12 Enzyme differences within species groups containing pest aphids

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Abstract

Many pest aphids belong to species groups or complexes, and are difficult to distinguish morphologically from other non-pest species within these groups. Pest species also show an exceptionally large range of intraspecific variation in morphology associated with season or host plant. The interpretation of such variation is difficult with our still very inadequate knowledge of the genetic structure of aphid populations.

Enzymes, particularly esterases, can show discrete differences in mobility between closely-related aphids, and help us not only to distinguish pest from non-pest species, but also to understand taxonomic relationships within such groups. This paper presents and discusses the findings of previously unpublished work on allozyme differences between closely-related species within the aphid genera *Myzus, Metopolophium, Macrosiphum,* and *Acyrthosiphon.*

Problems of pest aphid taxonomy

Many pest aphids are highly variable organisms, in which differences in host-plant-colonizing ability, life cycle, and morphology can be

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observed, raising questions about whether all the forms currently placed under a single name, such as *Aphis fabae* or *Myzus persicae*, can really be treated as single species. Such aphids usually have close relatives that look morphologically very much like them, but only feed on non-crop plants; hence, we need to be certain not only that these are in fact distinct species, but also to find reliable and convenient ways to distinguish them from the pest species.

Historically, aphid taxonomists recognized species by the association between a particular host plant and a particular morphology. As aphid life cycles were worked out, it became clear that aphid morphology changed greatly with the season, and often with the host plant. Experimental host-plant transfers became essential to clarifying taxonomic relationships. Some aphids previously described under different names from many different plant were subsequently recognized as single, polyphagous species, so that their currently valid names carry long lists of synonyms.

As the true extent of variation within species came to be appreciated, the morphological studies needed to discriminate between them became more complex. Aphids not only exist as discrete, recognizably distinct morphs — such as apterous and alate viviparous females, oviparous females, fundatrices, and so on — they also vary more or less continuously within morphs. Some of this variation can be ascribed to differences in general body size, and is then partly accommodated by using simple morphometric ratios to discriminate between species (Eastop 1985). There may, however, be more complex patterns of variation associated with aphid polymorphisms: for example, apterous females developing under certain conditions may show differing, lesser degrees of expression of characters that are fully expressed in the alate morph. Distinguishing between closely-related species on morphological grounds may then be rather difficult, requiring time-consuming multivariate techniques and complex discriminant functions that nevertheless may not be 100 per cent reliable for individual specimens (cf. Blackman 1987; Blackman and Paterson 1986).

Methods that circumvent these problems by focusing directly on gene products therefore offer considerable potential for discriminating between closely related aphid species. Standard techniques of electrophoresis using starch gels (Tomiuk *et al.* 1979; Tomiuk and Wöhrmann 1983; Eggers-Schumacher 1987), polyacrylamide gels (Loxdale *et al.* 1983), or cellulose acetate sheets (Easteel and Boussy 1987) are clearly the simplest way of looking at such gene products. Application of these techniques to studies of the genetic structure of populations within aphid species has been hampered by the apparent lack of variation in many of the enzyme systems most commonly

investigated (Tomiuk 1987). However, for species separation, all that is normally required is one or two diagnostic enzyme loci (Ayala 1983). Diagnostic loci have been found in almost all cases in which electrophoretic comparisons have been made between closely-related (but recognizably distinct) aphid species, and in some cases have indicated the presence of sibling species.

Aphids have one important advantage over most other small invertebrates for such studies. They can be reared as clones, so that the electrophoretic method does not have to be destructive of the genotype. Thus, there is no theoretical limit to the numbers or the amounts of enzymes available for analysis, nor of the number of buffer systems and staining procedures that can be employed on a single genotype. Correlations between allozyme data and morphological or cytological characters can also be investigated on the same genotype, avoiding the problem of genetic heterogeneity within sampled populations.

This chapter will review instances of the use of electrophoretic methods to discriminate between aphid populations at or around the species level, paying particular attention to species groups involving pest aphids, and including previously unpublished information on several species complexes. For the most part, we shall be looking simply at differences at diagnostic enzyme loci, as in most cases insufficient data are available on too few loci to examine genetic relationships quantitatively using measures of genetic identity or distance (e.g. Nei 1972). Nor are we concerned here with the application of electrophoresis to problems of classification at the genus level and above (e.g. Lampel and Burgener 1987), which is a more controversial use of allozyme data (see Buth 1984).

Diagnostic loci for pest aphids

1. Macrosiphum euphorbiae

The potato aphid is a typical example of a polyphagous pest aphid that is liable to taxonomic confusion with several other morphologically similar, but more host-specific species. This species is an important pest, transmitting more than 50 plant viruses in 35 different crops throughout the world. The number of taxa recognized within the *M. euphorbiae* group in Europe has varied from 4 (Hille Ris Lambers 1939) to 10 (Meier 1961). *M. euphorbiae* itself is apparently a North American species, unknown in Britain before 1917, and the actual and potential confusion with its close relatives in North America is probably even greater than in Europe.

Table 12.1 Species of the *Macrosiphum euphorbiae* group in Britain, their host plants and life cycles.

Species	Main host plant(s)	Life cycle[a]
M. euphorbiae (Thomas)	Polyphagous	Virtually anholocyclic
M. tinctum (Walker) (= *M. epilobiellum* Theobald)	*Epilobium* spp.	Virtually anholocyclic
M. penfroense Stroyan	*Silene maritima*	Virtually anholocyclic
M. centranthi Theobald	*Centranthus ruber*, *Valeriana officinalis*	Unknown; probably mainly anholocyclic
M. hellebori Theobald and Walton	*Helleborus* spp.	Mainly anholocyclic
M. euphorbiellum Theobald (= *M. amygdaloides* Theobald)	*Euphorbia* spp.	Mainly anholocyclic
M. stellariae Theobald	*Stellaria holostea* and other Caryophyllaceae	Partly anholocyclic
M. melampyri Mordwilko	*Melampyrum pratense*	Holocyclic
M. cholodkovskyi Mordwilko	*Filipendula ulmaria*	Holocyclic
M. gei Koch	*Geum* and Umbelliferae	Holocyclic
M. daphinidis Borner	*Daphne mezereum*	Holocyclic

[a] Most of these species, except *M. euphorbiae*, are holocyclic in continental Europe

Watson (1982) applied multivariate techniques (mainly canonical variate analysis) to British populations of the *M. euphorbiae* group, on the basis of which she concluded that 11 taxa were present (Table 12.1). Esterases of ten of these taxa were examined, using starch gel electrophoresis, and 11 other enzyme systems were looked at in 6 of them. For esterases, each species was found to have a characteristic pattern of bands, fully confirming the conclusions reached by multivariate morphometrics. A typical gel with six species is represented in Fig. 12.1(a). The complexity of the banding patterns did not permit any genetic interpretation of the loci involved, but most species were polymorphic for at least one esterase locus, and *M. gei* was markedly polymorphic. *M. euphorbiae* itself, however, showed no clear polymorphisms at esterase loci in any of the 75 samples

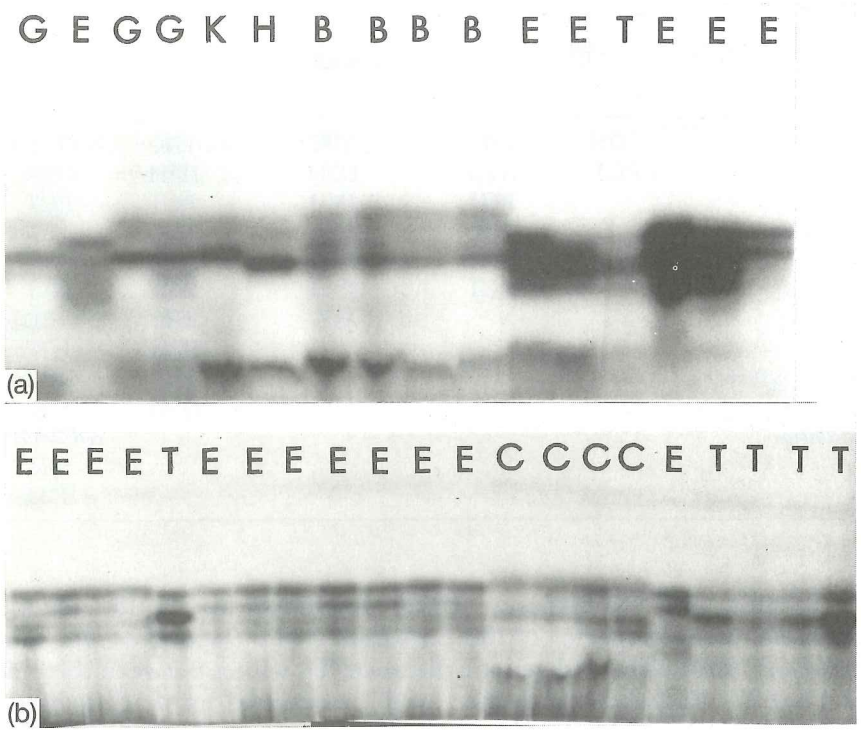

Fig. 12.1. Photographs of representative starch gels stained for esterases; species of the *Macrosiphum euphorbiae* group. (a) Samples of six species: E = *euphorbiae*, B = *euphorbiellum*, *G* = *gei*, H = *hellebori*, K = *cholodkovskyi*, T = *tinctum*. (b) 13 samples of *euphorbiae* (E), compared with four samples of *centranthi* (C), and five of *tinctum* (T).

examined, although the activity of individual allozymes varied greatly between samples (Fig. 12.1(b)).

The other enzyme systems examined provided at least one additional diagnostic locus for each species comparison (Table 12.2). *M. euphorbiae* and *M. tinctum* are the two species that are most difficult to separate morphologically and have most similar esterase patterns, yet they have different mobilities of 6-phosphogluconate dehydrogenase (6-PGD) and an isocitrate dehydrogenase locus (IDH-2). Contrary to the findings of Tomiuk and Wöhrmann (1983) in Germany, British populations of *M. euphorbiae* were found to be polymorphic at the IDH-1 locus. In North America, where *M. euphorbiae* is native, polymorphism has been found at a minimum of six loci, including IDH-1 and IDH-2 (Table 12.4; May and Holbrook 1978; Steiner *et al.* 1985*a*).

Table 12.2 Enzyme systems with diagnostic loci for six members of the *M. euphorbiae* group.

	tinctum	*hellebori*	*euphorbiellum*	*stellariae*	*gei*
euphorbiae	IDH	HK	HK	HK	α-GPDH
	6-PGDH	IDH	IDH	IDH	IDH
		PGI	PGI	PGI	PGI
tinctum		HK	HK	HK	α-GPDH
		IDH	IDH	IDH	IDH
		PGI	PGI	PGI	PGI
hellebori			HK	HK	α-GPDH
				IDH	HK
euphorbiellum				HK	α-GPDH
				IDH	HK
stellariae					α-GPDH
					HK
					IDH

Tomiuk and Wöhrmann (1983) examined up to 18 enzyme systems in three species of the *euphorbiae* group (*euphorbiae*, *gei*, and *hellebori*), and calculated genetic distance (D) values between them of 0.11–0.37. Eggers-Schumacher (1987) examined up to 12 enzyme systems in 8 species of the *euphorbiae* group (including one, *M. prenanthidis*, not included in Watson's study), and obtained values for the genetic distances between *euphorbiae*, *gei*, and *hellebori* comparable to those of Tomiuk and Wöhrmann. Both works also included data on *M. rosae* and *M. funestum*, two species which, in Watson's multivariate studies and according to most taxonomists, are placed outside the *euphorbiae* group. Again, the values of *D* they calculated between *rosae* and *funestum*, and between these two species and members of the *euphorbiae* group, agree fairly well. It is therefore instructive to compare phylogenetic trees produced from the two sets of data (Fig. 12.2(a,b)). The phylogeny produced by Tomiuk and Wöhrmann (Fig. 12.2(a)) accords with taxonomic relationships established on morphological grounds, the species of the *euphorbiae* group having a common ancestor not shared by *rosae* and *funestum*. A species of the genus *Sitobion*, which is sometimes considered as a subgenus of *Macrosiphum*, formed the nearest out-group. Tomiuk and Wöhrmann tried various methods of constructing phylogenetic trees, which for this group of taxa all produced similar results. The phylogeny obtained by Eggers-Schumacher, however, using another clustering technique, has *euphorbiae* less closely related to other members of the '*euphorbiae* group' than *rosae, funestum*, or another

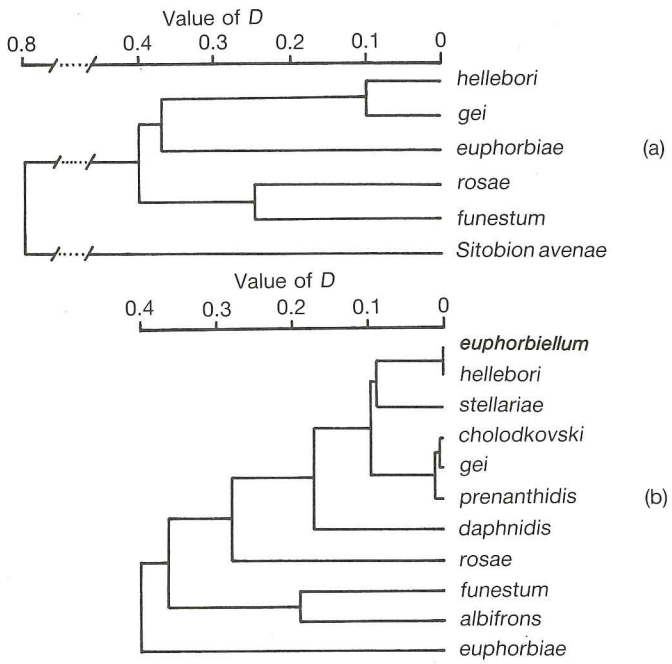

Fig. 12.2. Phylogenetic trees based on estimates of genetic distance (*D*) from allozyme data obtained by (a) Tomiuk and Wöhrmann (1980); (b) Eggers-Schumacher (1987).

species that is very different morphologically, *M. albifrons*. This illustrates the need for caution in constructing phylogenies on the basis of allozyme data alone, even at the intrageneric level of classification.

2. *Acyrthosiphon malvae*

A. malvae and its relatives colonize various plants in the families Geraniaceae and Rosaceae. Most species are of little economic importance, but some transmit viruses of cultivated strawberries, or infest zonal pelargoniums. As in the *M. euphorbiae* group, taxonomic confusion is rife. Eight taxa are recognized in the literature, most of which are usually given subspecific rank (e.g. Müller 1983). One of us (A.D.S.) has recently completed a morphometric and electrophoretic study identifying five new species, and demonstrating in particular that three separate taxa have been confused under the name of the strawberry aphid, *A. rogersii*.

Table 12.3 Enzyme systems with diagnostic loci for seven species of the *Acyrthosiphon malvae* group (letters apply to species listed in the legend to Fig. 12.3).

	C	D	E	F	H	I
A	HK	APH HK	HK	APH HK	HK MDH	HK
C		APH HK	HK	APH HK	HK MDH	
D			HK	APH HK	APH MDH	APH HK
E				HK	HK MDH	HK
F					APH HK MDH	APH
H						HK MDH

Attempts to use identical techniques of starch gel electrophoresis to those of Watson (1982) resulted in very poor resolution of bands, and polyacrylamide gels were therefore used. Differences in the relative quality of the results obtained with starch and polyacrylamide have been noticed for several different aphid genera, and are difficult to explain. Using polyacrylamide gels, 6 of the 16 enzyme systems investigated on nine taxa of the *A. malvae* group gave good staining and resolution. Esterase differences, despite the wealth of bands and the impossibility of genetic interpretation, again provided very good discrimination between the putative taxa recognized by multivariate morphometrics (Fig. 12.3; Table 12.3). A polymorphism of the fast-moving multimeric esterase of the species on *Geranium robertianum* was easy to recognize (Fig. 12.3,A), and not a source of confusion. The species that were most similar morphologically (B,C,G,H) had readily identifiable patterns of esterase mobility.

Of the other systems analysed, hexokinase was very polymorphic within species, but had species-specific patterns in all except two of the taxa examined. The newly recognized *Fragaria*-feeding species had two additional MDH bands in comparison with other species, and the new species distinguished on *Potentilla reptans* and *G. robertianum* had characteristic mobilities of APH-2.

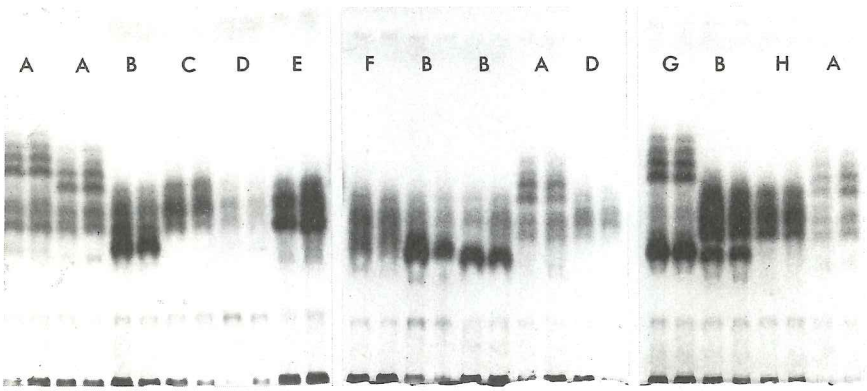

Fig. 12.3. Photographs of representative polyactylamide gels stained for esterases; species of the *Acyrthosiphon malvae* group. The pairs of samples consist of two individuals from a single clone. A = *Acyrthosiphon sp. nov.* from *Geranium robertianum*, B = *A. malvae* (Mosley), C = *A. geranii* (Kalt.), D = *A. poterii* Prior and Stroyan, E = *Acyrthosiphon sp. nov.* from *Geranium sanguineum*, F = *sp. nov.* from *Fragaria vesca*, G = *sp. nov.* from *Geranium palmatum*, H = *sp. nov.* from *Potentilla reptans*.

3. Acyrthosiphon pisum

The pea aphid is part of a complex of races or incipient species with different host-plant ranges and preferences. In Europe, several speciation events seem to be currently in progress, with parts of the original *pisum* genome in the process of being separated in aphids on *Ononis, Lotus, Sarothamnus*, and probably on *Pisum sativum* itself. The form introduced into North America and Australia is particularly destructive to alfalfa. In European populations, there is an MDH-1 polymorphism (Suomalainen *et al.* 1980), and at least in British populations there is also a polymorphism at the EST-3 locus (Fig. 12.4). The frequencies of alleles at this locus may vary between *pisum s. str.* and the forms on *Ononis* and *Sarothamnus*, respectively, but more samples are needed to confirm this. There is, however, a clear difference in mobility of glutamate-oxaloacetate transaminase (GOT) between *A. pisum* and the form (= *A. spartii*) on *Sarothamnus* (A. D. Seccombe, unpublished data). No allozyme data are available for populations outside Europe, although much work has been done in North America on the variability of *A. pisum* with respect to a variety of traits.

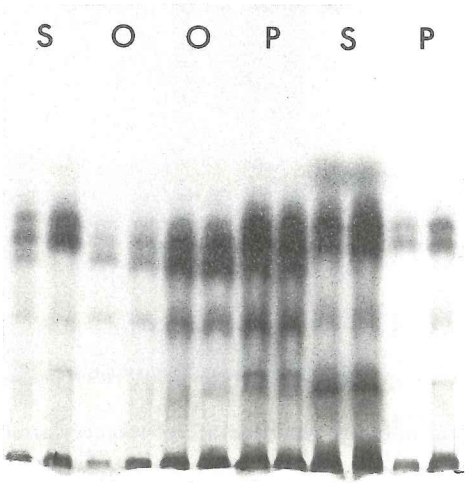

Fig. 12.4. Photograph of polyacrylamide gel stained for esterases; species of the *Acyrthosiphon pisum* group. Each pair of samples comprises two individuals of a single clone. O = *ononis* Koch, P = *pisum* Harris, S = *spartii* Koch.

4. Myzus persicae

The peach-potato aphid is well-known as a major crop pest throughout the world, and is probably the most polyphagous aphid species as well as the most important virus vector. In Europe, it has several close relatives with more specific host-plant associations. The problems of distinguishing alatae of *M. persicae* from those of *M. certus*, a common *Stellaria*- and *Viola*-feeding species in both Europe and North America, have long been recognized (Hille Ris Lambers 1959). Recently it has been shown that two other taxa, *M. antirrhinii* and *M. nicotianae*, can be separated from *M. persicae s. str.* using multivariate techniques (Blackman and Paterson 1986; Blackman 1987). However, morphological discrimination of these taxa is only possible using rather complex arithmetic functions involving several characters, and even then is not totally reliable for the identification of individual aphids.

Differences at esterase loci between *persicae* and *antirrhinii* have been noted by ffrench-Constant *et al.* (1988), and provide an easy method of distinguishing these taxa, especially as the esterase-4 activity of *M. persicae* is now routinely monitored because of its involvement with resistance to organophosphate and carbamate insecticides. *M. certus* also has a characteristic pattern of esterase bands, enabling it to be

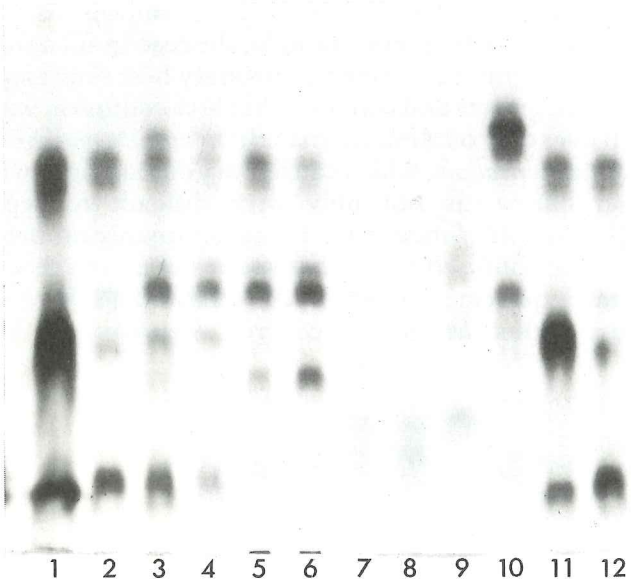

Fig. 12.5. Photograph of a polyacrylamide gel stained for esterases; clonal samples of *Myzus persicae* and its relatives. 1 and 11, *persicae* (insecticide resistant), with high EST-4 activity; 2 and 12, *persicae* (susceptible); 3 and 4, *antirrhinii*; 5 and 6, *certus*; 7 and 8, *cymbalariae*; 9, *ascalonicus*; 10, *ornatus*.

distinguished readily from these two species (Fig. 12.5). Other close relatives of *M. persicae* examined (including two not shown in Fig. 12.5: *M. myosotidis* on *Myosotis palustris* and *M. dianthicola* on *Dianthus caryophyllus*) all have characteristic esterases. The widely-distributed shallot aphid, *M. ascalonicus*, is often confused with the morphologically similar *M. cymbalariae*, and they often colonize the same plants (Brown 1983), yet they are easy to discriminate electrophoretically (Fig. 12.5).

No specific electrophoretic comparison has yet been made between *M. persicae* and the tobacco-feeding form *M. nicotianae*. However, May and Holbrook's (1978) survey of US populations of *persicae*, which amazingly failed to detect any variability at all at 19 enzyme loci, included specimens taken from tobacco in Maryland (population no. 2), and these were presumably *nicotianae*.

5. *Metopolophium dirhodum* and *M. festucae cerealium*

These cereal pests are relatively easy to tell apart, but each has close relatives on wild grasses that are virtually sibling species, and can

only be distinguished morphologically by use of multiple discriminant functions (Stroyan 1982). For example, the rose-grain aphid *M. dirhodum*, typically migrating between its primary host *Rosa* spp. and numerous species of grasses and cereals, is liable to confusion with *M. fasciatum*, which lives only on *Arrhenatherum elatius* and *Bromus carinatus*. Similarly, *M. festucae cerealium*, which can be very damaging to winter-sown oats and barley, is not only very difficult to separate morphologically from *M. festucae s.str.* living commonly on meadow grasses, but may be confused with other species of more specialized food and habitat requirements such as *M. albidum* and *M. tenerum*. Probably there are other, as yet undiscovered, *Metopolophium* species

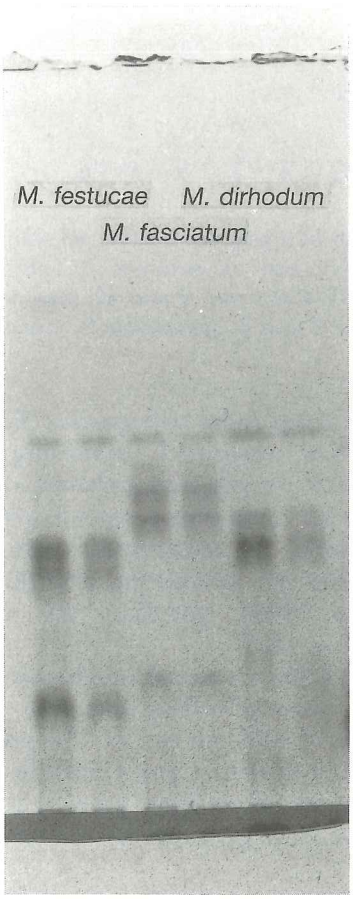

Fig. 12.6. Photograph of a polyacrylamide gel stained for esterases; samples of two clones of each of three *Metopolophium* species.

that will confuse the situation still further, especially in continental Europe where the group is in need of more detailed study.

One of us (C. F.) has examined the esterases of British populations of this group using polyacrylamide gel electrophoresis and isoelectric focusing. Differences in electrophoretic mobility of one or more esterases were found between *M. dirhodum, M. fasciatum, M. festucae, M. tenerum*, and *M. albidum* (Figs. 12.6, 12.7). Isoelectric focusing provided a much clearer separation, especially of *M. dirhodum* from *M. fasciatum* (Fig. 12.8), and of *M. festucae* from *M. tenerum*. The esterase system of *M. f. cerealium* was, however, not distinguishable from that of *M. festucae s. str.*

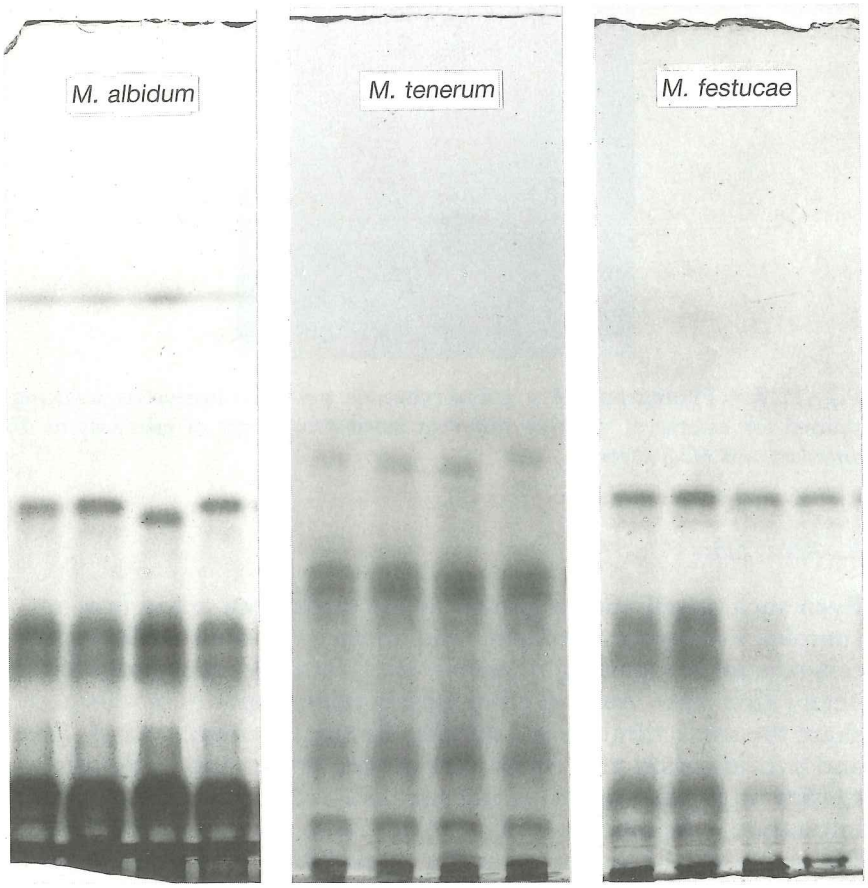

Fig. 12.7. Photograph of a polyacrylamide gel stained for esterases; samples of four clones of each of three *Metopolophium* species.

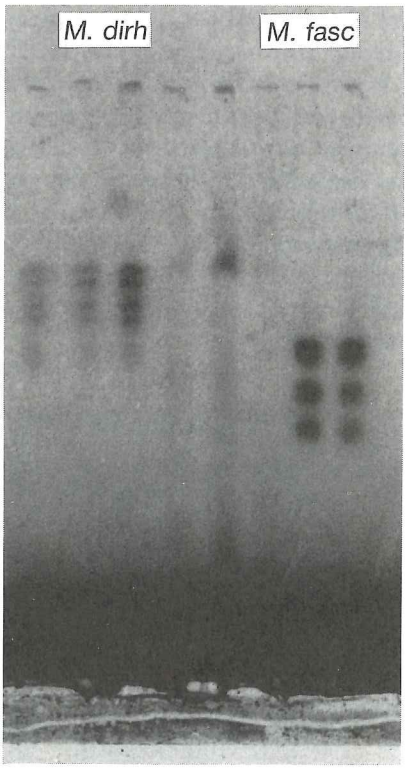

Fig. 12.8. Photograph of a polyacrylamide gel after isoelectric focusing, stained for esterases, showing different isoelectric points of esterases of *M. dirhodum* and *M. fasciatum*.

6. *Aphis fabae*

Even such a well known species as the black bean aphid presents a complex taxonomic problem, and in fact the only really reliable criterion for recognizing *A. fabae s. str.* is its ability to colonize broad bean (*Vicia faba*). Several other closely related species or subspecies share the same winter host (spindle, *Euonymus europea*) with *A. fabae*, and hybridization may sometimes occur (Müller 1982). Population studies of *A. fabae* on its winter host are used in Britain to predict infestation of field beans (Way *et al.* 1977), so it is important to clarify the taxonomy of the group as far as possible. *A cirsiiacanthoides* in particular is a common aphid on thistles and overwinters as eggs on *Philadelphus, Viburnum*, and *Euonymus*; on the latter host it is almost certainly still confused with *A. fabae* (Müller and Steiner 1986).

Furk (1979) collected samples of the black bean aphid group from *Tropaeolum*, *Calendula*, and *Dahlia*, none of which would transfer to *Vicia faba*, and compared their acid phosphatases, esterases, and total soluble proteins (the latter by isoelectric focusing) with those of 13 samples of *A. fabae s. str.*. The *Tropaeolum* aphids had a unique acid phosphatase pattern with additional bands that were not present in any of the other samples. This aphid was probably *A. mordwilkoi*, which is believed to colonize *Arctium lappa* and *Tropaeolum majus* in summer, and overwinter on *Viburnum opulus; A. barbarae*, described from *Arctium* and *Tropaeolum* in North America, may also be this aphid.

Isoelectric focusing revealed protein differences between *A. fabae s. str.* and the samples from *Calendula* and *Dahlia*; the *Calendula* aphids also showed an esterase difference. One of these samples may have been *A. cirsiiacanthoides*. Kiser (1979) found small differences between *A. fabae*, *A. cirsiiacanthoides* and *A. solanella* using thin-layer chromatography, and Odermatt (1981) found that *A. cirsiiacanthoides* had a diagnostic fast form of phosphoglucomutase.

However, the taxonomic significance of much of this variation is uncertain, since *A. fabae* itself also shows considerable variation between samples in the numbers and positions of certain bands. The *A. fabae* group clearly needs further detailed study in which allozyme data are correlated with the results of host-plant transference tests and morphometric analyses for numerous clonal samples from different sources.

7. *Aphis gossypii*

The melon or cotton aphid has a world-wide distribution on numerous plants, and pest populations probably comprise an indefinite number of permanently parthenogenetic lineages, some of which may have particular host plant associations. Until recently, the taxonomic situation with regard to *A. gossypii* in British glasshouses appeared to be relatively simple, with one form on cucumbers and another on chrysanthemums, only the latter having acquired resistance to insecticides. Furk *et al.* (1980) found that the esterases of these two forms could be distinguished, both by conventional starch gel electrophoresis and by isoelectric focusing.

One of us (C.F.) has shown recently that carbamate resistance is now present in populations on glasshouse cucumbers in England. The resistant aphids on cucumbers have the esterase pattern and morphology (V.F. Eastop, pers. comm.) of the chrysanthemum-colonizing form. In laboratory transference tests, the resistant

cucumber aphids have so far shown reluctance to feed on *Chrysanthemum*. Nevertheless, it seems likely that the chrysanthemum-feeding form has acquired the ability to feed on cucumbers, rather than the cucumber form having evolved insecticide resistance.

An available name for the insecticide-resistant aphid is *Aphis parva* Theobald, which was described from *Chrysanthemum* in Egypt; however, the application of this name outside Britain, where there may be numerous other parthenogenetic lineages of the *A. gossypii* group, may be difficult.

8. *Aphis pomi* and *A. spiraecola*

There has in the past been considerable confusion, particularly in North America, between the green apple aphid, *Aphis pomi* de Geer, and the more polyphagous citrus or spiraea aphid, *A. spiraecola* Patch. Both species form very similar colonies on woody Rosaceae, although they can be reliably distinguished by several morphological characters when viewed under the microscope. Probably a recent example of this confusion is the report by Singh and Rhomberg (1984) of a consistent association of electromorphs for GOT and two esterase loci in populations sampled from apple in southern Ontario. They concluded that two 'differentiated, non-interbreeding subpopulations' were present. It seems probable that one of these was *A. pomi*, and that the other was *A. spiraecola*.

Steiner *et al.* (1985*a*) found differences at three loci (EST, MDH, ALD) between samples of *A. spiraecola* collected on *Rumex* and *Spiraea*, but more extensive sampling would be required to show whether there was any consistent association between electromorph and host plant.

9. *Other species complexes involving pest aphids*

There are a number of other species complexes that have been looked at electrophoretically, and in some cases the allozyme data either confirm the separation of previously suspected sibling species or suggest the presence of unsuspected ones.

The Fordini, which colonize the roots of grasses and cereals as their secondary hosts, are mostly very specific in their choice of *Pistacia* species as primary host plants. However, aphids identified as *Geoica utricularia* produce galls on both *P. atlantica* and *P. palaestina* in Israel. Koach and Wool (1977) found that all samples from *P. atlantica* had an additional esterase band when compared with those from *P. palaestina*, the constancy of this difference making it seem certain

that two host-specific taxa were involved. The esterases of populations currently regarded as *G. utricularia*, on grasses and cereals in various parts of the world, have not been examined.

The willow-carrot aphid, *Cavariella aegopodii*, has a number of close relatives that share *Salix* species as primary hosts. One of these, *C. intermedia*, was for many years confused with *C. aegopodii*, and the apterous viviparae of the two species on *Salix* are difficult to separate morphologically (Hille Ris Lambers 1969), yet these species differ at at least five enzyme loci (Eggers-Schumacher 1987). The genus *Cavariella* appears to be a promising one for further electrophoretic study.

Detailed allozyme data might also contribute to the taxonomy of another genus, *Dysaphis*, in which morphologically similar species share primary hosts in the Rosaceae, some being pests of pome fruits. Eggers-Schumacher (1987) was able to find diagnostic loci for seven out of the eleven species he tested, and further electrophoretic work may help to show the extent of the natural hybridization thought to occur in this genus (Stroyan 1958).

The cherry blackfly, *Myzus cerasi* F., which has *Prunus cerasus* as primary host and various secondary hosts including *Galium* and *Veronica*, is one of a complex of species and/or subspecies with different host associations. Gruppe (1988) found populations on sweet cherry, *Prunus avium*, provisionally assigned to ssp. *pruniavium* Borner, to have a consistent esterase difference from *M. cerasi s. str.*

Without giving details of the enzyme systems involved, Eggers-Schumacher (1987) reported that the polyphagous aphid *Brachycaudus cardui* could be separated electrophoretically from closely related species living monophagously on *Myosotis* and *Malva*; that enzyme studies are potentially useful for sorting out the taxonomy and host plant relationships of the *Brachycaudus* subgenus *Appelia*; and that populations of *Pemphigus* spp. on roots of secondary host plants, which are almost impossible to identify to species by their morphology, can mostly be characterized using electrophoresis, and identified with the very different-looking aphids from galls on *Populus* spp. (the primary hosts). Concerning the last-named genus, Setzer (1980) obtained evidence of intergall migration in a North American *Pemphigus* species by analysing variation at an esterase locus.

Concluding points

The cases reviewed above illustrate the potential value of standard electrophoretic procedures for identifying aphid pest species and

helping to clarify their taxonomic relationships. Generally speaking, as might be expected, the loci that are most commonly polymorphic within species also most often show differences between closely related species. However, in some cases, closely related taxa have fixed monomorphic enzymes of different mobilities, and sometimes systems such as esterases show such complex patterns of bands that the homologies between species are unclear.

In Table 12.4, we list the enzyme systems shown to vary in at least some populations of the main pest aphid species involved in taxonomically difficult species complexes. The scope for further work is apparent. For example, the studies of Loxdale and co-workers on *Sitobion avenae* and *S. fragariae* in Europe, where these two species are morphologically distinct, could provide a sound basis for investigations of the taxonomy of cereal-feeding *Sitobion* in other parts of the world such as Australasia, where a third species of intermediate morphology, *S. miscanthi*, confuses the issue.

Esterases alone can often provide the diagnostic loci needed for the characterization and identification of species, especially when electrophoretic data are supported by morphological and biological information. The well-known lack of variability in many other enzyme systems of aphids — particularly the Group I enzymes involved in central metabolic pathways — may not therefore be such a problem when electrophoresis is used for taxonomic purposes, as compared with population genetics. Nelson and Hedgecock (1980) suggested that trophic generalists — of which *Myzus persicae* and several other pest aphids seem to be prime examples — would tend to be more variable in their Group II enzyme systems: enzymes such as esterases that function in peripheral metabolism and process a variety of substrates (see Smith and Fujio 1982, for discussion of this topic). However, there are plenty of variable Group I loci now known, for example, in *Macrosiphum euphorbiae*, which is a very polyphagous species.

If more variation is needed, this may be obtained by use of isoelectric focusing (e.g. Furk *et al.* 1980), which also has the advantage that it is less sensitive to experimental and interpretational error than electrophoresis (Johnson 1973), or by serial electrophoresis in either one or two dimensions using different gel concentrations and/or run times (e.g. Loxdale *et al.* 1985*a*). Clonal aphid populations are particularly amenable to the latter approach. Serial electrophoresis has, however, rarely revealed any hidden variability at loci that are monomorphic using standard techniques (Lewontin 1985).

Finally, at the risk of stating the obvious, it seems necessary to

Table 12.4 Enzyme variability within pest aphid species and species complexes.

Pest species/ species complex	Variable systems or loci	Reference
Acyrthosiphon pisum	EST-3, GOT	This work
	MDH, PGI	Tomiuk and Wöhrmann (1980) and
	MDH-1	Suomalainen *et al.* (1980)
	EST, SDH	Weber and Wöhrmann, in Tomiuk and Wöhrmann (1984)
Acyrthosiphon malvae	APH-2, EST (several loci), HK, MDH	This work
Aphis gossypii	EST (several loci)	Furk *et al.* (1980)
Aphis fabae	APH, EST	Furk (1979)
	EST (several loci)	Beranek (1974), Beranek and Berry (1974)
	EST, HK	Tomiuk and Wöhrmann (1980)
	MDH, PGM	Odermatt (1981)
Aphis spiraecola	ALD, EST, HK, MDH, 6-PGDH	Steiner *et al.* (1985*a*)
Cavariella aegopodii	ADK, IDH-1, IDH-2, MDH, 6-PGDH, PGI	Eggers-Schumacher (1987)
Hyalopterus pruni	CA-1, CA-2, GPI	Spampinato *et al.* (1988)
Macrosiphum euphorbiae	EST (several loci), HK, IDH-1, α-GPDH, 6-PGDH, PGI	Watson (1982) and this work
	AAT, IDH-1, IDH-2, 6-PGDH	May and Holbrook (1978)
	ALD, EST-1, G-6-PDH, HK, 6-PGDH, PGM	Steiner *et al.* (1985*a*)

Metopolophium dirhodum	EST	This work
Metopolophium festucae	EST	This work
Myzus cerasi	EST	Gruppe (1988)
Myzus persicae	EST (several loci)	Brookes and Loxdale (1987), ffrench-Constant *et al.* (1988), and this work
Rhopalosiphum padi	PGM, MDH	Steiner *et al.* (1985*a*)
	EST, PHOS, GOT, ME, 6-PGDH, α-GPDH, SORDH, PEP, MDH	Loxdale and Brookes (1988)
Rhopalosiphum maidis	GOT, MDH, PEP	Steiner *et al.* (1985*a*)
	ALD, GOT, HK, IDH-1, MDH, PEP, PGI, PGM	Steiner *et al.* (1985*b*)
Schizaphis graminum	EST	Volkova and Titova (1983)
Sitobion avenae	6-PGDH, PEP (3 loci)	Loxdale *et al.* (1985*a*)
	AK, EST, α-GPDH, GOT, G-6-PDH, PEP, PGM, PHOS, POD	Loxdale *et al.* (1985*b*)
	EST (several loci)	Stribley *et al.* (1983)
Sitobion fragariae	GOT	Loxdale and Brookes (1987)

stress that the taxonomy of any group of organisms can only be improved if full consideration is given to *the cumulative total* of available knowledge about them. While electrophoretic methods may be very useful diagnostic tools for distinguishing between closely related aphids, it is most unwise to rely on enzyme differences alone for definitive answers about taxonomic and phylogenetic relationships within species groups containing pest aphids. A proper understanding can only be achieved by adopting a multidisciplinary approach, bringing together available information on host plants, life cycles, morphology, and karyotype, and taking all these into account when interpreting the results of an electrophoretic study.

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