

ESTERASE ISOENZYME PATTERNS OF SOME ASTIGMATIC MITES

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----- ABSTRACT— Isoenzymatic patterns were obtained from the following astigmatic mites: *Acarus siro* L., *Tyrophagus putrescentiae* (Schrank), *Dermatophagoides farinae* (Hughes), *D. pteronyssinus* (Trouessart), *Glycyphagus ornatus* (Kramer), *Lepidoglyphus destructor* (Schrank), *Blomia tropicalis* Bronswijk *et al.*, *Austroglycyphagus* sp. and *Chortoglyphus arcuatus* (Troupeau). The zymograms with their corresponding densitometric curves are compared and discussed. -----

INTRODUCTION

Isoenzymes are proteins of different molecular structure, with similar enzymatic activities (Markert and Møller, 1959). Because of their different electric charges, these enzymatic proteins can be separated by zone electrophoresis. Each of these proteins is coded by a chromosomal gene locus and its frequency in a species population obeys to the Mendelian laws (Harris, 1969). More than 100 isoenzyme groups have been shown to exist in a large number of species of vegetals and animals, from protozoa to insects and mammals (Ann. N.Y. Acad. Sci., 1968, 151, 1-689). These isoenzymes are characteristic of each species and have been used to separate sibling species, subspecies and strains. In arthropods they have been applied as a useful tool in systematics of mosquitoes (Bullini and Coluzzi, 1973).

In the Acari (Arachnida), comparisons have been made on amino acid patterns of three ticks belonging to the genus *Dermacentor* (Micks & Gibson, 1957) and hemolymph proteins patterns of 6 ticks belonging to the genera *Hyaloma*, *Argas* and *Dermacentor* (Van Sande & Karcher, 1960). Species differences were reported to be "striking" in both articles.

The present study compares the esterase isoenzymatic patterns of nine astigmatic mite: two Acaridae, *Acarus siro* L., 1758 and *Tyrophagus putrescentiae* (Schrank, 1781); two Pyroglyphidae, *Dermatophagoides farinae* (Hughes, 1961) and *Dermatophagoides pteronyssinus* (Trouessart, 1897); four Glycyphagidae, *Glycyphagus ornatus* (Kramer, 1881), *Lepidoglyphus destructor* (Schrank, 1781), *Blomia tropicalis* Bronswijk *et al.*, 1973 and *Austroglycyphagus* sp. n. (which will be described later); one Chortoglyphidae, *Chortoglyphus arcuatus* (Troupeau, 1879).

MATERIAL AND METHODS

A. siro, *T. putrescentiae*, *L. destructor*, *G. ornatus* and *C. arcuatus* were reared from hay dust sent by Dr. J. Centner from South-Eastern Belgium; strains of *D. farinae* and *D. pteronyssinus* were sent by Dr. J. E. M. H. van Bronswijk from the Netherlands and maintained in our laboratory; *B. tropicalis* was reared from bean-dust stored in the Ministry of Health of Bujumbura (Burundi, Africa) and collected by one of us (A. F.); *Austroglycyphagus* sp. was reared from the guano of a Mollosidae bat colony, collected by one of us (A. F.) in the roof of a house in Bujumbura.

Twenty males and twenty females of each species were homogenized in 12 μ l of demineralized water and these homogenates absorbed by pieces of Whatman n° 3 filter paper (5 x 3 mm). The filter papers were inserted in a slit cut in a starch gel at a concentration of 11 to 12% (Smithies,

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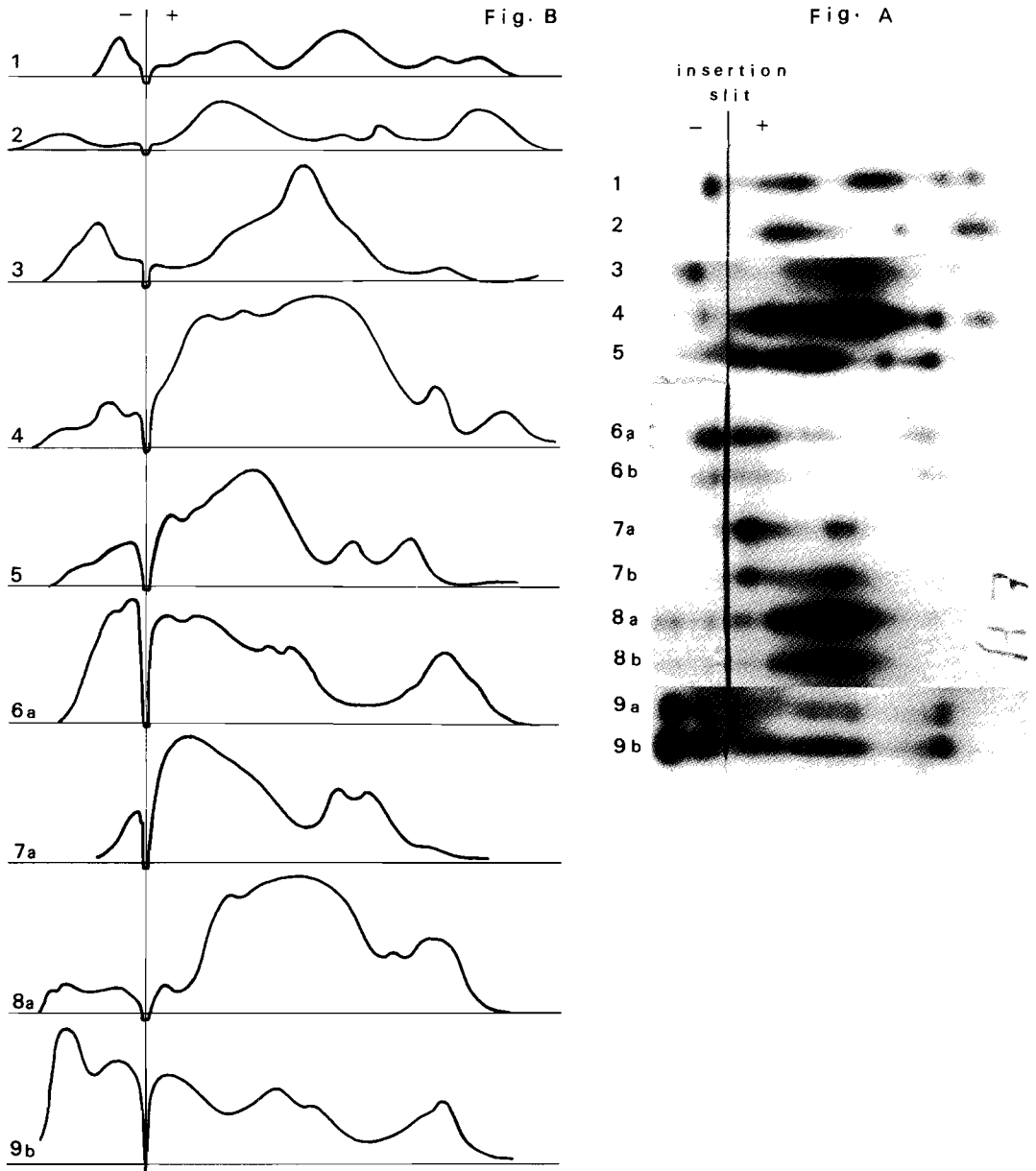


Fig. A (zymogram) and Fig. B (corresponding densitometric curves): 1, *Dermatophagoides farinae* ♀; 2, *D. pteronyssinus* ♀; 3, *Austroglycyphagus* sp. ♀; 4, *Glycyphagus ornatus* ♀; 5, *Blomia tropicalis* ♀; 6, *Tyrophagus putrescentiae* (a= ♀, b=♂); 7, *Chortoglyphus arcuatus* (a=♀, b=♂); 8, *Lepidoglyphus destructor* (a= ♀, b=♂); 9, *Acarus siro* (a=♂, b=♀).

1955). The buffer solutions (Selander *et al.* 1971) for the dilution of the starch (Connaught) and for the electrode baths were composed of TRIS-base (Sigma) and citric acid (Merck). A 5 Volt

current per cm of gel was maintained during 6 hours. Bromophenol blue drops, inserted in the slit, indicated the progress of the migration front. The gel is further (Pasteur et Sinègre, 1975) pre-incubated during 25 minutes in a buffer solution of 0.1 M di-sodium hydrogen phosphate (Merck) and 0.1 M potassium dihydrogen phosphate (Merck). This phosphate buffer was renewed before the esterase substrate, composed of a solution of 0.5% alpha- and 0.5% beta-naphthyl-acetate (Serva) in 75 ml acetone and 25 ml water, was added. Fast Garnett GBC salt (Serva) 100 mg, was after 15 min. added. Blue and red spots appeared rapidly after a few minutes. After about half an hour the gel was fixed in a 50% methanol and 10% acetic acid solution and 48 hours later, densitometric curves were taken (Quick Scan, Helena Lab., Texas).

RESULTS AND DISCUSSION

Seven to eight homogenates of males and females of each species were analysed, using 10 to 40 specimens in one homogenate. Constant distributions of the esterase isoenzyme spots in the gel were obtained for each species (these colored spot distributions are called "zymograms") (Fig. A). The intensity of coloration of the zymograms depended on the quantity of individuals homogenized. Ten individuals of the morphologically large species (Glycyphagidae) were enough to obtain a satisfactory zymogram, while, in the smaller species (Acaridae), 20 individuals were needed. Because males are smaller than females, zymograms of males were always less intensely colored than those of a same quantity of females (Fig. A: 6a to 9b).

In Diptera most of the isoenzymes migrate towards the positive pole, very few towards the negative one. In the present study of Acari, every species showed cathodal migrations: they were pronounced in the Acaridae (Fig. A: 6a, 6b, 9a, 9b) and were even the main spots in *Acarus siro*; they were barely present in *Chortoglyphus arcuatus* (Fig. A: 7a, 7b). The most distant anodal migration (about 5.5 cm for 6 hours migration) is reached by *Glycyphagus ornatus* (Fig. A: 4), the most cathodal one (about 1.5 cm) by the most distal of the three very distinct spots of *Lepidoglyphus destructor* (Fig. A: 8a, 8b). *Chortoglyphus arcuatus* (Fig. A: 7a, 7b) showed, close to the insertion slit, a heavy anodal spot and a weak cathodal one, which means that these isoenzymes are close to the isoelectric point; further this species shows only two isoenzymes more anodally. All these zymograms were quantitatively determined by their corresponding densitometric curve (Fig. B; numbering corresponding to Fig. A). The more intense the coloration of the spot on the zymogram, the higher the peak on the curve.

Striking indeed is the difference between the two species of the genus *Dermatophagoides* (Figs. A and B: 1, 2); *D. pteronyssinus* and *D. farinae* differed by their one cathodal spot: qualitatively by the migration distance (meaning a different electric charge due to a different molecular structure), and quantitatively by the intensity of colorations (meaning a greater quantity of enzymatic protein or a greater activity of it). These two species differed also obviously in their anodal isoenzymes. A first attempt may be done to compare the families: as mentioned already, the Acaridae (6a, 6b, 9a, 9b) show important quantity or/and very active cathodal isoenzymes, next to important groups of anodal ones, the Glycyphagidae (3, 4, 5, 8a, 8b) have several (less intense) cathodal isoenzymes but present mainly an anodal "big belly", followed by smaller but well distinct isoenzyme groups. The one Chortoglyphidae (7a, 7b) has few isoenzymes that remain the closest to the isoelectric point; the two Pyroglyphidae (1, 2) show "slender and long" zymograms.

CONCLUSION

Isoenzyme patterns (zymograms) are a useful tool to compare closely related and to help solve taxonomic problems. They can eventually be applied to compare generic and supra-generic groups with the purpose to establish phylogenetic relationships. We intend to study more species of one genus, in several groups, with the hope to achieve some of these aims.

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