Advances in PARASITOLOGY

VOLUME 29

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PREFACE

This volume starts with a paper by David Godfrey and his colleagues on a topic that they have made very much their own—the study of isoenzymic variants within the rather enigmatic subgenus *Trypanozoon* of the genus *Trypanosoma*. As a result of an immense amount of work over several years, they have analysed (with the aid of a computer) the electrophoretic profiles of 11 enzymes from (literally) hundreds of populations of trypanosomes. The results of this monumental task, a summing up of years of work, very probably provide a definite answer to the question, debated ever since the original descriptions of the species at the turn of the century, of the relationship between the members of the so-called *T. brucei* group.

David Kemp, Alan Cowman and David Walliker take us into the molecular minefield of a topic that they, too, have lately made very much their own—the genetics of the genus *Plasmodium*. They discuss antigenic diversity and its significance in the topical field of vaccine production, chromosome size polymorphisms, meiosis and genetic recombination, and—another topical and important aspect—drug resistance.

Masamichi Aikawa and Carter Atkinson review a fairly recently developed technique, immunoelectron microscopy, and its applications within the discipline of parasitology. After a full technical, explanatory introduction, they discuss applications of the process to parasites both protistan (*Plasmodium*, *Toxoplasma*, *Sarcocystis*, *Eimeria*, *Theileria* and *Trypanosoma*) and helminthic (*Trichinella* and other nematodes, *Schistosoma* and *Fasciola*). The review ends with detailed appendices describing the techniques involved.

Jane Huffman and Bernard Fried write on a topic more purely helminthological, reviewing an interesting group of parasites which perhaps suffer somewhat from underexposure—the echinostomes. The authors first help to clear up the rather confused systematics of the group, partly caused by the lack of host specificity, and then comprehensively review the biology, immunology, epidemiology, physiology and biochemistry. The use of members of the group as easily maintained laboratory models is stressed.

Finally, Hilary Hurd ends this volume with a subject which should interest both protistologists (as we must learn to call them) and helminthologists—the complex and fascinating interactions between parasites and their invertebrate hosts. This, again, is a topic which tends to be overlooked. Volumes have been written (including many reviews in this series of *Advances*) about the relationships between parasites and their vertebrate hosts, but this review considers the much less fashionable, though biologically no less important, aspects of their relationships with invertebrates—including those which serve as vectors of parasites of man and domestic animals. Dr Hurd has synthe-

sized information from a wide variety of sources and has attempted a conceptual rather than descriptive treatment. The complex manner in which host and parasite interact to limit harmful effects but to perpetuate the relationship are indicative of highly co-evolved associations.

J. R. BAKER
R. MULLER

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I. Introduction

The morphological similarity of the behaviourally different kinds of trypanosome (*Trypanosoma* spp.) within the subgenus *Trypanozoon* has led to considerable uncertainty regarding the taxonomic status of each (see Hoare, 1972). The uncertainty is only beginning to be resolved by studies on enzyme polymorphism and deoxyribonucleic acid (DNA) (e.g., Godfrey and Kilgour, 1976; Gibson *et al.*, 1980, 1985; Borst *et al.*, 1981, 1987; Tait *et al.*, 1985; Paindavoine *et al.*, 1986; Godfrey *et al.*, 1987). On the other hand, epidemiology has benefited from an appreciation of the geographical and hostal distribution of the different genetic forms (e.g., Gibson *et al.*, 1978, 1980, 1983; Mehlitz *et al.*, 1982; Gibson and Gashumba, 1983; Gibson and Wellde, 1985; Otieno and Darji, 1985; Boid, 1988).

So far, much of the characterizing work has been based on the enzyme polymorphisms seen after electrophoresis and specific staining. Since each enzyme in a sample may appear as several isoenzyme bands, the use of a number of enzymes results in a complex profile for every trypanosome population examined. Although this genetically controlled profile remains consistent in a clone, the wide variety encountered means that the assessment of relationships is best addressed by mathematical methods (Gibson *et al.*, 1980; Tait *et al.*, 1984, 1985). It is possible that a new enzyme profile may be generated after hybridization between two different isolates (Jenni *et al.*, 1986; Sternberg *et al.*, 1988), although the frequency of this phenomenon in nature remains unknown (Cibulskis, 1988).

One purpose of this review is to present and compare the enzyme profiles of a large number of trypanosome populations from many countries. The results were obtained over some years by associated workers using thin layer

starch gel electrophoresis under similar conditions with the same range of enzymes. Many published results are presented again here for comparision and for inclusion in the numerical analyses, which were used to group related trypanosomes and to devise a practical identification system.

The evaluation of any group rests on comparing its epidemiological and other non-enzymic attributes with those of other groups. Greater confidence can be placed on validity if similar groupings are produced by different numerical approaches. Consequently, two methods were used to determine relationships and groups. A similar dual approach has demonstrated three genetically distinct groups in *T. congolense*, which were later confirmed by differences in satellite DNA (Gashumba *et al.*, 1988; Gibson *et al.*, 1988). Unfortunately, the divisions within the subgenus *Trypanozoon* were not so striking and required detailed consideration, as described below.

II. Materials and Methods

A. TRYPANOSOMES

1. *Isolation and preparation*

Summaries of the origins of the primary isolates used are listed in Table A3. The references quoted in the text and tables (see Appendix) describe in detail the means of isolating and preparing the trypanosomes. In brief, the organisms were isolated from the original host by inoculating rodents, before cryopreservation and subsequent further multiplication in rodents to provide sufficient material for examination. Sometimes this was achieved by immunosuppression of the animals with cyclophosphamide or γ-irradiation. The bloodstream forms were obtained from the host's blood by column separation and centrifugation (Lanham and Godfrey, 1970), followed by extraction of the water-soluble enzymes. The extracts were stored in liquid nitrogen.

The observations on a number of stocks have been reported previously (Table A2). Most of the new results were obtained from isolates collected in Zambia and West Africa; details of these in relation to local epidemiology will be published later.

2. *Definitions*

For simplicity, the term "animal", when used in this publication to describe the original host, refers to a mammal other than man (or, in one case, to a bird).

Some primary isolates contained several populations of *Trypanozoon*, as shown by differences in enzyme profiles (Tables A3 and A5), and it is likely that other mixtures were undetected. The term "population" is used to describe the trypanosomes harvested for analysis on a particular occasion.

The generalizations in the text regarding location of isolates, etc., in East or West Africa are related to an imaginary line running from north to south down the middle of the continent. This simple division permits a clearer view of the observations than if further geographical terms had been included, such as central and southern Africa.

B. ENZYME ELECTROPHORESIS

1. *Techniques*

The methods for electrophoresis and specific enzyme staining in thin layer starch gel are described by Bagster and Parr (1973), Kilgour and Godfrey (1973) and Gibson *et al.* (1978).

The results were obtained with the following 11 variable enzymes, listed from the least to the most polymorphic: EC 5.3.1.9, glucose phosphate isomerase (GPI); EC 3.2.2.1, nucleoside hydrolase (NH); EC 1.1.1.103, threonine dehydrogenase (TDH); EC 1.1.1.42, isocitrate dehydrogenase (ICD); EC 1.1.1.37, malate dehydrogenase (MDH); EC 2.7.5.1, phosphoglucomutase (PGM); EC 2.6.1.1, aspartate aminotransferase (ASAT); EC 2.6.1.2, alanine aminotransferase (ALAT); EC 3.4.11, two peptidases, (i) substrate L-leucyl-L-alanine (PEP2) and (ii) substrate L-leucylglycineglycine (PEP1); and EC 1.1.1.40, "malic" enzyme (ME).

2. *Terminology*

After electrophoresis and staining, each enzyme gave a pattern consisting of one or several isoenzyme bands. Unlike earlier work, every pattern was coded with an arabic instead of a roman numeral after the enzyme abbreviation (e.g., ALAT-1, ALAT-2) (Fig. 1). This change occupies less computer space and avoids the errors that may arise when entering complex roman numerals.

Enzyme patterns were numbered in sequence as new ones were found. During the separate investigations, the same pattern was at times coded differently. The coding is now standardized but with the consequence that the sequence is incomplete with certain enzymes (Fig. 1); better rationalization is not possible because many pattern numbers are already published.

In a trypanosome population, the combination of patterns for the 11 enzymes is the "enzyme profile"; populations with the same profile belong to

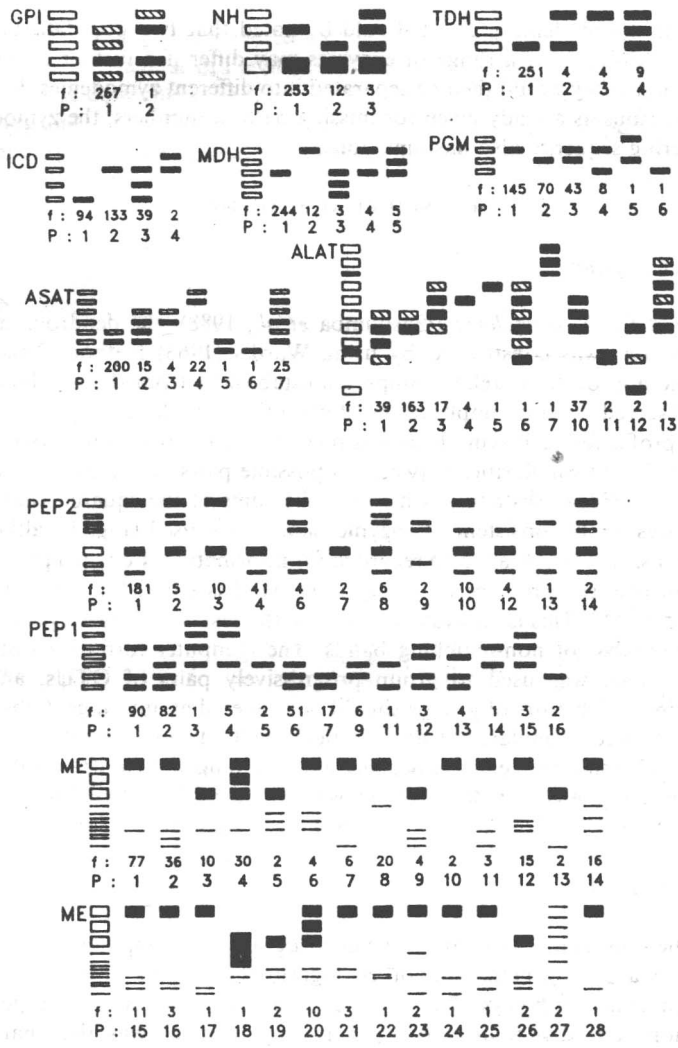

FIG. 1. Diagrams of enzyme patterns found in *Trypanozoon*. P, pattern number; f, number of zymodemes (total = 268) with pattern. All isoenzymes migrated towards the anode, represented by the top of each diagram. Faint or inconsistent bands are hatched. Only the bands that stained consistently were used in the numerical analyses. The full names of the enzymes are given in the text (Section I B 1, p. 4).

the same "zymodeme" (Z). It should be noted that two populations with identical profiles for a range of enzymes may differ if a further enzyme is introduced; they would then be separated into different zymodemes. For the same reasons as already given for missing pattern numbers, the zymodeme numbering sequence also has omissions.

C. NUMERICAL ANALYSES

1. *Dendrogram*

Like that for *T. congolense* (Gashumba *et al.*, 1988), the dendrogram for *Trypanozoon* was constructed by using Ward's (1963) method. This is a phenetic approach, as relationships are established from the attributes as now observed, with no implications of evolutionary relationships.

The profile for each zymodeme was regarded as an operational taxonomic unit (OTU). Dissimilarities between all possible pairs of OTUs were calculated as Euclidean distances, which are the sums of the squared character differences. Only consistent isoenzyme bands were used (Fig. 1), although the inconsistent bands are also recorded for information. For each pattern in every profile, the presence of a consistent band is entered as "1", and its absence as "0". This is equivalent to taking the distance between profiles to be the number of non-matching bands. The computer formed a distance matrix which was used to group progressively pairs of OTUs, and to determine which pairs of groups should be clustered at any stage. Gibson *et al.* (1980) used a similar, but not identical, method to construct a dendrogram for *Trypanozoon* enzyme profiles, as did Young and Godfrey (1983) for *T. congolense*. Another dendrogram was produced by Paindavoine *et al.* (1986), based on DNA digests of *Trypanozoon* after electrophoresis.

2. *Cladogram*

With the same raw data used for the dendrogram, the computer was used to construct a phylogenetic tree, or cladogram (Gashumba *et al.*, 1988), of developmental pathways that involved the minimal amount of genetic alteration. The dissimilarity between two zymodemes was the "patristic distance" (PD) between them through the cladogram. The measure of distance between OTUs was again the number of non-matching bands (Section II C 1). Since the dendrogram had nine obvious sections (Fig. 2), the cladogram was broken into the same number of clusters, by progressively cutting it to produce on each occasion the two best-resolved groupings of zymodemes.

When constructing the initial cladogram, the characters, or bands, were

weighted by using an iterative technique. Characters that changed many times during the traverse of the phylogenetic tree were given least weighting, while those seldom changing were considered potentially significant whenever they did alter. However, with so many characters available, this refinement did not appreciably modify the form of the cladogram.

The program was devised by R.D.B.

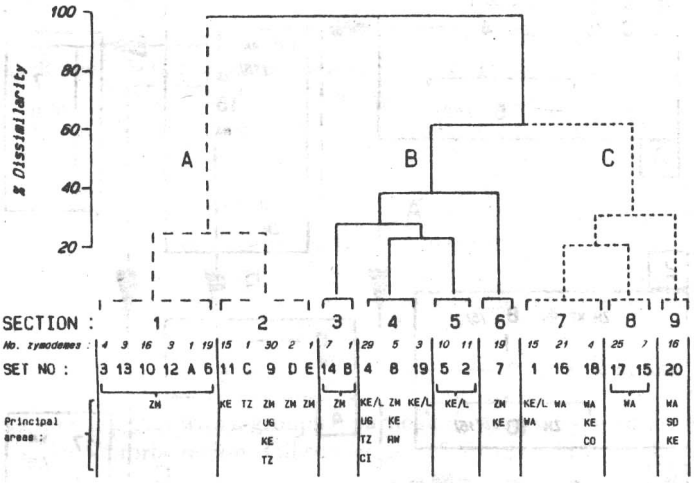

FIG. 2. Diagram representing the dendrogram of relationships between 268 *Trypanozoon* zymodemes. Three main divisions (A, B, C) are seen, and nine sections. See Table A1 for explanation of sets. CI, Ivory Coast; CO, Colombia, KE, Kenya; KE/L, Lambwe Valley, Kenya; RW, Rwanda; SD, Sudan; TZ, Tanzania; UG, Uganda; WA, West Africa; ZM, Zambia.

III. OBSERVATIONS AND COMMENTS

A. GENERAL

Altogether, 945 populations were examined, which were contained in the 268 zymodemes used in the analyses.

The enzyme patterns found are shown in Fig. 1, together with their frequency among the 268 zymodemes. The dendrogram is expressed diagrammatically in Fig. 2 and the cladogram in Fig. 3. Table A1 shows the zymodemes that remained together in both the dendrogram and the cladogram. These are numbered as "sets", and their positions can be found