Variation in the chromosomal distribution of amplified esterase (*FE4*) genes in Greek field populations of *Myzus persicae* (Sulzer)

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In northern Greece, insecticides have been used intensively against aphid populations of the *Myzus persicae* group, both on its primary host peach, on which annual sexual reproduction occurs, and on secondary host field crops such as tobacco, on which reproduction is entirely parthenogenetic. This has resulted in the selection of high levels of resistance based on the amplification of two genes encoding insecticide-degrading esterases, *E4* and *FE4*. We have used fluorescence *in situ* hybridization (FISH) to study variation in the number and distribution of loci with amplified esterase genes in clones established from field populations of *M. persicae*-group aphids sampled on various crops in northern Greece. All clones collected from peach, as well as most of the clones (74%) from tobacco and other secondary host plants, had amplified *FE4* genes and were of normal karyotype. Amplicon clusters containing *FE4* occurred at multiple sites which varied in number, zygosity and distribution between clones. Most loci were on autosome 1, which also had the only site that was consistently occupied by amplified *FE4,* situated near subtelomeric repetitive DNA. Possibly this was the original site of *FE4* gene amplification, and the location of the single-copy 'wild type' esterase genes. The rest of the clones from tobacco and other secondary hosts (26%) had amplified *E4* genes, and all those analysed by FISH had an amplicon cluster on autosome 3$^{\text{T}}$ at a site close to the breakpoint of an A1,3 translocation, confirming the close linkage of this translocation with, and its probable involvement in, E4-based resistance. Three translocated clones collected on capsicum at one site had both *E4* and *FE4* amplified, the first time that both esterase gene types have been found together in individual aphids from field populations.

Keywords: aphid chromosomes, FISH, gene amplification, insecticide resistance, multiple loci.

Introduction

The economically important pest aphid *Myzus persicae* (Sulzer) can combat organophosphate and carbamate insecticides by overproducing insecticide-degrading esterases, encoded by amplified genes (Devonshire & Field, 1991). Resistant aphids have one of two different but closely related amplified genes, *E4* or *FE4*, according to their karyotype. Aphids heterozygous for an autosomal (A) 1,3 translocation have amplified *E4*, whereas those without this translocation have *FE4* (Blackman *et al.*, 1995). Studies of the inheritance of these genes, coupled with *in situ* hybridization to map their locations on chromosomes (Blackman *et al.*, 1996), have shown that *E4*-containing amplicon clusters are usually

located at one site on autosome 3$^{\text{T}}$ closely linked to the A 1,3 translocation, whereas in clones with FE4-based resistance the amplicon clusters occurred at three to five sites on several different chromosomes.

Myzus persicae has a complex pattern of life cycle variation (Blackman, 1974). Populations on field crops reproduce by thelytokous (all-female), apomictic parthenogenesis, but in temperate regions a variable proportion of the population migrates in autumn to peach trees (*Prunus persica)*, where an annual bisexual generation occurs with meiotic recombination and overwintering of cold-resistant eggs. The proportion migrating to peach trees varies geographically according to (i) seasonal trends in photoperiod and temperature and (ii) the availability of peach. The karyotype (presence or absence of the A 1,3 translocation) and the type of esterase

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produced (E4 or FE4), appears to be associated with the life cycle category, in a way that is relevant to the evolution and inheritance of insecticide resistance. Translocated, E4-producing genotypes are widely distributed in warm temperate (and tropical) regions of the world, and in glasshouses in northern temperate regions. Mild winters allow such populations to reproduce all the year around by parthenogenesis, and most of them have lost the bisexual part of the life cycle. On the other hand, FE4-based resistance seems to have developed in peach-growing areas, in the absence of the translocation, where at least part of the population goes through the bisexual phase.

In some places, such as northern Greece, climate and availability of peaches favour both life cycle categories to a similar extent, and intensive use of insecticides in peach orchards and on field crops has resulted in high levels of resistance, so that genotypes with FE4-based resistance occur on peach trees in spring, and genotypes with either E4- or FE4-based resistance occur together in field crops. *Myzus persicae*-group populations in northern Greece also show much genetic variation in other respects, such as body colour and adaptation to different host plants; tobacco is widely grown, and populations on tobacco are sufficiently distinct to be recognized as a separate taxon (*nicotianae*; Blackman & Spence, 1992). Northern Greece therefore seemed to be an ideal place to examine the extent of variation in number and distribution of loci with amplified esterase genes that can occur in field populations of *M. persicae*-group aphids.

Materials and methods

Aphid collection and rearing

We sampled populations on peaches and some crop plants in northern and central mainland Greece in early May 1995, and populations on field crops in late May — mid April 1996 (Table 1). Clones were set up using the progeny of single parthenogenetic females collected from widely separated plants at each location. They were maintained at the Natural History Museum on excised leaves of potato (var. Pentland Crown) in a controlled environment room at 16 h photoperiod and 15°C.

Estimation of carboxylesterase activity

Assays for carboxylesterase activity were carried out on homogenates of individual aphids from each of the 1995 clones. The amount of detoxifying enzyme present was measured in a small fraction (one-fiftieth) of each homogenate by immunoassay using E4 antiserum in 96-well microtitre plates (Devonshire *et al.*, 1992). Three individuals of each clone were categorized as either susceptible (S) or resistant (R_1, R_2 or R_3, depending on the amount of

Table 1 Clones of aphids of the *Myzus persicae* group established from collections in Greece in 1995/96

Location	Date of collection	Host plant	Total no. of clones established (analysed by FISH)	Identification nos of clones analysed by FISH	No. of clones with A 1,3 translocation (analysed by FISH)
Alexandria (Valtos)	2/5/95	Peach	7 (7)	B 1-4, E 1-3	None
Alexandria (Valtos)	2/5/95	Sugar beet	1 (1)	VSB	None
Alexandria (Nissi)	2/5/95	Sugar beet	1 (1)	VN 3	None
Alexandria (Nissi)	2/5/95	Peach	1 (1)	VN 5	None
Alexandria (Nissi)	2/5/95	Tobacco	7 (6)	VN 7-12	None
Meliki (Paliambela)	3/5/95	Peach	6 (6)	P 1-6	None
Larisa (Falani)	5/5/95	Peach	10 (10)	F 1-10	None
Larisa	5/5/95	Peach	1 (1)	Lar '95	None
Volos	5/5/95	Peach	1 (1)	Volos	None
Alexandria (Nissi)	13/6/96	Brassica	22 (3)	BNI 7, 21, 22	2 (1)
Alexandria (Nissi)	13/6/96	Sugar beet	10 (1)	SBNI 1	None
Alexandria (Nissi)	12/6/96	Tobacco	55 (3)	TNI 26, 27, 43	19 (0)
Volos (Velestino)	28/5/96	Capsicum	18 (7)	9635-6, 9640-1, 9649, 9651, 9656	6 (3)
Volos (Dimini)	14/6/96	Capsicum	29 (4)	DIP 9, 11, 17, 26	1 (1)
Sofades (Anavra)	14/6/96	Tobacco	50 (4)	AN 3, 15, 39, 42	17 (1)

enzyme present compared with aphids from standard laboratory clones). Immunoassays were also performed on 1996 clones, but the data had to be discounted because of major inconsistencies between replicates.

Esterase gene characterization, karyotyping and FISH

The type(s) of esterase gene amplified in each clone (if any) was identified by a PCR-based diagnostic (Field *et al.*, 1996). In some key clones, especially those which had both *E4* and *FE4*, this was confirmed by probing Southern blots of genomic DNA digested with *Msp*I (Blackman *et al.*, 1996). Presence or absence of the A 1,3 translocation was observed in preparations of embryonic somatic cells viewed by phase contrast, which were then hybridized *in situ* to a biotinylated 8 kb genomic DNA probe including most of the *E4* gene and some 3′ flanking DNA. Under the conditions used, this probe hybridized to both *E4* and *FE4* sequences. Methods of chromosome preparation and fluorescence *in situ* hybridization have already been described (Blackman *et al.*, 1995).

Chromosome mapping

The chromosomes of Hemiptera are holocentric, so lack physical points of reference, and there have been no classical genetic studies of *M. persicae* (or any other aphid species) to provide linkage maps. In order to describe the locations of the amplified esterase sequences, we have divided the *M. persicae* haploid karyotype ($n = 6$) into 42 sections, and labelled the autosomal sections consecutively as A–Z on the longer autosomes 1–3, and a–f on the short autosomes 4 and 5 (Fig. 1a). The terminal sections of each autosome are defined as comprising telomeric and subtelomeric DNA; the latter largely consists of numerous copies of a 169 bp repeat (MpR: Spence *et al.*, 1998). After observation of a number of cells (usually six to 10) of each clone it is usually possible to assign the locations of the hybridized probe to specific chromosome segments. In order to apply this labelling scheme to the A1,3 translocation karyotype, we defined this translocation as involving breaks within section I of autosome 1 and section W of autosome 3, so that the subterminal section of autosome 1 was exchanged for sections S–V plus W (part) of autosome 3 (Fig. 1b, cf. Fig. 1a). This is supported by recent work indicat-
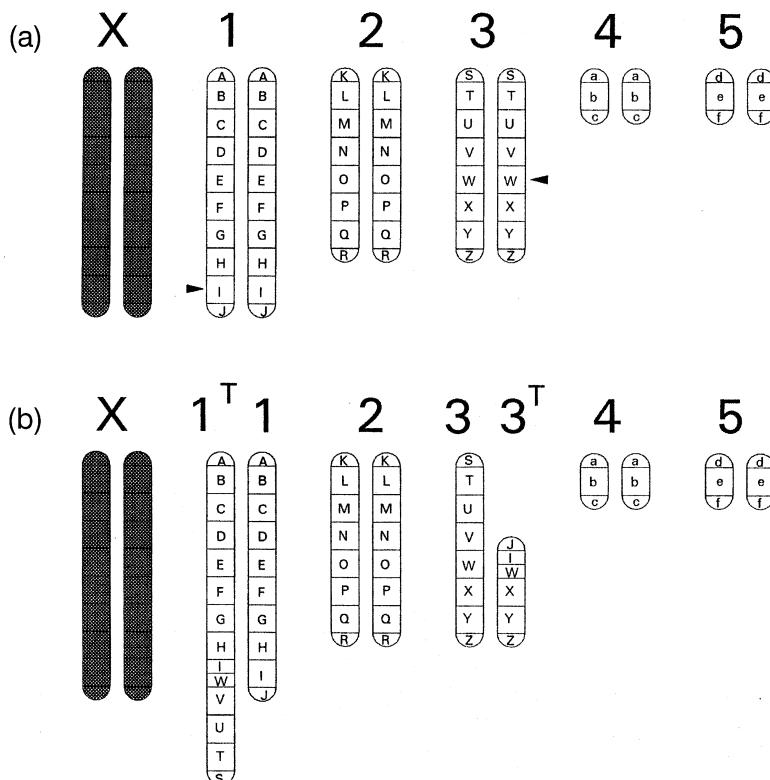

Fig. 1 System of sectional division of autosomes of *Myzus persicae*: (a) normal karyotype. Arrowheads indicate postulated breakpoints leading to (b) karyotype with autosomal 1,3 translocation (for fuller explanation see text).

ing that the subtelomeric repetitive sequence is relocated from autosome 1 onto autosome 3^T in A 1,3 translocation heterozygotes of *M. persicae* (Spence *et al*., 1998).

Results

All 35 clones established from the 1995 collections from peach in early May were of normal karyotype (i.e. none had the A 1,3 translocation), and all had amplified *FE4* genes. FISH revealed that *FE4* amplicon clusters occurred at six different loci (at least) on autosome 1 (although the maximum number discernible in any one clone was four), at two loci on autosome 3, and at one locus near the end of a short

autosome, probably autosome 5 (Table 2 and Fig. 2a–f). The last site was only found in one clone (F2), which had a total of eight sites with amplified sequences at six loci, the largest number of amplicon clusters so far recorded (Fig. 2a). Up to three of the loci on autosome 1 were homozygous for amplified *FE4* in any one clone (Fig. 2b). All six of the 1995 clones heterozygous for amplified *FE4* in segment W on autosome 3 were from the same peach orchard (at Falani), but these six clones differed with respect to the other loci amplified. Clones started from aphids collected on tobacco and sugar beet plants in fields adjacent to the sampled peach orchards had a range of distribution and number of *FE4* amplicon clusters similar to those from peach trees.

Table 2 Chromosomal locations of amplified esterase sequences in 1995/96 Greek clones of *Myzus persicae* (see Table 1 for collection data and Fig. 1 for labelling of autosome segments). Paired ticks represent loci appearing to be homozygous for amplified esterase genes, single ticks represent loci heterozygous for amplified esterase genes. Bold ticks are amplified *E4* (only found on autosome 3^T of translocated clones); others are all *FE4*

Autosome number and segment										Clones with amplified *E4*/*FE4* sites indicated
1						3		3^T	5	
B	C	D	E	H	I	W	Z		f	
✓		✓		✓✓	✓✓	✓			✓	F2
✓	✓			✓	✓✓					E3
✓		✓		✓✓	✓✓					VN 11
✓				✓	✓✓					P3
		✓✓		✓✓	✓✓		✓			E1
		✓✓		✓✓	✓✓					B3, F4, TNI 26
		✓✓		✓	✓✓					F3
		✓✓		✓	✓					VN 7, VN 8
		✓✓			✓✓	✓				F1
		✓	✓	✓✓	✓✓					E2
		✓		✓✓	✓✓	✓				F5, BNI 21
		✓		✓✓	✓✓					B1-2, B4, P2, P6, F6, VN 5, Lar '95, TNI27, TNI43, BNI 22
		✓		✓	✓					AN 15
		✓			✓✓					AN 39, 9640
		✓			✓					VN 9
		✓			✓✓	✓✓				DIP 17
		✓			✓✓	✓				F9, F10
		✓			✓			✔		9636, 9651, 9656
				✓✓	✓✓	✓				F7
				✓✓	✓✓					P1, Volos, VN 3
				✓	✓✓					F8
				✓	✓					P5, VN 10, VN 12, AN 42
					✓					VSB, SBNI 1, 9649
				✓✓		✓✓				DIP 9
				✓✓		✓				DIP 11
								✔		BNI 7, AN 3, DIP 26
No amplified genes (insecticide-susceptible)										9635, 9641

Immunoassays for carboxylesterase activity showed that all the 1995 clones, with from two to eight amplicon clusters, had moderate to high (R_2 or R_3) levels of insecticide resistance.

Of the 184 clones set up from 1996 collections on field crops, 46 (26%) had the A1,3 chromosome translocation. Most of these (78%) were from the two tobacco fields sampled. All of the translocated clones had amplified *E4*, but three clones originating from capsicum (9636, 9651 and 9656) also had amplified *FE4*. Clones with both amplified genes have been obtained in laboratory crosses (Blackman *et al.*, 1996), but this is the first time that they have been found together in the field. The body colour of the three clones with both *E4* and *FE4* was green, whereas all other clones with the A1,3 translocation were red. The clones of normal (untranslocated) karyotype included both red and green colour morphs, with green predominating (82%), but all had only *FE4* amplified, irrespective of body colour.

There was no correlation between colour and number of *FE4* amplicon clusters.

FISH to 22 of the 1996 clones did not reveal any amplicon clusters that were additional to those seen in the 1995 clones (Tables 1 and 2). Two of the clones from capsicum were apparently homozygous for amplified *FE4* at the W locus on autosome 3. The three clones from capsicum with both *E4* and *FE4* amplified had two sites (presumably *FE4*) on one homologue of autosome 1, plus the presumed *E4* site on autosome 3^T (Fig. 2f). Three translocated clones with only *E4* amplified, originating from three different crops, all had a single amplicon cluster on autosome 3^T (Table 2), as found previously in other E4-producing, translocated clones from the U.K. and the U.S.A. (Blackman *et al.*, 1995).

In FE4-producing clones, the only locus that was invariably amplified on at least one homologue was that in segment 1I, the site closest to the end of autosome 1 (Table 2).
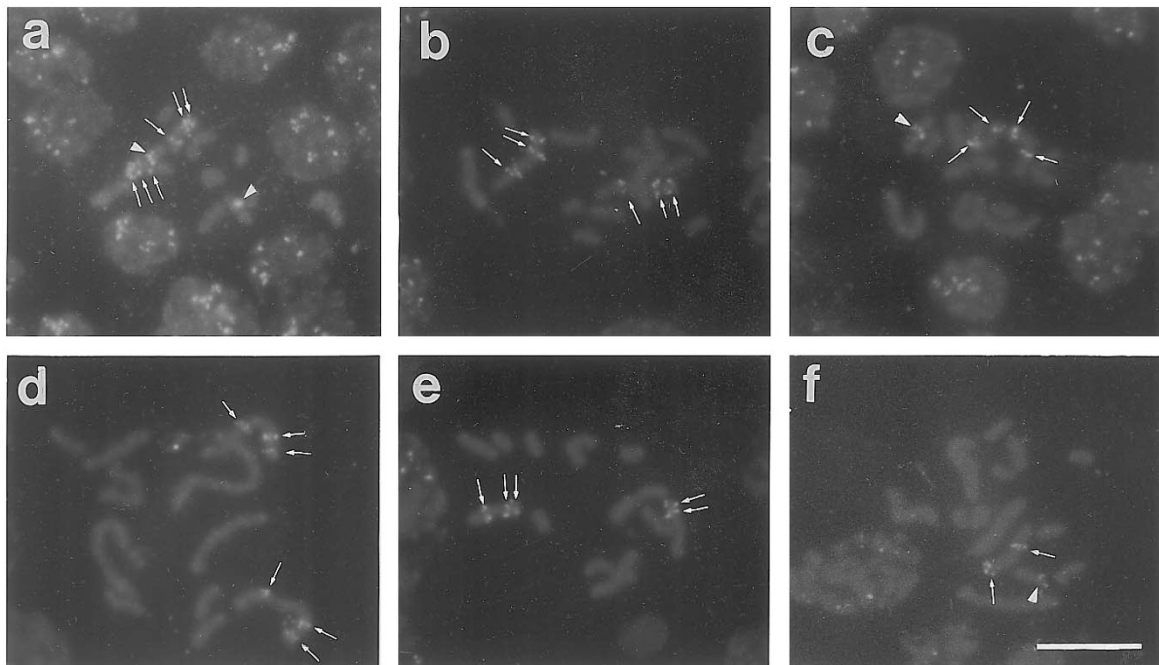

Fig. 2 *In situ* hybridization of an esterase (E4) DNA probe to chromosomes of insecticide-resistant *Myzus persicae* from Greece, showing location of amplified esterase genes. *FE4* amplicon clusters on autosome 1 are indicated by small thin arrows, those on other autosomes by arrowheads: (a) clone F2, with eight amplicon clusters, including sites on autosomes 3 and 5; (b) clone B3, with six amplicon clusters on autosome 1, interpreted as three homozygous loci; (c) clone F1, with five amplicon clusters, interpreted as two homozygous loci on autosome 1 and an additional site on autosome 3; (d) clone E2, with six amplicon clusters on autosome 1, interpreted as two homozygous loci plus additional sites in segment D of one homologue and segment E of the other; (e) clone B1, with five amplicon clusters on autosome 1 at three loci — the most frequent combination of sites found in the populations studied; (f) clone 9656, which has the autosome 1,3 translocation found in genotypes with amplified *E4*, and also has amplified *FE4*. From previous work it is probable that the two amplicon clusters on one homologue of autosome 1 are *FE4*, and that the cluster at one end of the short element (3^T) is *E4*. Bar represents 10 μm.

Discussion

It is only possible to map the approximate positions of the amplified *FE4* genes so, if anything, the number of separate locations may be underestimated. However, one can have some confidence in the single identity of the most commonly occupied sites (1D,1H, 1I, 3W), as one would expect these to become homozygous for amplified *FE4* in sexually reproducing populations. The patterns of presumed homozygosity/heterozygosity at these sites (Table 2) conform to this expectation.

Our FISH technique was not sufficiently sensitive to locate the single-copy 'wild-type' *FE4* locus in susceptible aphids, so we do not know whether amplification has occurred at the original locus. In Chinese Hamster ovary (CHO) cell lines, initial amplification of dihydrofolate reductase (*DHFR*) genes occurs on the same chromosome as the parental single-copy gene but usually at some distance (at least 50 Mb) away from it (Trask & Hamlin, 1989). In *M. persicae*, in spite of all the variation among clones in the number and distribution of *FE4* amplicon clusters, there is only one site (1I) where they occur consistently on at least one homologue, suggesting that this is the original site of the amplification, and possibly of the single-copy parental *FE4* and *E4* genes, which are thought to occur adjacent to each other in a head-to-tail arrangement, with *E4* upstream of *FE4* and ≈ 19 kb of intervening sequence (Field & Devonshire, 1998).

Contrary to our initial conclusions based on studies of laboratory clones (Blackman *et al.*, 1995), it now seems that, at least in Greek populations of *M. persicae*, other locations of *FE4* amplicon clusters occur most commonly on the same chromosome (autosome 1) as the putative original amplification site, similar to the situation found in CHO cells. Subsequent relocation of *FE4*-containing amplicons to other sites on autosome 1 may have occurred by inversion, and to other autosomes by reciprocal interchange. The postulated original locus (1I) is situated near subtelomeric heterochromatin containing numerous copies of the 169 bp repeat MpR that also occurs subtelomerically on all the other autosomes of *M. persicae* (Spence *et al.*, 1998), suggesting that interchange of subtelomeric sections may occur relatively frequently.

The MpR subtelomeric repeat has been shown to have relocated from autosome 1 onto autosome 3^T in clones with the A 1,3 translocation. An interchange event with similar breakpoints, followed by recombination in a subsequent sexual phase, could have led to the occurrence of *FE4* amplicon clusters at site 3W. Transposable elements might be involved in any or all of such rearrangements. In this context, a non-LTR retrotransposon-like sequence associated with the telomeric DNA of *M. persicae* has recently been isolated (Spence, unpublished results), and inverted repeat sequences have been found within the *E4* amplicon (Field, unpublished data).

If *FE4* gene amplification was a unique event, then this most likely happened in *M. persicae* populations on peaches in north-western U.S.A., where organophosphate resistance was first reported in the mid-1950s (Anthon, 1955). This was more than 10 years before the first reports of resistance in peach-feeding populations in some parts of the Mediterranean area (Baranyovits, 1973). However, such resistance was not noted in peach- and tobacco-growing areas of northern mainland Greece until about 1985, and in 1989 it still seemed to be limited to two localities (G. Michalopoulos, personal communication). The large variation in number and distribution of *FE4*-containing amplicon clusters now present may therefore be a rather recent development. In contrast to results with clones of known parentage reared in the laboratory (Blackman *et al.*, 1996), no correlation was found in 1995 Greek field-collected clones between the number of amplicon clusters and carboxylesterase activity, suggesting that *FE4* gene copy number may be less variable than the number of sites at which it is located. It will be of interest to examine populations with *FE4*-based resistance in other countries and other parts of the world, to see whether different patterns of amplicon cluster distribution occur outside Greece, indicating that multiple loci have arisen independently. That this may be the case is suggested by one clone from France used in studies of the inheritance of resistance (French R; Blackman *et al.*, 1995); this had an amplicon cluster at the 1I site as usual, but was also homozygous for amplified *FE4* at a subtelomeric locus on autosome 2, a site not occupied by amplified *FE4* genes in any of the Greek clones studied.

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