Electrophoretic distinction between the peach-potato aphid, *Myzus persicae*, and the tobacco aphid, *M. nicotianae* (Homoptera: Aphididae)

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Abstract

The electrophoretic mobility of the enzyme glutamate oxaloacetate transaminase (GOT) on cellulose acetate plates was compared among sibling species of the *Myzus persicae* (Sulzer) group (*M. persicae*, *M. nicotianae* Blackman, *M. antirrhinii* (Macchiati)). *M. persicae* itself is monomorphic for GOT-1 (genotype **ff**), whereas European populations of *M. nicotianae* are polymorphic for this enzyme, with two forms of slightly different mobility (alleles **s**, **f**). In the samples of *M. nicotianae* examined, *M. persicae*-like **ff** genotypes were rare and heterozygotes (**sf**) were in large excess, even in samples from Greece where *M. nicotianae* has a regular holocycle (i.e., annual sexual reproduction). In North America, where *M. nicotianae* is probably entirely anholocyclic, samples of both red and green colour morphs of this species were found to be heterozygous for GOT-1. The enzyme difference can thus provide a means of distinguishing most individual specimens, including trapped alatae, of *M. persicae* and *M. nicotianae*. The anholocyclic taxon *M. antirrhinii* appears to be a fixed heterozygote for GOT-1 and thus resembles most *M. nicotianae*, but can be distinguished electrophoretically from both *M. persicae* and *M. nicotianae* by its distinctive pattern of esterases.

Introduction

Morphometric studies of numerous samples of aphids of the *Myzus persicae* (Sulzer) group from four continents showed that samples from tobacco (*Nicotiana tabacum*) can be distinguished by canonical variate (CV) analysis. They have consistently different mean CV scores when compared with samples collected from other host plants, even after they have been reared in the laboratory on excised leaves of plants other than tobacco (Blackman, 1987). On the basis of this evidence the tobacco-feeding form was described as a distinct species, *M. nicotianae* Blackman. The two taxa were believed to be genetically isolated from one another because *M. nicotianae* was thought to be anholocyclic, therefore lacking the ability to produce sexual morphs and interbreed with *M. persicae*.

Aphids of the *M. persicae* group feeding on tobacco in some parts of the world (Japan, Central Asia) do however produce sexual morphs, and the taxonomic status of such populations is unresolved (Blackman, 1987). It was found recently that a migration from peach (*Prunus persica*) to tobacco occurs in Greece (G. Michalopoulos, pers. comm.). Peach is the normal primary host of *M. persicae*; that is, the plant on which sexual reproduction occurs. If *M. nicotianae* can also have a sexual phase on peach, then interbreeding might occur which would be expected to reduce or eliminate the morphological distinction between the two taxa. Nevertheless, when included in CV analysis, samples of *M. nicotianae* originating from tobacco in Greece, and also samples that originated from spring populations on peach in tobacco-growing areas, consistently group with samples from anholocyclic populations of *M. nicotianae* in other parts of the world (Blackman & Spence, unpublished data, and discussion section of this paper).

The morphological differences between the two taxa are very slight, and can only be demonstrated reliably by CV analysis involving 11–14 characters. Discriminant functions involving 2–4 characters have been computed from a large data set and included in keys (Blackman,

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Table 1 Clones of *Myzus persicae* (M.p) and *Myzus nicotianae* (M.n) from which samples were taken for GOT analysis

Species	NHM clone no.	Other ident.	Country of origin	Original host	Life cycle category	A1,3 trans-location present	Colour in life	Carboxyl-esterase activity	GOT-1 mobility
M.p	3978	405D	UK	sugar beet	anholocyclic	no	green	moderate	ff
M.p	3980	794J	UK	sugar beet	androcyclic	yes	green	high	ff
M.p	3985	'French R'	France	peach	holocyclic	no	green	moderate	ff
M.p	3986	Reading diet	UK	brussel sprout	anholocyclic	no	green	low	ff
M.p	4105	800F	Italy	peach	holocyclic	no	green	high	ff
M.p	4108	'Larisa'	Greece	peach	holocyclic	no	green	high	ff
M.p	4154	931D	USA	sweet potato	unknown	yes	green	high	ff
M.p	4155	932B	USA	chrysanthemum	unknown	yes	green	high	ff
M.p	4156	#17	UK	beet (clamp)	androcyclic	yes	green	moderate	ff
M.p	4157	#21	UK	beet (clamp)	unknown	no	green	high	sf
M.p	4158	#24	UK	cauliflower	androcyclic	yes	green	high	ff
M.p	4186	US1L	UK	sugar beet	androcyclic	no	green	low	ff
M.p	4187	'Dorobek'	Germany	unknown	androcyclic	no	green	low	ff
M.p	41		Holland	unknown	unknown	no	green	low	ff
M.n	4107	'Meliki'	Greece	peach	holocyclic	no	green	high	sf
M.n	4128	926B	Greece	tobacco	holocyclic	no	green	high	ss
M.n	4129	926C	Greece	tobacco	unknown	no	green	high	sf
M.n	4130	926F	Greece	tobacco	unknown	no	green	high	ss
M.n	4131	927B	Greece	tobacco	unknown	no	green	high	ff
M.n	4138	933D	USA	tobacco	unknown	no	green	low	sf
M.n	4139	935E	USA	tobacco	androcyclic	yes	green	?	sf
M.n	4153	934E	USA	tobacco	unknown	yes	red	moderate	sf
M.n	4182	Kecskemet	Hungary	tobacco	unknown	no	green	moderate	sf

1987), but these have proved unreliable when applied to new samples, and are almost useless when trying to identify single specimens, especially when the possible presence of a third sibling species, *M. antirrhinii* (Macchiati), has to be taken into account.

Diagnostic enzyme loci have proved useful in other aphid groups to resolve differences between morphologically similar or sibling species (Blackman *et al.*, 1989). Extensive surveys for enzyme variation in *M. persicae* (e.g. May & Holbrook, 1978; Brookes & Loxdale, 1987) have found virtually no detectable enzyme variation, except in esterases. With relevance to the present study, Brookes & Loxdale (1987) found that the enzyme glutamate oxaloacetate transaminase (GOT) was monomorphic in 512 clones of *M. persicae* set up from populations sampled on various crops and weeds in south-east England. Here we report a polymorphism of GOT in *M. nicotianae*, which should enable this species to be distinguished from *M. persicae* with 90–100% reliability.

Materials and methods

The study material comprised 25 clonal samples of *M. persicae* and 9 clonal samples of *M. nicotianae*, plus 83 individual adult virginoparae of *M. nicotianae* collected in a tobacco field in Hungary (all apterae), and 100 *M. nicotianae* (89 apterae, 11 alatae) from a tobacco field in Greece. The 25 *M. persicae* clones came from diverse localities and host plants in Europe (15) and North America (10), and represented different karyotypes (presence or absence of an autosomal 1,3 translocation; Blackman & Takada, 1975), different levels of activity of the carboxylesterase that is associated with resistance to organophosphorus insecticides (Devonshire, 1989), and different life cycle categories (holocyclic, androcyclic, anholocyclic; Blackman, 1971). The nine *M. nicotianae* clones came from tobacco in Greece (5), Hungary (1) and N Carolina, USA (3), and included representatives of different karyotypes (translocated, untranslocated), colour morphs (red, green) and life cycle categories (table 1). The analysis also included five laboratory clones of *M. antirrhinii*, representing four different karyotypes.

In a preliminary study of the inheritance of GOT, males from one clone of *M. nicotianae* ('Meliki') were crossed with oviparae of one clone of *M. persicae* ('French R'). Mating readily occurred and eggs were laid, which were overwintered in an outdoor insectary. Fundatrices hatching in early spring were matured on radish plants (*Raphanus sativus*) and separated into excised leaf cages when matured to rear F_1 clones.

Specimens for electrophoresis were deep-frozen at −80°C. Homogenates were prepared of two adult apterae from each clone in 10 µl of grinding buffer (50 ml Tris HCl pH 8.0, 25 µl Triton X-100). In the case of field-collected specimens, single specimens were homogenized in 7 µl of this buffer. 2 µl of each homogenate was loaded onto a cellulose acetate plate pre-soaked in electrode buffer (25 mM Trizma base and 192 mM glycine at pH 8.5), using a 'Super-Z' 12-sample applicator (Helena Laboratories). Electrophoresis was carried out at 200 V for 12 min. The staining mixture was 5 ml of a solution of 0.2 mM pyridoxal-5-phosphate, 17 mM L-aspartic acid, 8.9 mM α-ketoglutaric acid in 0.1 M

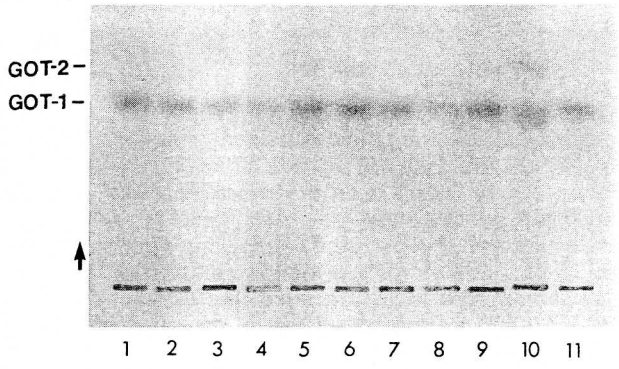

Fig. 1. Photograph of a cellulose acetate plate with 11 clonal samples of *Myzus persicae*, stained for glutamate oxaloacetate transaminase (GOT). Clone 4157 (see text) is sample 10.

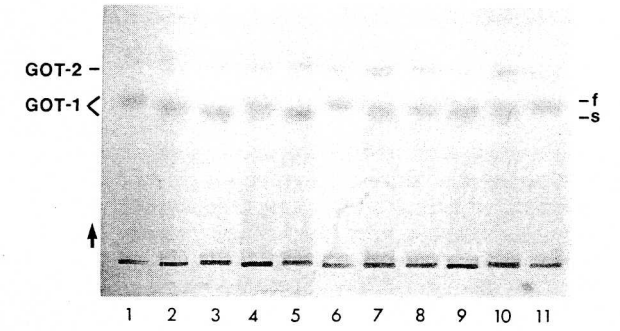

Fig. 2. Photograph of a cellulose acetate plate stained for glutamate oxaloacetate transaminase (GOT), with 9 clonal samples of *Myzus nicotianae* (2–10) between 2 clonal samples of *M. persicae* (1, 11). Samples 2–6 are from Greece, 7–9 from USA (North Carolina), and 10 from Hungary.

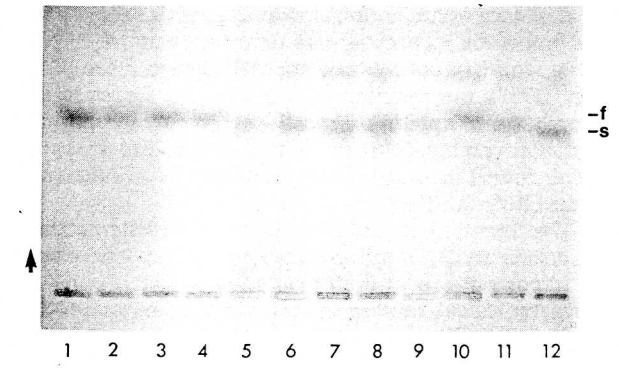

Fig. 3. Photograph of a cellulose acetate plate stained for glutamate oxaloacetate transaminase (GOT), with clonal samples of *Myzus persicae* (**ff**, 1–4), *M. antirrhinii* (**sf**, 5–9), and examples of the 3 GOT-1 genotypes of *M. nicotianae* (**ff**, **sf**, **ff**; 10, 11 and 12, respectively).

Table 2 Numbers of GOT-1 genotypes in samples from two field populations of *Myzus nicotianae*, and in the F1 generation of a cross between *M. nicotianae* (sf) and *M. persicae* (ff).

	ss	sf	ff
Greece	25	67	8
Hungary	1	82	–
Hybrids sf × ff	–	5	4

sodium phosphate buffer at pH 7.0, adjusted to pH 7.4 before adding 1 ml of saturated Fast Blue BB salt solution and 2 ml of warm agar (Hebert & Beaton, 1989).

Results

Using the above technique there appeared to be two loci for GOT in *M. persicae* and its relatives (GOT-1, GOT-2; figs 1–3), although only one was noted in previous work on *M. persicae* using polyacrylamide gels. The differences described in this paper are in the allozyme we are calling GOT-1; GOT-2 was represented by a single, faster-moving, invariant band in all samples studied.

Twenty-four of the 25 clonal samples of *M. persicae* had GOT-1 of identical mobility (fig. 1). The exception was a clone (RLB 4157, Rothamsted #21), originating from a beet clamp in Norfolk, England; this clone was classified on morphological grounds as *M. persicae*, but was peculiar in other ways (dark green colour, high insecticidal resistance but normal karyotype), and is being investigated further.

In contrast, *M. nicotianae* was polymorphic for GOT-1, with two allelomorphs (**s** and **f**), differing in their mobility on cellulose acetate plates by about 4%. Heterozygotes (**sf**) showed a broad, more diffuse band of intermediate mobility between the two homozygotes, (**ss** and **ff**), indicating a dimeric enzyme in which the hybrid band could not be fully resolved (fig. 2). The single GOT-1 band found in *M. persicae* clearly corresponded to the **ff** genotype in *M. nicotianae*. Most clones of *M. nicotianae*, including all three from North America, were heterozygous (table 1). Populations sampled from tobacco in Hungary and Greece (table 2) both had large excesses of heterozygotes ($P < 0.001$). Only one clone and 0–8% of field-collected individuals of *M. nicotianae* were homozygous **ff** like *M. persicae*.

Oviparae of *M. persicae* mated with males of *M. nicotianae* produced numerous eggs, but mortality of eggs and 1st instar fundatrices was high, and only nine F_1 clones were obtained. The hybrids segregated as **sf** or **ff** according to expectations (table 2).

The five clones of *M. antirrhinii* all showed identical electromorphs, closely resembling the **sf** heterozygote of *M. nicotianae*, except that a fainter, slower-moving band was also present (fig. 3).

Discussion

The marked excess of GOT-1 heterozygotes in *M. nicotianae* indicates that even in Greece, where sexual reproduction occurs annually, part of the population consists of clones reproducing all year round by parthenogenesis.

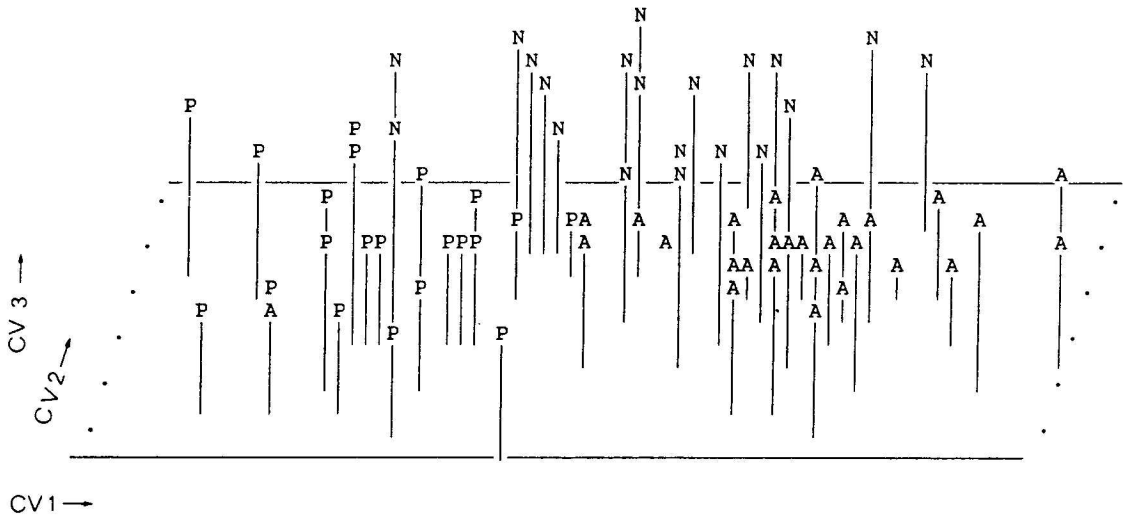

Fig. 4. Plot of mean scores on the first three canonical variates of 69 samples of apterous viviparae of the *M. persicae* group from Europe and North America, based on measurements of 11 characters in 10 individual apterae of each sample. **A** = *M. antirrhinii* (identified by karyotype, 2n = 13 or 14); **N** = *M. nicotianae* (2n = 12, on tobacco); **P** = *M. persicae* (2n = 12, on plants other than tobacco).

In many tobacco-growing areas of the world, sexual reproduction is probably entirely absent. Linkage disequilibrium is characteristic of asexual and unisexual populations, and heterozygote excesses may occur, particularly in populations of cyclic parthenogens in which there is only intermittent sexual reproduction, separated by extended periods of parthenogenesis, as in the cladoceran *Daphnia magna* (Hebert, 1982). However, it is difficult to show whether a heterozygote excess at a particular locus in a unisexual population is due to selection on that locus or on the genotype as a whole. In *M. nicotianae*, clones differing in colour, insecticidal resistance and karyotype are all heterozygous for GOT-1, whereas clones of *M. persicae* displaying similar differences are homozygous for the fast form of the enzyme. This seems to point to selection acting specifically at the GOT-1 locus, which differs somewhat from the conclusion of Loxdale & Brookes (1990) that GOT alleles in *Sitobion fragariae* (Walker) populations were selectively neutral or, at most, weakly selected. Further work on more samples of *M. nicotianae* is required, and needs to include a study of relative fitness of the different GOT genotypes of *M. nicotianae* in relation to host-plant. Ideally this might then eventually be related to the functions of different forms of the enzyme at the molecular level.

Thus, taken on its own, the difference in GOT between *M. nicotianae* and *M. persicae* is not good evidence of a taxonomic distinction, because it could be explained by preferential selection of particular, host-adapted genotypes within a single species. It only acquires validity as a taxonomic criterion when considered in the context of all the other evidence from colour, insectide resistance, karyotype and multivariate morphometrics. The strong preponderance of heterozygotes for GOT-1 (the **sf** genotype) in *M. nicotianae*, especially where populations are anholocyclic, should provide a useful means of distinguishing most individual aphids of this species from individuals of *M. persicae* – something which cannot be done reliably with morphometric techniques – and the electrophoretic method could also be applied to trapped alatae. *M. antirrhinii* is difficult to distinguish from the **sf** genotype of *M. nicotianae*, but is easily separable from both *M. persicae* and *M. nicotianae* if esterases are also analysed (ffrench-Constant *et al.*, 1988).

Many more samples of *M. persicae*-group aphids have now been studied morphometrically, and they continue to cluster as *M. antirrhinii*, *M. nicotianae* or *M. persicae* when the mean scores on the first three canonical variates are compared (fig. 4), although some samples would be misclassified if one relied solely on morphological data. Having established that holocyclic populations of *M. nicotianae* occur on tobacco in Greece, it seems likely that holocyclic tobacco aphids reported from other parts of the world (Japan, Kazakhstan, Uzbekistan; see Blackman (1987) for references) are also *M. nicotianae*. In Japan, tobacco aphids are mostly red in colour, and have particular combinations of esterase alleles that are rarely if ever found in *M. persicae*-group aphids on other host plants (Takada, 1986).

The retention of multiple host-related differences by *M. persicae* and *M. nicotianae*, even in regions where holocyclic populations of both taxa have a sexual phase on peach, indicates a mechanism for partial or total reproductive isolation. The nature of this is so far unknown. Mating takes place readily in the laboratory and fertile eggs are laid. The high mortality cannot be regarded as significant, because intraspecific matings in *M. persicae* commonly show similar egg mortality. Prezygotic isolation by behavioural or phenological differences may occur under field conditions, or F_1 hybrid clones may have reduced viability; this latter possibility has not yet been tested.

M. nicotianae seems to be rapidly extending its range; it was recently found causing serious damage to tobacco in southern Brazil (C.L. Costa, pers. comm.).

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