TGT CCA CCA G	550
159	GAG CCC GTA G	480
165	GAA GGC ACT G	290, 340, 390, 520, 650, 950, 1000
166	ACT GCT ACA G	520
168	CTA GAT GTG C	200, 300, 370, 550, 750, 900
169	ACG ACG TAG G	700, 800, 1000, 1500
170	ATC TCT CCT G	900
171	TGA CCC CTC C	700, 750, 800, 950, 1000
175	TGG TGC TGA T	390, 450, 900, 1000
176	CAA GGG AGG T	200, 300, 400, 500, 750, 1000, 1500
177	TCA GGC AGT C	200, 250, 280, 300, 500, 700, 1000, 2000, 3000
178	CCG TCA TTG G	250, 300, 350, 400, 450, 550, 600, 800, 850, 950, 1000
179	TCA CTG TAC G	300, 390, 500, 750, 800, 1000
180	GGG CCA CGC T	150, 450, 500, 700, 800, 900, 1000
181	ATG ACG ACG G	150, 200, 250, 300, 350, 400, 700, 800
182	GTT CTC GTG T	140, 150, 350, 390, 750, 900, 1000
183	CGT GAT TGC T	300, 350, 400, 550, 800, 1000, 1300
184	CAA ACG GCA C	220, 280, 300, 500, 1000, 1600
186	GTG CGT CGC T	170, 250, 290, 390, 450, 500, 550, 700, 750, 800, 900
187	AAC GGG GGA G	300, 400, 500, 1000, 1500, 2000
188	GCT GGA CAT C	200, 400, 500, 1000
189	TGC TAG CCT C	150, 200, 300, 350, 700, 1000, 1500
190	AGA ATC CGC C	350, 700, 1000, 1500
191	CGA TGG CTT T	200, 250, 700, 800, 1000, 1500, 2000
192	GCA AGT CAC T	300, 330, 370, 390, 600, 700, 800, 900, 1200, 1700
193	TGC TGG CTT T	280, 380, 450, 600, 700, 800, 900, 1500

DISCUSSION

The presence of the highly resistant variants R_2 and R_3 has been correlated with control failures both in potato crops sprayed with a range of compounds (ffrench-Constant and Devonshire 1988) and field experiments repeatedly sprayed with pyrethroids (ffrench-Constant et al. 1988). Their occurence in Wisconsin therefore indicates a potential for control failures within potato crops given appropriate conditions for aphid resurgence late in the season (ffrench-Constant *et al.* 1988; Harrington et al. 1989).

The absence of any variability in the RAPD markers among any of the aphid clones, representing all four resistance variants (S, R_1, R_2 or R_3), stands in contrast to a recent paper where two primers (OPA-02 and OPA-07) were reported to show interclonal variation in *M.persicae* (Cenis et al. 1993). However, in our hands, following repeated analysis of any primer/DNA combinations showing variable bands, in a larger number of clones and with a larger number of primers, we were not able to show *any reproducible* variability. Any variation found between clones was attributable to differences in primer: DNA concentration ratios. This result shows that genetic variability between

M. persicae clones is also very low at the DNA, as well as the allozyme, level. The main documented genetic differences between between insecticide resistant clones are therefore the differences between the DNA flanking the E4 and FE4 genes *themselves* (Field et al. 1993). Although the precise genetic relationship (in relation to number and location of amplification events) between the E4/FE4 mobility variants has not been described, they have been postulated to have been originally allelic before their independent duplication and divergence (Field et al. 1993). This absence of genetic variability between resistant clones thus supports the hypothesis that E4 or FE4 gene duplications have recently arisen within a very few clones that are highly related. However, this similarity of both E4 sequences and RAPD markers, however, does not preclude the possibility that the *number of locations* in the genome at which these genes have been amplified may differ between clones.

The apparent absence of FE4 variants from Wisconsin populations is extremely interesting. Although this observation obviously needs confirmation across the rest of the USA, one explanation is that the absence of these variants from North America is due to a 'founder effect'. That is to say that FE4 variants have simply not colonized the US from Europe and that resistant variants in North America are thus all descended from E4 clones. The hypothesis that aphids within the *Myzus* group may have originated from a limited number of locations and spread worldwide is not unprecedented. Thus detailed morphological analysis of tobacco-adapted populations has suggested that populations from various parts of the world share a common origin and that the species *M. nicotianae* was only introduced into America as recently as over 40 years ago (Blackman 1987). Further, other examples of insecticide resistance evolution in similar resistance conferring amplified esterase genes in *Culex* mosquitoes (Raymond et al. 1991), and an altered γ-aminobutyric acid receptor conferring cyclodiene resistance in *D. melanogaster* (ffrench-Constant et al. 1993), support the contention that resistance can arise a limited number of times (probably only *once* in the case of cyclodiene resistant *D. melanogaster*) and spread by migration. In contrast to sexually reproducing mosquitoes and fruit flies, this would be particularly applicable for an aphid whose predominant mode of reproduction in temperate regions appears to be asexual and clones of which can readily be transmitted on plants and cuttings. These results may therefore have important implications for the exclusion of insecticide resistance strains or clones via quarantine.

ACKNOWLEDGMENTS

We thank L. Kabrick and J. Wyman for their support and help with field collections. This work was supported by a grant to R.ff-C from the United States Department of Agriculture (NCRPIAP).

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