Experimental & Applied Acarology, 8 (1990) 97–104
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Differentiation of Mite Species by using Cellulose Acetate and Equilibrium Polyacrylamide Gel Electrophoresis of Isoenzymes

B.J. HART[1], A. FAIN[2] and D.T. HART[3]

[1]*University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS (Great Britain)*
[2]*Institut Royal des Sciences Naturelles de Belgique, Rue Vautier, 29, B-1040 Bruxelles (Belgium)*
[3]*King's College London (KQC), University of London, Department of Biology, Campden Hill Road, London W8 7AH (Great Britain)*

(Accepted 11 October 1988)

ABSTRACT

Hart, B.J., Fain, A. and Hart, D.T., 1990. Differentiation of mite species by using cellulose acetate and equilibrium polyacrylamide gel electrophoresis of isoenzymes. *Exp. Appl. Acarol.*, 8: 97–104.

The isoenzymes of various species of medically and economically important mites were studied using cellulose acetate and equilibrium polyacrylamide gel electrophoresis. Interspecific differences in isoenzymes were found, which were species-specific. Intraspecific differences in isoenzymes were also found when individual mites were examined. The efficiency of each technique, and their use in various fields of acarology, are discussed. In addition, possible phylogenetic relationships as revealed by these techniques are suggested.

INTRODUCTION

The extremely powerful technique of isoenzyme electrophoresis is currently used to distinguish species with similar morphological characteristics, as well as to establish evolutionary relationships between species (e.g. Miles et al., 1979). This technique has as yet, however, scarcely been exploited to differentiate between mite species which are often extremely difficult to distinguish morphologically (e.g. Hart and Fain, 1988). Silberstein et al. (1979), Cicolani et al. (1981) and Dujardin et al. (1981) developed starch and amidon gel electrophoresis techniques suitable for use with mites and cellulose acetate plates, and gradient polyacrylamide gels have also been used, primarily for analysing gut contents of mite predators (Murray and Solomon, 1978; Solomon et al., 1985; Van der Geest and Overmeer, 1985; Heitmans et al., 1986; Easteal and

0168-8162/90/$03.50

Boussy, 1987; Dicke and de Jong, 1988). We have investigated cellulose acetate electrophoresis (CAE), as well as gradient equilibrium polyacrylamide gel electrophoresis (E-PAGE) as methods for differentiating between various species of medically and economically important mites, using their isoenzyme-banding patterns. The E-PAGE technique exploits the equilibrium migration of native proteins, separated by molecular mass (MM) and not exclusively by charge differences.

MATERIALS AND METHODS

Mite samples

A total of 16 mite species, representing 4 families and 11 genera of the suborder Astigmata, were involved in this study. These were reared in laboratory conditions of $25 \pm 2°C$ with 75% relative humidity, as described previously by Hart and Fain (1988). Adult males, females and tritonymphs were found to exhibit identical isoenzyme-banding patterns, although the bands from males and tritonymphs were less intense. Thus, fifty adult females of each species were freed of food particles and homogenised in 10 μl buffer for CAE or in 100 μl for E-PAGE. The homogenisation buffer consisted of 40m*M* tris acetate buffer and 2m*M* EDTA at pH 7.4, with 10% glycerol and 0.1% bromophenol blue. Homogenisation was performed on ice, where samples were sonicated (8 microns peak-to-peak) for 2 sec, followed by centrifugation for 1 min at 12 500 g. This procedure was repeated three times to ensure complete homogenisation, with the final centrifugation for 5 min to remove particulate material. The same procedure was used for the individual mite samples, but in these, individual adult females were homogenised in 2 μl or 20 μl buffer for CAE and E-PAGE respectively.

Cellulose acetate isoenzyme electrophoresis

Cellulose acetate plates (Helena, Beaumont, Texas, U.S.A.) were soaked for 30 min in the running buffer (40m*M* tris acetate, 2m*M* EDTA, pH 7.4) before application of 2 μl of mite sample (1 or 10 mites) using a Super CPK 8-sample applicator. The loaded CAE plate was then placed onto a Helena horizontal tank and run at 200 V, 2 mA for 30 min at $\pm 4°C$. After this time the plates were removed from the tank and stained for carboxylesterases for 1 h at room temperature with 100m*M* phosphate buffer, pH 6, containing 0.1% napthyl acetate and 0.1% fast garnet GBC salt.

Polyacrylamide equilibrium gel isoenzyme electrophoresis

Pre-cast polyacrylamide gels with a gradient of 4 to 30% (Phamacia, Milton Keynes, Great Britain) were used with a Pharmacia vertical gel apparatus. To optimise separation of isoenzymes, 4% polyacrylamide loading wells were cast and 20-μl samples (1 or 10 mites) were added. Gels were run using the tris

acetate buffer described above, with 100 V constant voltage at 10°C for 15 h, before staining for carboxylesterases as described for CAE. Molecular-mass markers were stained overnight using Coomassie blue and destained using 10% acetic acid, 20% methanol. The molecular-mass markers used were Thyroglobulin (MM 670 kDa), Ferritin (MM 440 kDa), Catalase (MM 232 kDa), Bovine Serum Albumin (MM 67 kDa) and Ovalbumin (MM 43 kDa) (Pharmacia gel filtration markers).

Densitometric scans

Polyacrylamide equilibrium gels were fixed in 5% glacial acetic acid, and densitometric scans were taken using a Chromoscam densitometer (Hart et al., 1987).

RESULTS

As can be seen in Fig. 1, CAE resulted in an acceptable separation of carboxylesterase isoenzymes. Obvious differences in both positive and negative isoenzyme migration were seen between each species. Ten mite samples gave easily detectable band intensity; however, isoenzymes from single mites could not easily be detected using this technique (data not shown).

Figure 2 illustrates the superior separation of isoenzymes obtained using the E-PAGE technique. Isoenzyme separation via molecular mass as well as charge reveals a completely different banding pattern to that seen in Fig. 1, and sheds

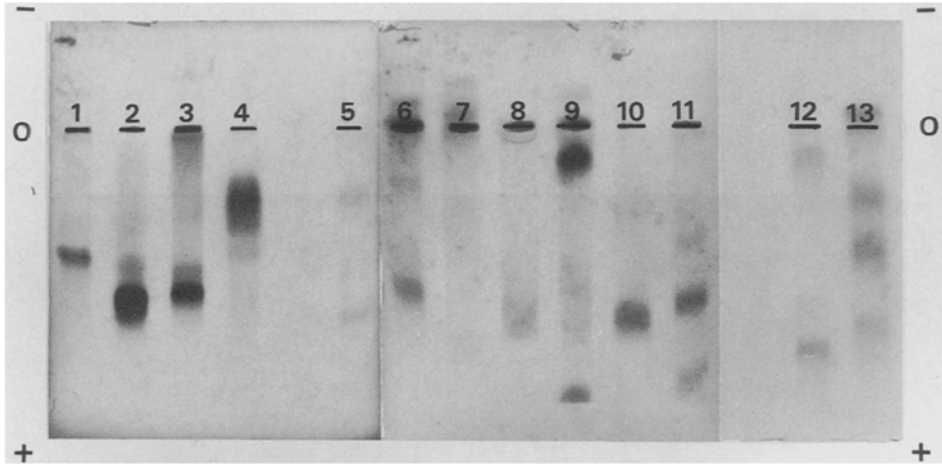

Fig. 1. Carboxylesterase isoenzymes separated from 13 mite species using cellulose acetate electrophoresis. (a) *Austroglycyphagus asthmaticus*; (2) *A. hughsae*; (3) *A. malaysiensis*; (4) *Lepidoglyphus destructor*; (5) *Blomia tropicalis*; (6) *Tyrophagus putrescentiae*; (7) *Acarus siro*; (8) *Carpoglyphus lactis*; (9) *Diamesoglyphus intermedius*; (10) *Euroglyphus maynei*; (11) *E. longior*; (12) *Dermatophagoides pteronyssinus*; (13) *De. farinae*.

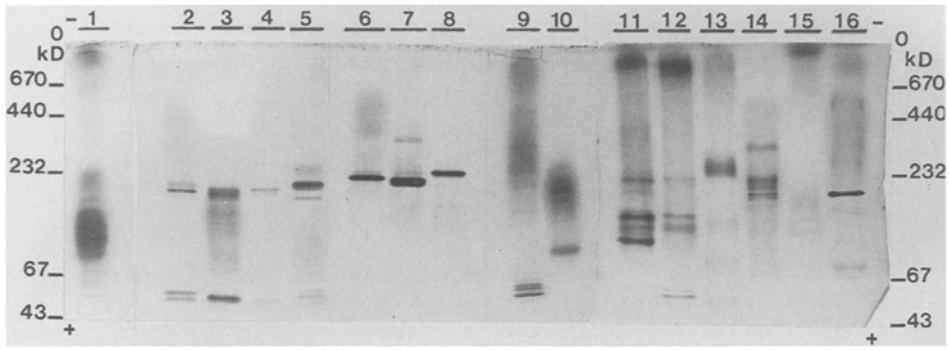

Fig. 2. Carboxylesterase isoenzymes separated from 16 mite species using equilibrium polyacrylamide gel electrophoresis. (1) *Blomia tropicalis*; (2) *Dermatophagoides pteronyssinus*; (3) *De. farinae*; (4) *Euroglyphus maynei*; (5) *E. longior*; (6) *Austroglycyphagus malaysiensis*; (7) *A. hughsae*; (8) *A. asthmaticus*; (9) *Glycyphagus domesticus*; (10) *Lepidoglyphus destructor*; (11) *Tyrophagus putrescentiae*; (12) *T. tropicus*; (13) *Acarus siro*; (14) *Suidasia pontifica*; (15) *Carpoglyphus lactis*; (16) *Diamesoglyphus intermedius*.

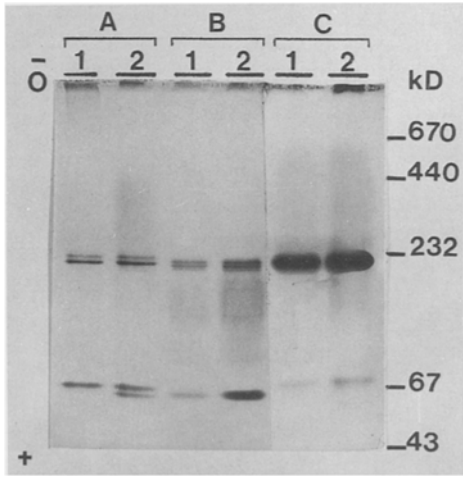

Fig. 3. Carboxylesterase isoenzymes separated from individual mites using equilibrium polyacrylamide gel electrophoresis. (A1) *Dermatophagoides pteronyssinus* individual mite; (A2) *D. pteronyssinus* pooled from 10 mites; (B1) *D. farinae* individual mite; (B2) *D. farinae* pooled from 10 mites; (C1) *Austroglycyphagus malaysiensis* individual mite; (C2) *A. malaysiensis* pooled from 10 mites.

new light on phylogenetic relationships. Although with this technique positively charged isoenzymes are not seen, the electrodes may be reversed to investigate these. Indeed, under the conditions used no major bands were lost, suggesting that for analysis of carboxylesterases of these mites, this technique was adequate. By the use of molecular-mass markers, E-PAGE also enabled

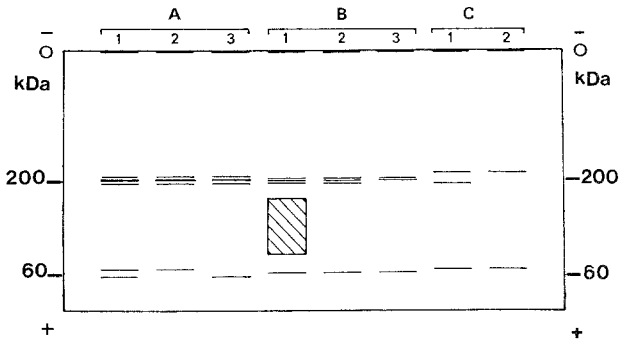

Fig. 4. Zymogram of carboxylesterase isoenzyme differences found between individuals of 3 species of mites, using equilibrium polyacrylamide gel electrophoresis. (A1-3) *Dermatophagoides pteronyssinus* individuals; (B1-3) *D. farinae* individuals; (C1-2) *Euroglyphus longior* individuals.

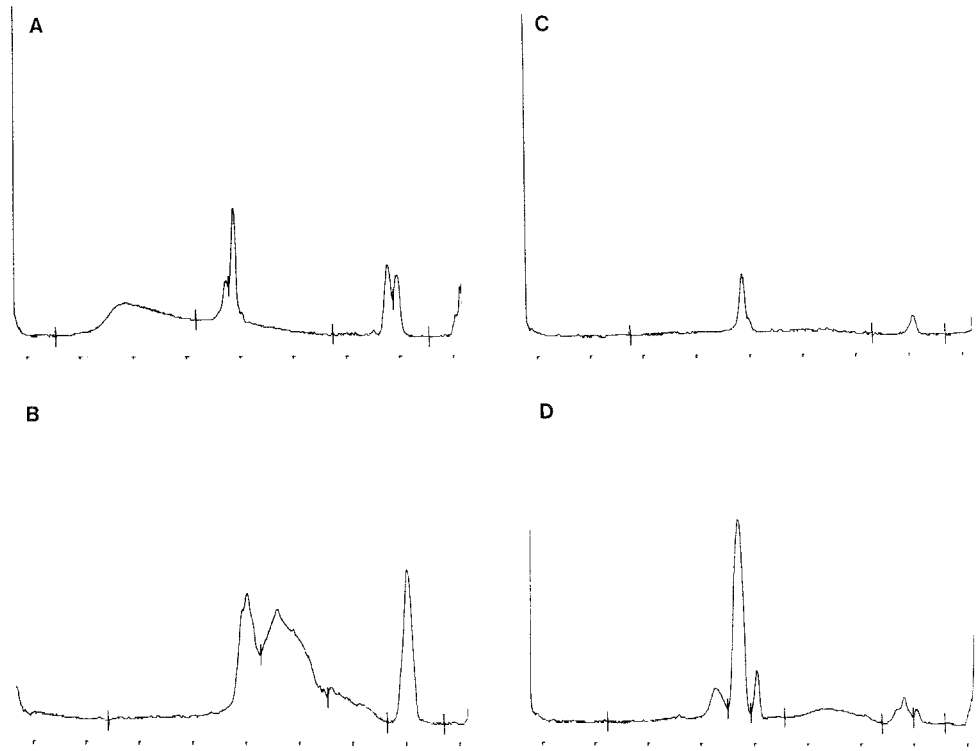

Fig. 5. Densitometric scans of carboxylesterase isoenzymes separated from *Dermatophagoides pteronyssinus* (A); *D. farinae* (B); *Euroglyphus maynei* (C); *E. longior* (D), from gels as shown in Fig. 2.

the estimation of molecular mass of the isoenzymes, as can be seen in Figs. 2–4.

Isoenzymes from single mites were clearly seen using this technique, as illustrated in Fig. 3. The isoenzymes in single *Dermatophagoides pteronyssinus*, *D. farinae*, *Euroglyphus maynei* and *E. longior* mites were further studied. Using a total of 20 individual mites, very interesting intraspecific differences were found in all species except *E. maynei*. The zymogram presented in Fig. 4 illustrates the differences found.

Interestingly, using the equilibrium isoenzyme technique, isoenzymes from the four species of Pyroglyphidae were found to be species-specific, but also showed striking similarities, in that all had carboxylesterase isoenzymes of approximately 200 and 60 kDa. In addition, the three *Austroglycyphagus* species all had a similar, very intense isoenzyme of 220 kDa. Finally, the two species of *Tyrophagus*, which are extremely difficult to distinguish morphologically, were easily distinguishable by isoenzymes at 90 and 65 kDa, although their close taxonomic relationship was illustrated by similar isoenzymes at 140 and 210 kDa.

Examples of densitometric scans of *D. pteronyssinus*, *D. farinae*, *E. maynei* and *E. longior* carboxylesterase isoenzymes separated using E-PAGE are shown in Fig. 5. These scans provide a useful quantitative analysis of the isoenzyme profiles. For example, it can be calculated from the integration of the area under each peak that *E. longior* has, in total, 4.7-fold higher carboxylesterase activity than *E. maynei*. We are presently using this technique to quantify common isoenzymes between species. In addition, we are currently using internal standards so that the scans can be used to accurately determine the molecular mass of individual isoenzymes.

DISCUSSION

The results presented herein have provided the first insight into the species-specific carboxylesterase isoenzymes of many mites, including such medically and economically important species as *Euroglyphus maynei* and *Glycyphagus domesticus*.

Of the two techniques developed for mites, CAE is extremely simple and quick and is therefore useful in taxonomic aetiology investigations. In particular, this technique could have promising potential for routine analysis of house dust, if background esterase activity was sufficiently low, and could be most useful for clinical diagnosis in desensitisation studies. E-PAGE, using pre-cast gels, is also applicable to these studies, but is rather more expensive and time-consuming. Nevertheless, the isoenzyme separation achieved by E-PAGE is far superior to that obtained with CAE, and therefore, E-PAGE is a better tool for closely examining inter- and intraspecific differences in mite isoenzymes and their molecular mass.

The new mite-homogenisation procedure developed during this study could be widely useful whenever soluble and particulate mite proteins are required. For example, a modified version of this technique has been used in immunological studies, where solubilised samples of allergenic mites were required for immunization of rabbits, cross-immunoelectrophoresis and Western blotting (Le Merdy et al., 1988).

The techniques of starch and amidon gel electrophoresis have previously been used to examine mite carboxylesterases; however, both CAE and gradient polyacrylamide gels used here and in other studies resulted in greatly improved resolution of these isoenzymes, using fewer mites. In CAE this is probably due to the very thin layer of cellulose used, and in PAGE, the use of gradient gels concentrates the isoenzymes to provide optimum resolution. Dujardin et al. (1981) described some differences in banding within populations of *D. farinae*. Our studies confirm these observations and, indeed, the improved resolution of the individual bands unequivocally confirm the occurrence of this interesting phenomenon.

Our results also provided interesting clues to the phylogenetic relationships between species of mites belonging to the same genus and between closely related genera. The Dermatophagoidinae and Pyroglyphinae had strikingly similar isoenzymatic-banding patterns, and therefore presumably they have a close phylogenetic association. Within each of these subfamilies, the species had very similar complements of carboxylesterase, again suggesting a recent speciation from a common ancestor.

The above-mentioned phylogenetic relationships corresponded to the similar morphology of the relevant four species. However, mites of the genera *Austroglycyphagus*, *Lepidoglyphus* and *Glycyphagus*, which are very similar morphologically and belong to the family Glycyphagidae, revealed strikingly different carboxylesterase isoenzymes, suggesting a more ancient divergence of these genera from a common ancestor or from different ancestors. Nevertheless, within the genus *Austroglycyphagus*, the three species studied had some isoenzymes in common, indicating their common ancestry.

Finally, *Tyrophagus* and *Acarus* mites are similar morphologically, but again the isoenzymes of each genus were quite distinct, suggesting a less recent or independent phylogenetic ancestry. *Tyrophagus putrescentiae* and *T. tropicus* are extremely difficult to distinguish morphologically, but isoenzymatic differences between these species were clearly seen using E-PAGE. Despite these differences, their speciation from a common ancestor was suggested by certain similar isoenzymes.

Thus, isoenzyme electrophoresis of mites may be beneficial not only to taxonomists, but also to phylogeneticists and ecologists. It is hoped, therefore, that the techniques described herein will encourage the further exploitation of this invaluable tool in these and other fields of acarology. Indeed, we are cur-

rently using both CAE and E-PAGE to study a range of isoenzymes with a view to furthering our knowledge of mite biochemistry and metabolism.

ACKNOWLEDGEMENTS

B.J. Hart was supported during this study by the Royal Society, London. We thank Dr. F.R. Opperdoes (ICP, Brussels) for the use of electrophoresis equipment.

REFERENCES

Cicolani, B., Bianchi Bullini, A.P. and Bullini, L., 1981. Morphological and genetic differentiation between *Macrocheles glaber* and *Macrocheles perglaber* (Acarina: Mesostigmata) Int. J. Acarol., 7: 221-224.

Dicke, M. and de Jong, M., 1988. Prey preference of the phytoseiid mite *Typhlodromus pyri*. 2. Electrophoretic analysis. Exp. Appl. Acarol., 4: 15-25.

Dujardin, J.P., Fain, A. and de Deken, B., 1981. Une microméthode d'électrophorèse en plaque d'amidon applicable à l'étude d'individus isoles de microarthropodes. Ann. Soc. Belge Med. Trop., 61: 467-475.

Easteal, S. and Boussy, I.A., 1987. A sensitive and efficient isoenzyme technique for small arthropods and other invertebrates. Bull. Entomol. Res., 77: 407-415.

Hart, B.J. and Fain, A., 1988. Morphological and biological studies of medically important house-dust mites. Acarologia, 29: 285-295.

Hart, D.T., Bouduin, P., Opperdoes, F.R. and de Duve, C., 1987. Biogenesis of the glycosomes in *Trypanosoma brucei*: Synthesis, translocation and turnover of glycosomal polypeptides. EMBO J., 6: 1403-1411.

Heitmans, W.R.B., Overmeer, W.P.J. and van der Geest, L.P.S., 1986. The role of *Orius vicinus* Ribaut (Heteroptera; Anthocoridae) as a predator of phytophagous and predacious mites in a Dutch orchard. Z. Angew. Entomol., 102: 391-402.

Le Merdy, L., Guerin, B. and Hart, B.J., 1988. Cross-reacting antigens and allergens of mites from house dust. In: A.L. de Weck and A. Todt (Editors), Mite Allergy — A Worldwide Problem. UCB Inst. of Allergy, Brussels, pp. 42-45.

Miles, M.A., Povoa, M.M., de Souza, A.A., Lainson, R. and Shaw, J.J., 1979. Some methods for the enzymic characterization of Latin-American *Leishmania* with particular reference to *Leishmania mexicana amazonensis* and subspecies of *Leishmania hertigi*. Trans. R. Soc. Trop. Med. Hyg., 74: 221-237.

Murray, R.W. and Solomon, M.G., 1978. A rapid technique for analysing diets of invertebrate predators by electrophoresis. Ann. Appl. Biol., 90: 7-10.

Silberstein, A.J., Fain, A. and Herin, A., 1979. Esterase isoenzyme patterns of some astigmatic mites. Int. J. Acarol., 5: 1-4.

Solomon, M.G., Murray, R.W. and van der Geest, L.P.S., 1985. Analysis of prey by means of electrophoresis. In: W. Helle and M. Sabelis (Editors), Spider Mites, their Biology, Natural Enemies and Control, Vol. 2. Elsevier, Amsterdam, pp. 171-173.

Van der Geest, L.P.S. and Overmeer, W.P.J., 1985. Experiences with polyacrylamide gradient gel electrophoresis for the detection of gut contents of phytoseiid mites. Meded. Fac. Landbouww. Rijksuniv. Gent, 50: 469-471.