Electrophoretic variation in esterases and phosphatases in eleven wild-type strains of *Metarrhizium anisopliae*[1]

E. de Conti, C. L. Messias, Hebe Myrina L. de Souza and J. L. Azevedo

Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, 13.100 Campinas/S. Paulo (Brasil), 9 April 1979

Summary. 11 wild-type strains of *Metarrhizium anisopliae* were electrophoretically tested for phosphatases and esterases. Phosphatase was homogeneous for all strains. For esterase it was possible to distinguish 5 different patterns with which it is possible to characterize the strains analyzed.

The electrophoretic patterns of soluble enzymes and other proteins from fungi represent a direct manifestation of the cell's genetic constitution, and can be specially valuable when utilized for taxonomic classification of these organisms. These patterns exhibit a lesser intraspecies than interspecies variation, but are still useful for the characterization of isolates within a species[2].

The objective of the present note was to characterize different isolates of *Metarrhizium anisopliae* var. *minor* in terms of esterase and phosphatase production. This entomogenous fungus is currently being employed in the biological control of the sugar cane spittlebug *Mahanarva posticata* (Homoptera, Cercopidae) in several regions in Brazil, and is a good candidate for the control of some pasture pests such as the spittlebugs *Zulia entreriana* and *Deois flavopicta* (Homoptera, Cercopidae).

Material and methods. 11 isolates of *Metarrhizium anisopliae,* var. *minor,* were analyzed by the electrophoretic method of enzymatic systems. 7 of the isolates, A_4, A_6, A_8, A_{18}, A_{19}, A_{20} and A_{21}, were collected in the state of Bahia; isolates E_6 and E_9 are from the state of Espírito Santo; and isolates C and K are from the states of Pernambuco and São Paulo, respectively (figure 1).

Mycelia were grown for 7 days at 28 °C in test tubes containing 5 ml of minimal medium ($NaNO_3$ 6 g, KCl 0,52 g, $MgSO_4$ $7H_2O$ 0.52 g, KH_2PO_4 1.52 g, $FeSO_4$ $7H_2O$ and $ZnSO_4$ traces), and glucose 10 g in 1000 ml distilled water at 4 °C according to the method described in de Souza et al.[3].

Horizontal electrophoresis on starch gel was used as described by Bush et al.[4]. Optimal resolution for phosphatase and esterase was obtained using 0.3 M borate buffer, pH 8.2, for the electrode, and 0.076 M Tris (hydroxymethyl) amino methane and 0.005 M citric acid buffer, pH 8.7, for the gel. Each gel was prepared with 46.5 g. hydrolyzed starch (Sigma Chemical Co.) in 300 ml gel buffer. To prevent heat denaturation and loss of enzymatic activity, the electrophoretic run was carried out at 4 °C. Migration was 12 cm for both types of enzymes starting from the point of sample application. Gels were sliced and incubated at 37 °C in appropriate staining solutions for esterase and phosphatase[5]. Stained gels were fixed in 5:1:5 solution of methanol, acetic acid and distilled water, respectively.

Results and discussion. Figures 2 and 3 schematically illustrate the electrophoretic patterns of the phosphatase and esterase systems for isolates of *M. anisopliae.* For the phosphatase system the gels were homogeneous for the isolates analyzed, all showing three isozymic bands. In contrast, the patterns obtained for the esterase system differed in band number and mobility among some of the isolates. It was possible to distinguish 5 isozymic esterase patterns, as shown in figure 3. Pattern 1 was detected in 6 out of the 7 isolates from Bahia: A_4, A_6, A_8, A_{18}, A_{20}, and for A_{21}; pattern 2 was detected in isolate A_{19}; pattern 3 in isolate K; pattern 4 in isolate C; and pattern 5 in isolates E_6

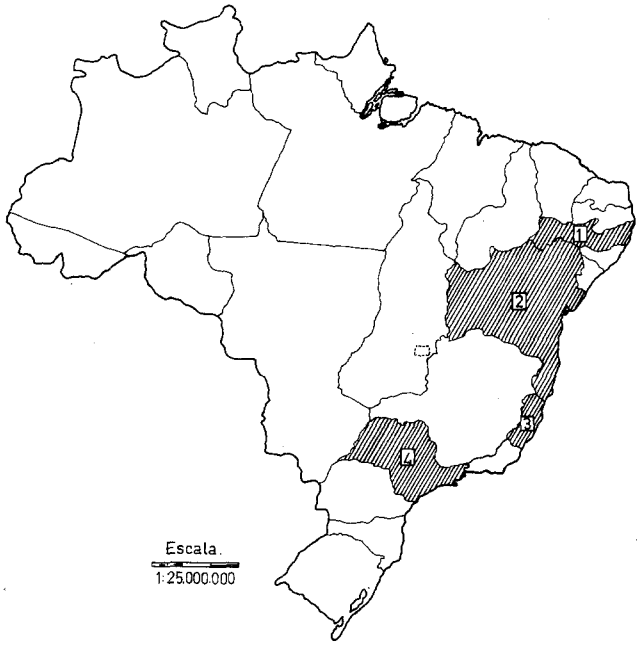


Fig. 1. Brazilian map showing the states where the strains were isolated. *1* Pernambuco; *2* Bahia; *3* Espírito Santo and *4* São Paulo.

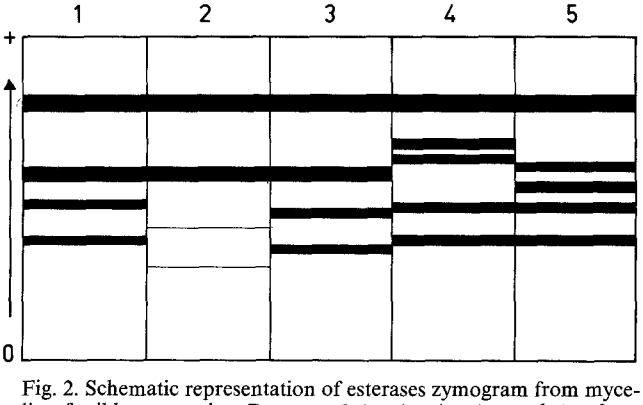


Fig. 2. Schematic representation of esterases zymogram from mycelia of wild-type strains. Patterns: *1* A_4, A_6, A_8, A_{20} and A_{21} from Bahia; *2* A_{19} from Bahia; *3* K from São Paulo; *4* C from Pernambuco and *5* E_6 and E_9 from Espírito Santo.

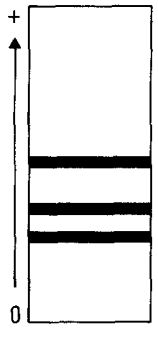


Fig. 3. Schematic representation of esterases zymogram from mycelia of 11 wild-type strains of *M. anisopliae.*

and E_9. Patterns 1, 2 and 3 were very similar, differing only in the mobility of bands 1 and 2. Patterns 4 and 5 differed in the mobility of bands 3 and 4. The only band which showed no differences in any of the patterns was the fast one, the most distant from the origin in figure 3.

These results show that the detected variability in the EST system of *M. anisopliae* permits characterization of the different isolates. Characterization is also possible through the use of other biochemical methods such as immunoelectrophoresis, as shown by Fargues et al.[6]. The possibility of the electrophoretic characterization of different strains of *M. anisopliae* is particularly promising for the improvement of pest-control techniques, since some of these isolates may well exhibit different pathogenicity.

1 Acknowledgment. The authors are thankful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and to the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) for financial assistance.

2 B.G. Clare, N.T. Flentje and M.R. Atkinson, Aust. J. biol. Sci. *21*, 275 (1968).
3 H.M.L. de Souza, I.R. Baracho, C.L. Messias and J.L. Azevedo, Experientia *33*, 1433 (1977).
4 G.L. Bush and R.N. Huettel, A manual of techniques - Project phase I. Int. Biol. Program, 1978.
5 S. Toledo and L.E. Magalhães, Ciência Cultura *25*, 1148 (1973).
6 J.M. Fargues, T. Duriez, S. Andrieu, R. Popeye and M.P. Robert, C.r. Acad. Sci. Paris *281* (1975).

Tetraploidy in *Protopterus* (Dipnoi)[1]

A. Vervoort

Institut Agronomique et Vétérinaire Hassan II (APESA), Département de Biologie Animale, 6, rue Mohammed Triki, Rabat-Agdal (Marocco), 17 May 1979

Summary. Comparative DNA values obtained by Feulgen cytophotometry for 3 species of *Protopterus,* as well as karyotype analysis, show the existence of a diploid-tetraploid relationship within the genus *Protopterus.*

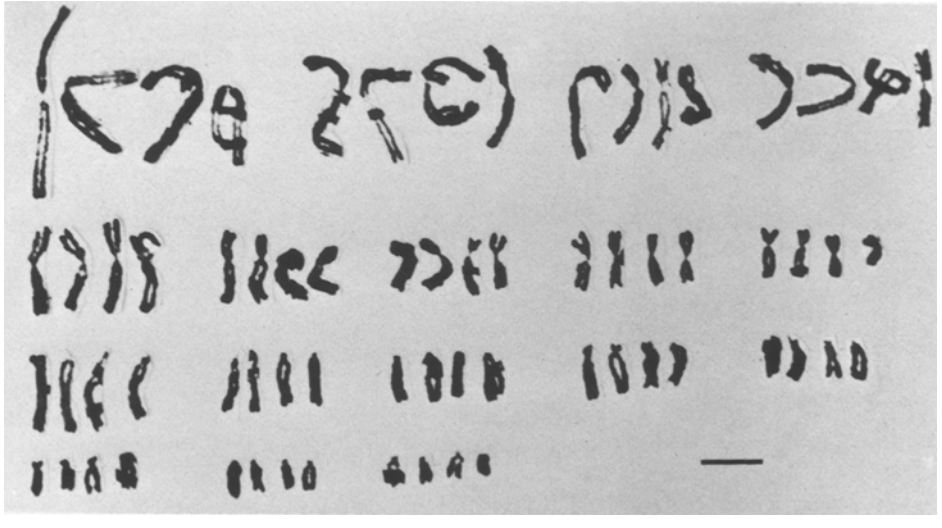
Lungfish (Dipnoi) today posses very large amounts of nuclear DNA (100–284 pg)[2–4] but rather few (32–38)[3,5,6] chromosomes. It has therefore been postulated[7] that their ancestors increased their genome size exclusively by tandem duplication, not by polyploidy.

In the case of the African genus *Protopterus,* with 4 species and 7 subspecies[8], 2 DNA values were reported: 100 pg for a non-identified species[2] and 284 pg for *P. aethiopicus*[4]. The question arises whether the discrepancy between the 2 values reflects biological variation or if it results from a difference in methods of measurement as was suggested[4].

In this study the DNA contents of *P. aethiopicus, P. dolloi* and *P. annectens* have been measured by Feulgen cytophotometry; in addition, the karyotype of *P. dolloi* has been studied for comparison with the previously reported[6] chromosome complement of *P. annectens.*

Materials and methods. 3 specimens of *P. dolloi* Boulenger, 1901, 3 specimens of *P. aethiopicus congicus* Poll, 1961, caught near Kinshasa, Zaire, and 4 specimens of *P. annectens annectens* (Owen), 1839, from the region of Dakar, Senegal, were used.

The Feulgen stain content of erythrocyte nuclei was measured with a Zeiss dual beam microspectrophotometer (UMSP I)[9] at 560 nm with a matched pair of 100× objectives. Blood samples which were to be compared in the same experiment were obtained simultaneously by heart-puncture from anesthetized fish and processed without storage. Drops of blood from 2 species (*P. dolloi* + *P. annectens* and *P. dolloi* + *P. aethiopicus*) were mixed on a slide before smearing; mixed cells could be distinguished as *P. dolloi* had much larger cells than the other species. Composite slides with chicken and *Protopterus* blood were also made. Slides were fixed with ethanol/acetic acid (3:1) for 30 min, hydrolysed with 5 N HCl at room temperature and Feulgen stained following the procedure of de Tomasi[10]. Because of the high density of *Protopterus* nuclei –



Karyotype of *Protopterus dolloi* (2n = 68); the bar equals 10 µm.