

THE SUBSPECIFIC TAXONOMY OF *TRYPANOSOMA BRUCEI*

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Summary :

Trypanosoma brucei was first seen by David Bruce in 1894, in the blood of a cow in South Africa, and named in his honour in 1899. Trypanosomes seen in the blood of an Englishman in The Gambia in 1901 were named *T. gambiense* in 1902. Finally, in 1909, trypanosomes from the blood of an Englishman in Zambia ("Rhodesia") were named *T. rhodesiense*. Since then there has been continuous debate about the interrelationships of these three "species". Studies of the molecular biology of these trypanosomes, mainly analyses of their isoenzymes and deoxyribonucleic acid, now appear to have shown that *T. "rhodesiense"* cannot be distinguished from *T. brucei brucei* by any valid and consistent criterion, while *T. "gambiense"* probably does constitute a valid subspecies of *T. brucei*. There is still doubt whether populations of *T. brucei* are predominantly clonal or sexual. While some form of genetic exchange undoubtedly can occur in this species, its nature and frequency are unknown and there is evidence that the population structure of *T. brucei* is essentially clonal.

KEY WORDS : *Trypanosoma brucei*. history. subspecific taxonomy. population structure.

Résumé : LA TAXONOMIE SOUS-SPÉCIFIQUE DE *TRYPANOSOMA BRUCEI*

Trypanosoma brucei a été observé pour la première fois en 1894 par David Bruce, dans le sang d'une vache d'Afrique du Sud, et ainsi nommé en son honneur en 1899.

Les trypanosomes observés dans le sang d'un anglais en Gambie en 1901 furent appelés *T. gambiense* en 1902. Enfin, en 1909, les trypanosomes provenant du sang d'un anglais en Zambie ("Rhodésie") furent appelés *T. rhodesiense*. Depuis, les interrelations entre ces trois "espèces" ont fait l'objet d'un débat continu. L'étude de la biologie moléculaire de ces trypanosomes, principalement l'analyse de leurs isoenzymes et acide désoxyribonucléique, semblent avoir montré que *T. "rhodesiense"* ne peut être différencié de *T. brucei brucei* par aucun critère valable et permanent, alors que *T. gambiense* constitue vraisemblablement une sous-espèce de *T. brucei*. Il persiste un doute quant au caractère principalement clonal ou sexuel des populations de *T. brucei*. Bien qu'une mutation génétique puisse indubitablement survenir chez cette espèce, sa nature et sa fréquence sont inconnues et il est démontré que la structure démographique de *T. brucei* est essentiellement clonale.

MOTS CLES : *Trypanosoma brucei*. histoire. taxonomie sous-spécifique. structure démographique.

BACKGROUND

In November 1894, just over 100 years ago, David Bruce (Fig. 1) (then a captain in the British army and subsequently knighted as Sir David) and his wife Mary arrived in a small village ('a magistrate's office and a few mud huts' : Joubert *et al.*, 1993) called Ubombo (*Obonjeni* in the local language, according to Joubert *et al.*, 1993) in KwaZulu, Natal Province, South Africa. They had been sent there by the military authorities to try to discover the cause of a disease known as *nagana* which was devastating the cattle of the inhabitants of the region and the horses used by the administrators of what was then a British colony.

Bruce and his wife established their home (and laboratory) in a rectangular building (Fig. 2), built mainly of mud on a framework of wattle (interlaced branches of small trees and shrubs) apparently with a

foundation of stones (Joubert *et al.*, 1993; Cook, 1994).

Bruce started his investigation by examining daily the blood of a 'brown cow' suffering from *nagana*. He observed bacteria, which he at first thought were probably the cause of the disease, but on the sixth day he recorded in his notes the presence of 'Haematozoa' (MacArthur, 1955). He subsequently admitted (Bruce, 1915) that he originally thought these 'Haematozoa' were small filariae, but within a short time (probably before the end of 1894) Bruce had concluded that the haematozoa (Fig. 3) were the cause of *nagana* and also of 'tsetse fly disease' of humans, which until then had been thought to be a separate entity (Bruce, 1895, 1896). A few years later the 'Haematozoon' was named by Plimmer and Bradford (1899) as *Trypanosoma brucei* [sic], Bruce having sent an infected dog to England. The specific name, whether printed thus due to a typesetter's error or a lapse on the part of the authors, was soon corrected to *T. brucei* (Nabarro, 1907, p. 112 footnote 1, stated that the change was made by Laveran and Mesnil, but cited no reference).

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Fig. 1. – David Bruce (photograph taken probably in 1917, and kindly supplied by courtesy of the Administrator, Royal Society of Tropical Medicine and Hygiene).

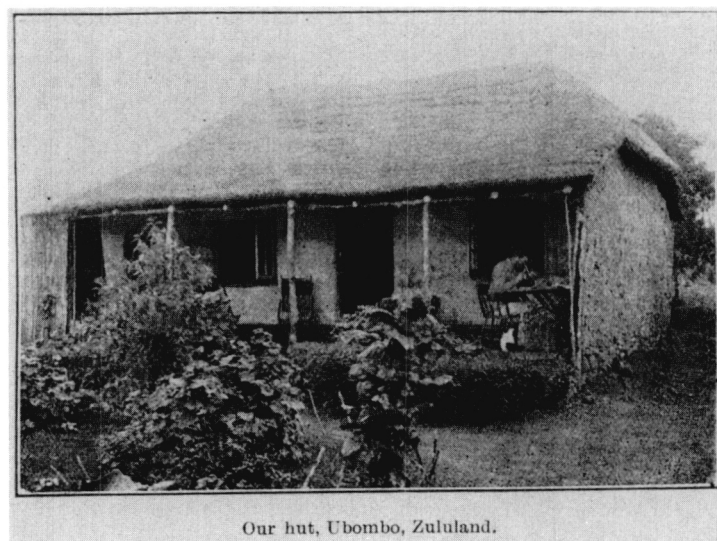
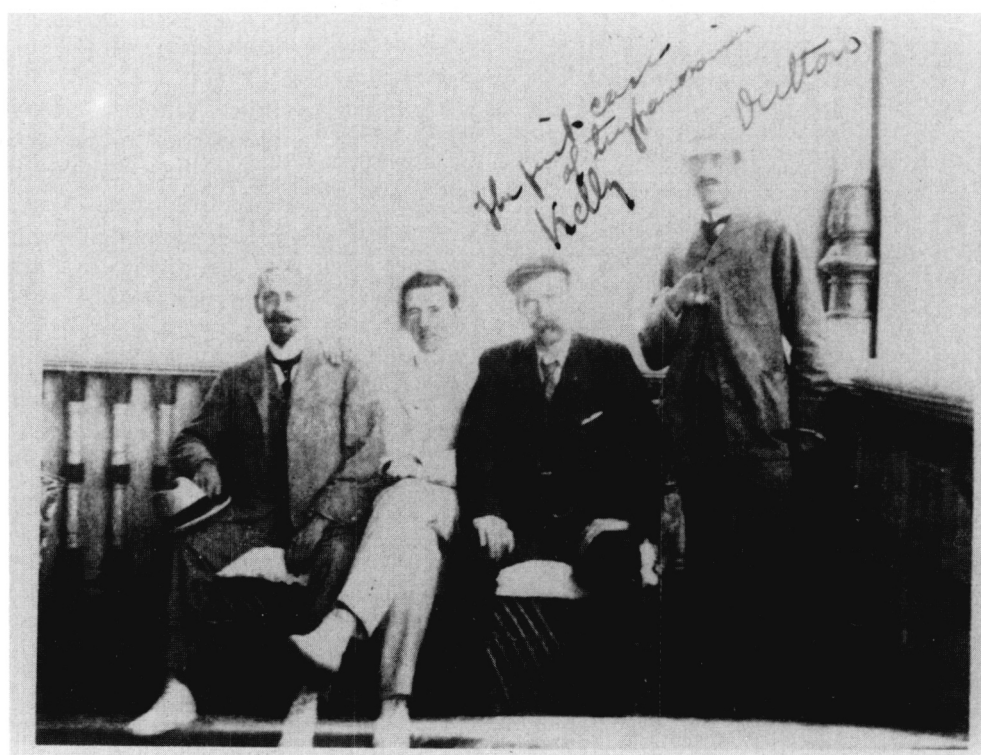


Fig. 2. – David Bruce's hut in Ubombo, 1894-1895 (from Bruce, 1915 : *Lancet*, ii, 1-6; photograph kindly supplied by Dr G.C. Cook [see Cook, 1994]).

Fig. 3. – Photograph of Dr J. E. Dutton (right) with, next to him, Mr H. Kelly, the first known case of human trypanosomiasis (1901). One of the other two men is Dr R. M. Forde; the identity of the second is not known. The original photograph is annotated in Dutton's handwriting 'The first case of trypanosomiasis Kelly Dutton'. Photograph kindly supplied by courtesy of Miss Patricia Miller, archivist, and the Photographic Department of the Liverpool School of Tropical Medicine.



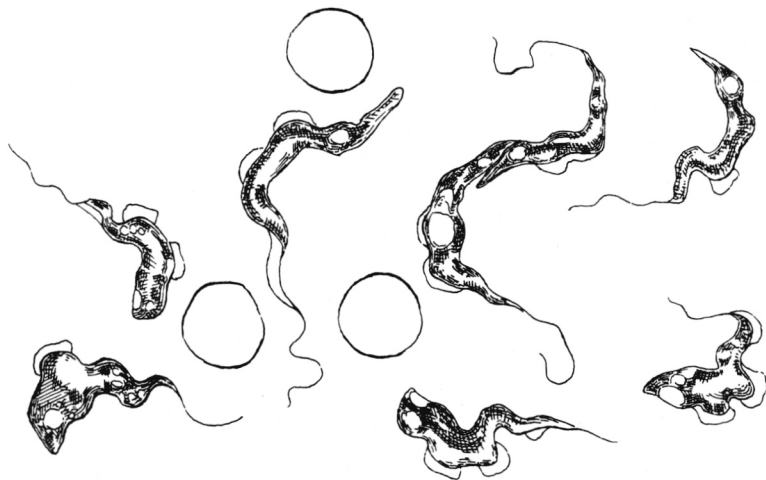


Fig. 4. – Sketches of 'Haematozoa' in the blood of a dog, from Plate V of Bruce (1895). Later, coloured drawings of trypanosomes in Bruce's reports were made by Mrs (later Lady) Bruce; these sketches are cruder, and may have been drawn by Bruce himself (photograph kindly supplied by courtesy of Ms Mary Gibson, bibliographer, and the Visual Aids Department of the London School of Hygiene and Tropical Medicine).

Only two years after the naming of *T. brucei*, in May 1901, a doctor in the British colonial service in The Gambia named R. M. Forde¹ saw, in the blood of a 42 years old Englishman ('H. K.'), subsequently identified from the archives of the Liverpool School of Tropical Medicine as H. Kelly; see fig. 4), who was master of a government steam boat on the Gambia river, what he described as 'small worm-like, extremely active bodies' which he first thought were filariae. However, later in 1901 J. E. Dutton, of the Liverpool School of Tropical Medicine, was visiting Bathurst in The Gambia. Forde showed his patient, and the 'worms' in his blood, to Dutton, who recognized them as trypanosomes (Forde, 1902). Dutton (1902) subsequently named the parasite *T. gambiense*. (Dutton actually wrote 'At present then it is impossible to decide definitely as to the species, but if on further study it should be found to differ from the other disease producing trypanosomes I would suggest that it be called *Trypanosoma gambiense*' (p. 467); although this is a 'conditional proposal' in the terms of the International Code of Zoological Nomenclature (third edition, 1985, article 15), such proposals made before 1961 do not prevent availability of the name.)

On 17 November 1909 trypanosomes were seen in the blood of a 26 years old Englishman who had been travelling along the valley of the Luangwa river in Zambia (then north-east Rhodesia); he was thought to have become infected there in September 1909. On 2 December he was admitted to the Royal Southern hospital in Liverpool, under the care of Dr

Ronald Ross (who is now more famous for his collaboration with Patrick Manson on the mosquito transmission of malaria). The case history of this patient was reported by Ross and Thompson (1910, 1911); he was subjected to a horrifying battery of attempted therapies for his condition, including atoxyl, quinine, methylene blue, trypsin, amylopsin, succinamide of mercury, 'izal oil' [presumably a commercial household disinfectant of that name], trypan red, potassium iodide, 'vaccines' prepared from his own trypanosomes grown in rats, and an extract of rat peritoneal leucocytes. On one occasion he was injected with 1×10^9 'dead' trypanosomes in rat's blood; this, the doctors reported, 'seemed to cause no harm' (Ross and Thompson, 1911). Perhaps not surprisingly, the unfortunate patient died on 29 June 1910. He was identified by Ross and Thompson (1910), and by Stephens and Fantham (1910), only as 'W. A.', a native of Northumberland in the UK. Duggan (1970) named him as W. Armstrong, but gave the date of his infection as 1908; this seems to be a mistake.

Before his death, trypanosomes were isolated from W. A.'s blood and studied (in rats) by Drs Stephen and Fantham at the Liverpool School of Tropical Medicine. Stephens and Fantham (1910) noted the virulence of this strain, and reported the appearance of posternuclear trypomastigotes in the rats' blood which (they thought) distinguished it from *T. gambiense*; they discussed the possibility that it was (i) a 'variety' or 'local race' of *T. gambiense*, (ii) a subspecies of *T. gambiense*, or (iii) – 'our own view' – a new species, *T. rhodesiense*.

It is interesting that, even at the outset, there should have been some doubt in the minds of the describers

1. Robert Michael Forde was born at Cloyne, County Cork, Ireland in 1862 and died 'at his home in Worthing [Sussex, England] on March 27 [1942] at the age of 86' (C.A.H., 1948).

of *T. rhodesiense* about its validity as a separate species. These doubts have survived until the present day, with scientific opinion swaying first one way and then another.

HOW MANY SPECIES OF 'POLYMORPHIC' TRYPANOSOMES IN AFRICA ?

There were two early schools of thought. One, which might be called the 'British' school, concluded that *T. brucei* and *T. rhodesiense* were indistinguishable, though they recognized that not all 'strains' of *T. brucei* infected humans (Kingham and Yorke, 1912; Bruce *et al.*, 1913). The opposite view, that the two were distinct species, was held by a group of German workers (Taute, 1913; Kleine, 1914, 1923; Beck, 1914), whose view was supported by the indisputable fact that six isolates of *T. brucei* were later shown by direct experimental inoculation not to be infective to humans (Taute and Huber, 1919), who included themselves among the 131 recipients of trypanosome-infected blood).

Duke (1921) adhered to the 'British' view, suggesting that *T. rhodesiense* and *T. brucei* were merely 'biological races' of *T. brucei*, and Wenyon (1926), in his great monograph, discussed the different opinions and concluded that, while *T. gambiense* was a distinct species, '*T. rhodesiense* is merely a strain of *T. brucei* in man.'

Gradually, however, the 'separatist' German view became generally accepted. The question seemed to be settled by the historically famous 'Tinde experiment'. This experiment, which was conducted by a series of workers at the small experimental station at Tinde in northern Tanzania (then Tanganyika), consisted of the serial passage of a strain of trypanosomes, isolated in 1936 from a human patient, alternately through *Glossina morsitans* and sheep, with periodical testing of its infectivity to humans either by blood inoculation or tsetse fly bite. The experiment continued for 23 years, and the strain, which was considered to be *T. rhodesiense*, never lost its ability to infect humans (apart from a few sporadic failures, attributed to faults in technique) (Willett and Fairbairn, 1955; Ashcroft, 1959).

With hindsight, it is easy to regret that the converse experiment – serially passaging a strain of *T. brucei* known not to be infective to humans, through *G. morsitans* and sheep – was not conducted simultaneously. As pointed out by Rickman (1977), it would have been interesting to see whether such a strain would have spontaneously acquired the ability to infect humans.

For many years epidemiological evidence had been building up to suggest that human-infective strains of *T. brucei sensu lato* existed in wild 'game' mammals, and this was conclusively demonstrated when Heisch *et al.* (1958) isolated such a strain from *Tragelaphus scriptus* in Kenya. Eight years later, Onyango *et al.* (1966) isolated a similar strain from a domestic cow in the same country. However, this was not perceived as undermining the current concept of the distinction of the two 'species' – *T. brucei* and *T. rhodesiense*. In the book 'The African trypanosomiasis' (Mulligan & Potts, 1970) – in many ways a summing-up of the British contribution to trypanosomiasis research in Africa during the then rapidly ending 'colonial' era – Hoare (1970a) referred to the three species *T. brucei*, *T. rhodesiense* and *T. gambiense*, although he qualified this by adding that, 'from the zoological point of view', they could be classified as subspecies of *T. brucei* and in the next chapter of the same book he wrote that *T. rhodesiense* represented 'merely a virulent race of *T. gambiense*' (see Hoare, 1970b). In 1925 (quoted by Hoare, 1972) and in 1943 Hoare had suggested that *T. rhodesiense* and *T. gambiense* were merely races of *T. brucei*. He later (Hoare, 1966) elevated them to subspecies. In 1970 (Hoare, 1970a, b) his ideas seem to have been in a fluid state, but finally, in his 'magnum opus', Hoare (1972) no longer treated *T. rhodesiense* as a distinct subspecies, but merely as one of two 'nosodemes' of *T. brucei gambiense*.

In 1967, human African trypanosomiasis was newly reported in south-western Ethiopia and, as a result of investigations there, my colleagues and I (Baker *et al.*, 1970; McConnell *et al.*, 1970) concluded that the disease was, indeed, a new occurrence in that area. In discussing its origin, we considered three possibilities: that it had always been covertly present; that it had been newly introduced from endemic areas to the south; or that it had arisen from enzootic *T. brucei* which had 'recently acquired the ability to infect man'. Although we regarded the last possibility as 'interesting', we were rather dismissive of it and concluded that introduction from the south was the most likely explanation (the quotations are from Baker *et al.*, 1970).

However, the idea that these trypanosomes need not necessarily be categorized into rigid divisions began to take root. Hoare (1967) suggested that the *T. brucei* group, which he had redefined (Hoare, 1964) as forming the subgenus *Trypanozoon* Lühse, 1906, should be regarded as 'complexes of morphologically identical populations' or, in the terminology of Huxley (1963), 'polytypic species'. This concept was developed, independently, by two Canadian workers

(Bardsley and Harmsen, 1973) who were studying the trypanosomes of anuran amphibia. These workers concluded that the trypanosomes of *Anura* form 'a species complex composed of a number of clonal aggregates of varying degrees of separateness [one or two of which] can be recognized as separate species...'; they added the cautionary note that 'We must... be prepared for the possibility of finding one or more central cores of highly variable, polymorphic species which may have to remain as unresolved species complexes in the eyes of all except the most ardent "splitters"'. This is a concept which I think should be borne in mind when considering the subgenus *Trypanozoon* today, uncomfortable though it may be for ardent 'splitters'.

Experimental investigation of the interrelationships of the *T. brucei* complex was bedevilled for many years by the lack of any means of determining the ability, or inability, of a particular strain to infect humans other than its experimental inoculation into a human volunteer. The situation changed when Rickman and Robson (1970) described the 'blood incubation infectivity test' (BIIT). If trypanosomes of *T. brucei sensu lato* were incubated under controlled conditions with human blood (or serum), some strains lost their infectivity for rats while others retained it; and the phenomenon appeared to correlate well with their inability or ability to infect human beings.

It was shown by Rifkin (1978) that the trypanocidal factor was associated with the serum high density lipoproteins (HDL), and later work has shown it to be located in several different HDL populations (Lorenz *et al.*, 1994). Studies by Hajduk and his collaborators (Hager *et al.*, 1994; Hajduk *et al.*, 1994; see also Rowe, 1994) have shown that the lytic activity resides in a minor subspecies of HDL. This component may bind to a surface receptor in the flagellar pocket of *T. brucei* and subsequently enter the parasite's lysosomes. The lysosomal membranes are then disrupted, resulting in autodigestion of the parasite cell. Why this does not occur in the human-infective trypanosomes is currently being investigated.

At first, the BIIT seemed to support the distinction between *T. brucei* and *T. rhodesiense*. However, more detailed studies by Rickman (1977) showed that different antigenic variants of a strain initiated by inoculating a single trypanosome into a rat (a cloned strain) could show different BIIT responses, suggesting that the property of infectivity or noninfectivity to humans was not necessarily fixed. This led Rickman (1977) to suggest that human-infective trypanosomes could 'emerge' among a population of hitherto noninfective (serum sensitive) individuals.

Loss of human infectivity by strains of *T. rhodesiense*

during serial non-cyclical passage (i.e., passage by syringe inoculation) through laboratory rodents had long been known to occur. What was surprising, however, was that Rickman's work (1977) seemed to show that the change could occur in the opposite direction – from not infective to infective; in other words, that *T. brucei* could spontaneously change into *T. rhodesiense*.

This heretical idea was given some support by the fact that, in 1975, a medical student working in a laboratory in Edinburgh, Scotland, became accidentally infected with a strain of *T. brucei sensu lato* which had been isolated from a *Glossina pallidipes* in south-east Uganda 15 years earlier (Robertson *et al.*, 1980). This strain had been frequently handled in laboratories without special precautions, and no accidental infection had occurred; it was therefore assumed to be non-infective to humans – although, unfortunately, this had never been deliberately tested. A cloned strain had been prepared from a single trypanosome of the original isolate, and from this 12 different variable antigenic types had been selected and cryopreserved. A serological study of the infected student showed that he had been infected with type ETat 10 (ETat = Edinburgh trypanosome antigenic type) (Herbert *et al.*, 1980), which was found to be the only one of this series of 12 which was resistant to human serum *in vitro* (Van Meirvenne *et al.*, 1976), and therefore, presumably, the only one to be infective to humans. Happily, the unfortunate student fully recovered after treatment with suramin. The most likely explanation of this event seems to be that human infectivity had been spontaneously developed by ETat 10, possibly by mutation, since the other eleven types (all of which had originated from the same trypanosome) were rendered non-infective by human serum.

EVIDENCE FROM RECENT MOLECULAR AND GENETIC STUDIES

The modern, molecular approach to a study of relationships within the subgenus *Trypanozoon* (*T. brucei sensu lato*) originated with the painstaking studies by Godfrey and his co-workers of the isoenzyme profiles of about one thousand trypanosome populations, as revealed by starch gel and cellulose acetate electrophoresis. The enormous amount of data thus produced was then analysed with the aid of a computer to determine the degree of relatedness of the various isoenzymic groups (termed zymodemes; WHO, 1978). This work, when first reviewed by Gibson *et al.* (1980), indicated that there could be 'no doubt... that *Trypanozoon* stocks form a homoge-

neous group [and] that *T. brucei*, *T. rhodesiense* and *T. gambiense* are too closely related to deserve separate species status...' (Gibson *et al.*, 1980, p. 199). The study also strongly suggested that *T. evansi* – a 'species' not transmitted by *Glossina* and chiefly associated with camels – should be included in this 'homogeneous group'. Gibson *et al.* (1980) proposed distinguishing six subgroups, to which they gave names without taxonomic significance. One of these groups ('gambiense') correlated fairly well with the 'classical' *T. gambiense*, but no one of the other groups could be related clearly with the classical '*T. rhodesiense*' or '*T. brucei*'.

A later, shorter review by Gibson (1986) of this and other work concluded that there did exist a group of stocks which corresponded more or less to the classical concept of '*T. gambiense*' as infective to humans, of low virulence to experimental animal hosts, and generally confined to Central and West Africa. This group Gibson (1986) referred to as 'group 1 *T. b. gambiense*'; she also recognized a 'second, less easily defined group with greater... heterogeneity' which she provisionally referred to as 'group 2 *T. b. gambiense*'. Group 2 had been isolated only from Burkina Faso and Côte d'Ivoire.

Summarizing and extending this work, Godfrey *et al.* (1990) and Stevens and Godfrey (1992) concluded that most or all of the zymodemes of *Trypanozoon*, when numerically analysed by the construction of both a dendrogram and a cladogram, fell into three major divisions or pathways, which corresponded, 'albeit imperfectly'. These divisions did not, however, correspond with the three classical species or subspecies, but were separated more on geographical criteria. One division was predominantly East African, one was mainly West African, and the third formed a 'residual group' linked with the Lake Victoria region. Using the accepted nomenclature, Godfrey *et al.* (1990) concluded that '*T. evansi* and *T. b. gambiense* appeared to be distinct entities.... The status of *T. b. rhodesiense* and *T. b. brucei* was particularly uncertain...'. These authors finally concluded that it may be 'reasonable' to retain the name *T. b. rhodesiense* for their West African strain groups, whether or not they are infective to humans, and the name *T. b. brucei* for an essentially West African strain group, predominantly but not exclusively non-infective to humans.

These conclusions have been broadly supported by the work of other authors. Tait *et al.* (1984, 1985), also using isoenzyme electrophoresis as a tool to investigate the complex relationships within the subgenus *Trypanozoon*, concluded that *T. b. gambiense* was a valid subspecies which could be distinguished from other stocks of the subgenus which they refer-

red to as '*T. brucei* (non-gambiense)' (in the 1984 paper). They concluded that the so-called *T. b. rhodesiense* was 'a set of variants' of *T. b. brucei* rather than a true subspecies (Tait *et al.*, 1985).

Paindavoine *et al.* (1986, 1989) examined the deoxyribonucleic acid (DNA) of some 70-80 populations of *Trypanozoon* by means of digestion with restriction endonucleases, hybridization and electrophoresis. These authors concluded that all the *T. b. gambiense* stocks which they examined had a conserved, specific DNA band pattern and could be 'non-ambiguously identified', forming a single homogeneous population. The other stocks they examined, in contrast, yielded highly variable DNA patterns and were distributed amongst several heterogeneous groups. *T. b. gambiense*, they concluded, was therefore a 'real subspecies' whereas '*T. b. rhodesiense*' was not, and they suggested that the heterogeneity indicated that the 'non-gambiense' trypanosomes were evolving more rapidly than *T. b. gambiense* itself (Paindavoine *et al.*, 1986). In their later paper (Paindavoine *et al.*, 1989), these authors reported that studies using four DNA probes had shown that all 49 *T. b. gambiense* stocks examined possessed the same combination of alleles and were homozygous for the four markers used. This study supported the view that the 'non-gambiense' stocks were diploid and could undergo genetic exchange (see below). *T. b. gambiense*, however, they thought was genetically isolated and did not exchange genetic material with other stocks of the subgenus *Trypanozoon*. This study also supported the claim by Dero *et al.* (1987) that the genome of *T. b. gambiense* contained only about 70% of the amount of DNA in the genome of the non-gambiense stocks, and only about 50% of the latter's variable antigen gene repertoire.

CLONAL OR SEXUAL POPULATION STRUCTURES ?

In spite of sporadic publications claiming to have detected sexual reproduction among trypanosomes (reviewed by Hoare, 1972, pp. 48-51), it was generally accepted until about 15 years ago that genetic exchange did not occur among trypanosomes of the subgenus *Trypanozoon* (nor, indeed, among any other groups). However, in 1980 Tait again raised the possibility of genetic exchange occurring, supporting his claim with evidence of apparent hybrid formation detected by isoenzyme electrophoresis. This work has been considerably extended and confirmed by several workers (for example, Jenni *et al.*, 1986; Schweitzer *et al.*, 1988; Sternberg *et al.*,

1988, 1989; Gibson, 1989; Paindavoine *et al.*, 1989; Pearson & Jenni, 1989). It now seems indisputable that some form of genetic exchange can occur during that part of the life cycle which takes place within *Glossina*, at some stage before the production of metacyclic trypomastigotes. The details of the process, however, are unclear, and – perhaps suprisingly – no confirmatory cytological evidence of the process has yet been obtained.

It is also clear that this genetic exchange is not an obligatory process and, although it may be frequent between certain stocks (Sternberg *et al.*, 1989), its occurrence is by no means universal. It has been suggested by Pearson & Jenni (1989) that some system of mating types may be involved, as is well known to be the case with *Paramecium*. The DNA of the kinetoplast appears not to take part in this process; Sternberg *et al.* (1988) showed that inheritance of the kinetoplast was uniparental.

There also seems little doubt that trypanosomes (*Trypanozoon*) are diploid throughout most of their life cycle. This was first suggested by Tait (1980), and confirmed by Gibson *et al.* (1985), Paindavoine *et al.* (1989) and Pearson & Jenni (1989).

However, Cibulskis (1988), while agreeing that the 'range of genotypes in *T. brucei* is most readily explained by genetic exchange', rightly pointed out that the frequency of its occurrence was (and is) not known; it 'may not occur sufficiently frequently, or in such a way as to break up associations between loci'. Cibulskis (1988) concluded that 'it is not clear that sex exerts any control over the relative proportions of genotypes at individual loci.' This conclusion is in accord with the views of Tibayrenc and his co-workers (Tibayrenc *et al.*, 1990, 1991; Truc & Tibayrenc, 1993; Mathieu-Daudé & Tibayrenc, 1994) who, from a study of population genetics, concluded that trypanosomes (and many other parasitic protists) have an essentially clonal population structure, rather than one which is based on sexual reproduction; their analysis has shown that segregation and recombination, which are necessary consequences of sexual reproduction, are rare or absent in natural populations of the organisms concerned. This does not exclude the possibility that sexual processes may occur, but merely indicates that they may occur infrequently and are not generally of genetic significance in these populations (Tibayrenc *et al.*, 1990, 1991; Mathieu-Daudé & Tibayrenc, 1994; Mathieu-Daudé *et al.*, 1994).

A consequence of this view is that the clones, many of which appear to be stable over space and time, are the basic taxonomic unit rather than the conventional Linnean species and subspecies. The three 'subspecies' of *T. brucei* were regarded by Tibayrenc *et al.*

(1990) merely as 'pathotypes', the species being composed of 'numerous clones, some of which have become specialized to human hosts'. The *T. gambiense* group 1 of Gibson (1986) is a 'genetically homogeneous... successful, ubiquitous human-host clone' (Tibayrenc *et al.*, 1990). In a later paper (Mathieu-Daudé and Tibayrenc, 1994) this group of workers used the 'deme' terminology introduced into the trypanosomiasis literature by Hoare (1955) and referred to the so-called *T. b. brucei* and *T. b. rhodesiense* as 'nosodemes' rather than actual genetic clades; *T. b. gambiense* group 1 (Gibson, 1986) was regarded as a 'group' of clones [not, now, a single clone] consisting of most of the stocks of human-infective trypanosomes from Central and West Africa. In another paper, Mathieu-Daudé *et al.* (1994) showed that a group of 12 stocks of human-infective trypanosomes from Central and West Africa were closely related by the use of a specific kinetoplast deoxyribonucleic acid probe, and they identified this group as Gibson's (1986) group 1 *T. b. gambiense*.

Truc and Tibayrenc (1993) also equated one group of zymodemes, among the 23 which they identified in 55 stocks of *T. brucei sensu lato* isolated in West Africa, with the 'classical' *T. b. gambiense*. The various zymodemes, they suggested, were equivalent to 'natural clones (or a family of closely related clones), stable in space and time.'

CONCLUSIONS

The inescapable conclusion from the foregoing brief review seems to me to be the fact that it is futile to attempt to maintain the distinction between the erstwhile subspecies (or species) *T. b. brucei* and *T. b. rhodesiense*. These organisms must be regarded as a collection of populations (whether clonally or sexually derived), all fairly closely related – Mathieu-Daudé & Tibayrenc (1994) pointed out that the total genetic variability detected in a study of 18 isoenzyme loci amongst 78 stocks of *T. brucei sensu lato* was less than that seen in the 'single' species *T. cruzi* – some of which had developed the ability to resist destruction by HDL in human blood; this distinction, though often durable for quite long periods, cannot be thought of as immutable. I am not in favour of the proposal by Godfrey *et al.* (1990) to redefine *T. b. brucei* and *T. b. rhodesiense* in terms other than their infectivity (or non-infectivity) to human hosts. This course of action would, I think, be confusing since the two subspecific names have for so long been used to denote precisely that characteristic.

The situation is different with *T. b. gambiense*. All the recent work discussed above has shown that there

exists a core of 'typical' human-infective *Trypanozoon* stocks producing chronic human African trypanosomiasis, based on (but not restricted to) West Africa, which can fairly certainly be distinguished from other stocks of the *T. brucei* complex. The argument has thus, in a sense, swung around a full circle : *T. rhodesiense* is not a valid species, or even subspecies, not because it is indistinguishable from *T. b. gambiense*, as the early British workers thought, but because it is merely a group of zymodemes (or clones) of *T. b. brucei*.

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