CellPress

Trends in Parasitology

Opinion

# African trypanosome strategies for conquering new hosts and territories: the end of monophyly?

Julius Lukeš [1,2,*], Ambar Kachale [1,2], Jan Votýpka [1,3], Anzhelika Butenko [1,2,4] and Mark C. Field [1,5]

***Trypanosoma brucei*** **parasites are the causative agents of African trypanosomiasis in humans, as well as surra, nagana, and dourine in animals. According to current widely used nomenclature, *T. brucei* is a group of five (sub)species, each causing a distinct disease and possessing unique genetic marker(s) or a combination thereof. However, minimal nuclear genome differences, sometimes accompanied by ongoing genetic exchange, robustly support polyphyly resulting from multiple independent origins of the (sub)species in nature. The ease of generating such (sub)species in the laboratory, as well as the case of overlapping hosts and disease symptoms, is incompatible with the current (sub)species paradigm, which implies a monophyletic origin. Here, we critically re-evaluate this concept, considering recent genome sequencing and experimental studies. We argue that ecotype should be used going forward as a significantly more accurate and appropriate designation.**

## Highlights

*Trypanosoma brucei* is a complex of five ecotypes, namely, *T. brucei* f. *brucei*, *T. b.* f. *gambiense*, *T. b.* f. *rhodesiense*, *T. b.* f. *equiperdum*, and *T. b.* f. *evansi*, which are highly evolvable and appear to conquer new hosts and territories relatively easily as a result of just a few simple mutations in their genomes, which can be induced even in laboratory conditions.

The subspecies status of *T. brucei* lineages is incompatible with the accumulating evidence pointing at the significant genetic similarities, apparent polyphyly, as well as overlapping hosts and disease symptoms.

The ecotype concept fits the data accumulated in the area of African trypanosome research much better than the subspecies nomenclature.

## Context: species and molecular data

Representing many of the most ancient lineages, protists are hugely successful unicellular eukaryotes contributing the majority of known extant eukaryotic diversity [1]. Most lack mineralized components, making fossilization extremely rare, albeit that the fossil record reflects only morphology and is limited for determining fine-grained lineage distinctions. In some instances, genome sequences can be correlated with geological events, allowing in part a molecular clock to be inferred. For parasites, fossils of hosts/vectors provide valuable temporal calibration, at least for inferring the earliest possible times of infections of plants, invertebrates, and vertebrates.

According to Mayr, 'species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups' [2]. Hence, species can be considered to be initiated via a speciation event and ended by either extinction or further speciation. While 80 years later the species concept has yet to reach a complete consensus, the basic definition of a separately evolving population, delimited in some manner, is, mainly for practical reasons, widely accepted even in protists (for review, see [3]). Furthermore, cryptic species are increasingly being identified by genome sequencing, indicating that overt morphological changes do not always occur even for metazoan speciation events, let alone protists. Furthermore, the level of genetic divergence required to provide a reproductive barrier can vary considerably, as can population size or the rapidity by which traits can become fixed [4]. Difficulty in defining species has been recognized for trypanosomes, with acceptance of discrete typing units for the South American trypanosome, *Trypanosoma cruzi* [5]. Here, we argue that a similar revision is in order for African trypanosomes.

[1]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic
[2]Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic
[3]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic
[4]Faculty of Science, University of Ostrava, Ostrava, Czech Republic
[5]School of Life Sciences, University of Dundee, Dundee, UK

*Correspondence: jula@paru.cas.cz (J. Lukeš).

 https://doi.org/10.1016/j.pt.2022.05.011

*T. brucei* is generally considered as a group of closely related **(sub)species** (see Glossary and Box 1) [6,7], which, mostly for pragmatic reasons, remain frequently labeled as separate species [8,9]. Each is associated with a distinct disease and discrete host/vector range and distinguished, at the molecular level, by the presence/absence of a set of genes or even a single gene. Trypanosome populations are capable of extensive hybridization both in the laboratory and in the field and can conduct meiotic reproduction, although the major reproductive mode is asexual [7,8]. Various *T. brucei* (sub)species are recognized based on their host(s), the course of disease they cause, the vectors by which they are transmitted, and, most important, mutual well-documented genetic differences. These latter have been ascribed in many cases to specific associations with host/vector and disease. We propose that these genetic differences are insufficient to justify *bona fide* establishment of either separate species or subspecies and that an alternative definition is more compatible with data from both the field and dozens of genomes of *T. brucei* available in the databases. Moreover, we highlight an overlooked connection between currently recognized *T. brucei* (sub)species in the wild and strains generated in the laboratory that are genetically similar, if not identical, with naturally occurring forms. We argue that while pragmatic reasons justify association of each (sub)species with a specific host and pathology, the five *T. brucei* (sub)species nomenclature is untenable (see later). We believe that the **ecotype** concept, as defined by the International Code of Zoological Nomenclature, best fits the available data (Box 1). From here on, the ecotype nomenclature will be used throughout this text, unless the reference to a (sub)species rank is explicit.

## The transition to parasitism and diversification

The monoflagellated trypanosomes and related parasitic trypanosomatids are descended from a free-living heterotrophic biflagellate bodonid-like ancestor (Figure 1) [10]. Significantly, the switch to parasitism probably occurred on several occasions as various bodonids periodically enter the alimentary tracts of (in)vertebrates, including tsetse flies [11], seemingly testing the barriers to attaining parasitism. Comparative genomics and phylogenetics robustly implicate the ancestral trypanosomatid lifestyle as involving a single host (monoxenous), and repeatedly developing into the two-host (dixenous) mode [12]. The earliest hosts were most likely arthropods (Figure 1), among which trypanosomatids were transmitted by numerous mechanisms, in common with their extant descendants [13]. It is reasonable to assume that the dixenous life cycle was rapidly established following the massive radiation of vertebrates, among which parasites were transmitted by blood-feeding arthropods. Although it most likely occurred much earlier, a transmission via insects is documented from the mid-Cretaceous [~110 million years ago (MYA)] fossil record by an amber-trapped *Leishmania*-like flagellate [14], already possessing the lineage-defining **kinetoplast DNA** (**kDNA**) disk [15]. Moreover, within the insect vector, the parasite is encircled by nucleated erythrocytes [16], indicating long cohabitation with vertebrates

### Box 1. Characterization of an ecotype in the context of *T. brucei* evolution

Terms used in intraspecific taxonomy often do not have a universal, widely accepted definition and are ambiguously applied in the literature, with the 'ecotype' being no exception [81]. Here, we define an ecotype as a group of organisms within a species that is adapted to particular environmental conditions and therefore exhibit genetic and often behavioral, structural, or physiological differences from other species members (compare with the definition of subspecies in the Glossary). Since distinct ecotypes differ in the resources they use, they do not drive each other to extinction, whereas selection is expected to eliminate diversity within but not between ecotypes [82]. It is important to note that the same ecotypes may emerge repeatedly in nature, as well as be generated experimentally. *T. brucei* ecotypes emerge recurrently, which is reflected in paraphyly and polyphyly on phylogenetic trees (see later), resulting from a parallel evolution often observed in multicellular organisms [83]. From this perspective, *T. b. evansi* types A and B can be viewed as independently emerging cases of the 'evansi' ecotype. Phenotypic characteristics demonstrated by the representatives of a certain *T. brucei* ecotype often depend on multiple host-related factors, such as host genotype, physiology, immune status, and/or coinfections. Formally, ecotypes are recognized as forms (sometimes bioforms) and in the Latin name of the organism, the abbreviation 'f.' is used (e.g., *T. b.* f. *evansi*).

### Glossary

**Ecotype:** a group of populations within one species adapted to certain environmental conditions that exhibit behavioral, structural, or physiological differences through variations in traits and allele frequencies. Ecotypes occur throughout the geographic range of a species in similar ecological niches (e.g., different hosts). When similar niches occur in widely separated places, identical ecotypes can arise independently and do not form monophyletic groups.
**Haptoglobin-hemoglobin receptor (HpHbR):** a surface molecule mediating heme uptake in trypanosomes. Represents a VSG paralog.
**Human African trypanosomiasis (HAT):** also called 'sleeping sickness', a vector-borne disease endemic to sub-Saharan Africa and caused by *Trypanosoma brucei* f. *gambiense* and *T. brucei* f. *rhodesiense*.
**Kinetoplast DNA (kDNA):** the complex mitochondrial DNA of kinetoplastid flagellates, in trypanosomatids represented by a dense network of interlocked circular DNA molecules of two types: maxicircles carrying protein-coding genes and minicircles encoding guide RNAs, templates for editing maxicircle transcripts.
**Nagana/surra/dourine:** vector-borne or sexually transmitted diseases caused by *Trypanosoma brucei* f. *brucei*, *T. brucei* f. *evansi*, and *T. brucei* f. *equiperdum*, respectively.
**Serum resistance-associated (SRA) protein:** a variant surface glycoprotein paralog responsible for resistance of *T. b.* f. *rhodesiense* to trypanolytic factors present in some host sera.
**Subspecies:** under the International Code of Zoological Nomenclature, to which protists belong, it is a taxonomic rank below the species level, commonly used to recognize morphological and/or geographically distinct populations; the subspecies is considered to represent an initial stage of the species formation and thus should be genetically distinct and constitute a monophyletic group.
**Variant surface glycoprotein (VSG):** surface molecule of African trypanosomes covering the cell as a dense coat. VSG switching is an effective mechanism used for evading the vertebrate host immune system.

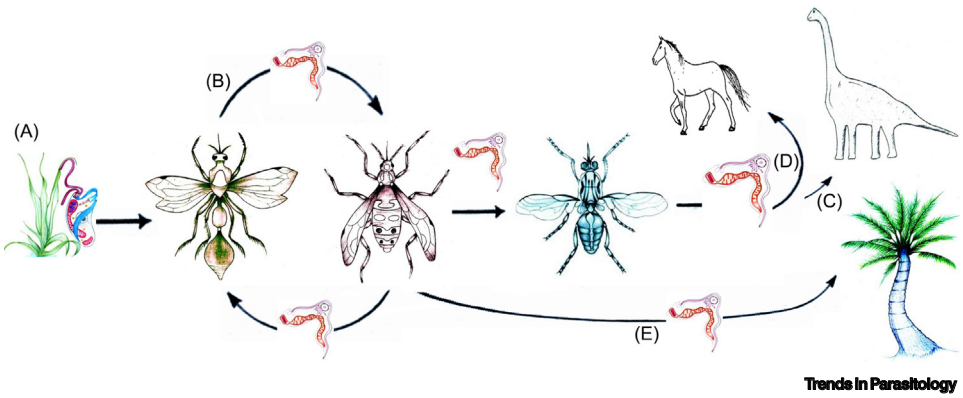


Figure 1. Putative evolutionary history of host–parasite life cycles of trypanosomes. (A) The predecessor of trypanosomatids was a free-living bodonid. (B) Trypanosomatids initially circulated among insects. (C) Blood-feeding insects, possibly ancestral tsetse flies, transmitted trypanosomatids into vertebrates (e.g., a dinosaur), establishing trypanosomes, characterized by the dixenous life cycle. At that time, tsetse were globally distributed, providing unrestricted distribution to trypanosomes. (D) Tsetse and other blood-sucking invertebrates transmitted trypanosomes to all vertebrate groups (e.g., mammals). (E) More recently, plant sap-sucking heteropteran insects transmitted trypanosomatids into plants, establishing another dixenous life cycle of phytomonads.

and thus coevolution between dixenous trypanosomatids and their hosts. The invasion of plants by the ancestor of *Phytomonas* was significantly more recent [17]. New and Old World trypanosomes, represented by *T. cruzi* (subgenus *Schizotrypanum*) and *T. brucei* (subgenus *Trypanozoon*), respectively, separated ~100 MYA, coincident with tectonic separation of Africa and South America [18].

## The emergence of African sleeping sickness

It is plausible that the ancestors of *T. brucei* infected Gondwanan fauna long before the supercontinent's fragmentation ~100 MYA. Ancestral tsetse flies had a global distribution 30–40 MYA [19], and tsetse-like fossils indicate a presence in Europe 10 MYA [20]. Since the tsetse origin likely predates the Gondwanan breakup [21], trypanosomes may have been present in the proto-African region of Gondwana and already established their relationship. However, perhaps due to a complex life cycle producing very low numbers of progeny and significant climatic changes, the distribution of tsetse progressively shrunk to a belt in sub-Saharan Africa and the southern tip of the Arabian Peninsula (Box 2). A reduced tsetse range would increase the selective advantage of accessing additional niches, associated with penetration beyond the tsetse belt and/or infection of a wider spectrum of hosts. While we will speculate on the specific trigger(s), it may also have been serendipity and/or ingrained flexibility of trypanosomes that allowed diversification into a range of life strategies.

African trypanosomes have both unique adaptations [22] and can parasitize every group of vertebrates [23], with a capacity to quickly acquire new hosts when introduced into a novel niche [24]. The antigenic variation system also provides an efficient mechanism for immune evasion [25] and a response to an extracellular lifestyle within mammalian hosts. Perhaps because of its complex life cycle that imposed multiple bottlenecks and hence accelerated fixation of genotypes, *T. brucei* particularly stands out in some of these aspects. Recent interactions between *T. brucei* and humans can be linked to historical events and thus relatively accurately timed [26]. Weir and colleagues [27] estimated that human-infective *T. brucei* emerged within the past 10 000 years, coincident with establishment of a settled agricultural population, likely favoring human–human and human–domestic animal transmission. kDNA-based

phylogenies indicate that strains with kDNA replication defects (see later) appeared before 5000 YA also possibly concurrent with access to domestic animals [18].

## Beyond classical taxonomy

Ancestral forms of *T. brucei* circulated among tsetse and vertebrate African fauna for tens of millions of years [17], a considerable period for multiple strategies and adaptations to arise. Indeed, this occurred, but in the absence of significant morphological diversification. Humans (genus *Homo*) emerged ~2.5 MYA, becoming omnipresent within the African ecosystem and inevitably turning into a highly relevant host. Over this relatively short period, *T. brucei* evolved two distinct pathologies in humans, and, based on geographic range, the causative agents were accorded species status, with *Trypanosoma rhodesiense* and *Trypanosoma gambiense* responsible for the Western and Eastern **human African trypanosomiasis (HAT)**, respectively, complemented with *T. brucei*, specifically causing **nagana** in animals [28].

### Box 2. Characteristics of *T. brucei* (sub)species/ecotypes

(i) *T. b.* f. *brucei* is transmitted by tsetse (genus *Glossina*; e.g., *Glossina morsitans*, *Glossina pallidipes*, and *Glossina fuscipes*) (Figure I), and causes nagana, a relatively mild and protracted disease in many animals but frequently more severe in non-native species [84]. *T. b.* f. *brucei* is lysed by sera from several primates, including humans, due to the presence of apolipoprotein 1 (APOL1) complexed with lipids and other proteins forming trypanolytic factor (TLF). TLF lyses trypanosomes following endocytosis and delivery to the lysosome [61,62,85] and effectively precludes human infection by *T. b.* f. *brucei* – anthropocentrically a huge distinction, supporting the classical differential between (sub)species.

(ii) *T. b.* f. *rhodesiense* causes an acute form of trypanosomiasis, a zoonotic disease infecting both humans and wild and domestic animals in Eastern Africa [86]. It is mostly transmitted by *G. fuscipes*, and although wildlife was regarded as its natural reservoir [87], *T. b.* f. *rhodesiense* can also circulate between cattle and humans [88]. Human infectivity is due to a unique SRA protein, a VSG mutant that neutralizes TLF [89], albeit remaining capable of being lysed by some TLF variants [61,90]. *T. b.* f. *rhodesiense* infections are geographically distinct from *T. b.* f. *gambiense*, supporting the concept of a distinct species or subspecies (Figure I).

(iii) *T. b.* f. *gambiense* causes, in Western Africa, mild and often asymptomatic chronic disease, with apparent mainly human–human transmission lacking a significant animal reservoir [91]. There is also rare congenital transmission [92]. *Glossina palpalis* is the major known vector and has very low infection rates, mirrored by laboratory studies indicating inefficient infection. *T. b.* f. *gambiense* is resolved into two groups [58,93,94]; group 1 includes genetically homogeneous strains meeting requirements for anthroponotic transmission, whereas group 2 is genetically heterogeneous and infects multiple mammalian species, potentially serving as reservoirs for human infection [95,96]. TLF-mediated lysis is avoided via **haptoglobin-hemoglobin receptor (HpHbR)** mutations decreasing ligand affinity [68,97]. Additional mechanisms for lower *T. b.* f. *gambiense* pathogenicity have been proposed [69,70]. A truncated VSG, namely, TgsGP, is specific to *T. b.* f. *gambiense* group 1 [53,98], but no equivalent marker is available for group 2 [58]. However, the very distinct course of disease has been sufficient for at least subspecies designation, despite an otherwise near genetic identity between *T. b.* f. *brucei*, *T. b.* f. *gambiense*, and *T. b.* f. *rhodesiense.*

(iv) *T. b.* f. *equiperdum* is sexually transmitted between equids. Significantly, tsetse-mediated transmission is replaced by blood exchange during coitus, albeit mechanic transmission via blood-feeding flies cannot be excluded [99]. Such a direct mammal–mammal route eliminated sexual reproduction (which takes place in tsetse) and released all constraints for retention of kDNA [6,78], leading to progressive loss, although the kDNA minicircle component is retained [41].

(v) *T. b.* f. *evansi* infects horses, cattle, goats, buffalos, dogs, camels, and wild game. It is transmitted through the bite of stable, horn, and tabanid flies or occasionally vampire bats or by ingestion of raw meat [47]. Mechanical transmission allowed the parasite to use various vectors and migrate out of Africa. Driving forces for emergence seem to be similar to *T. b.* f. *equiperdum*, but the trajectory toward kDNA loss has been either faster or more thorough, leading to complete lack of kDNA maxicircles and in most cases minicircles [6]. Both *T. b.* f. *evansi* and *T. b.* f. *equiperdum* are monomorphic, with the mitochondrial import of tRNAs [100] and the protein machinery for kDNA replication and maintenance intact and fully functional [32]. Furthermore, they both carry mutations in the nucleus-encoded γ-subunit of the $F_1F_O$-ATPase, which fully compensates for the loss of otherwise essential kDNA [60,101].

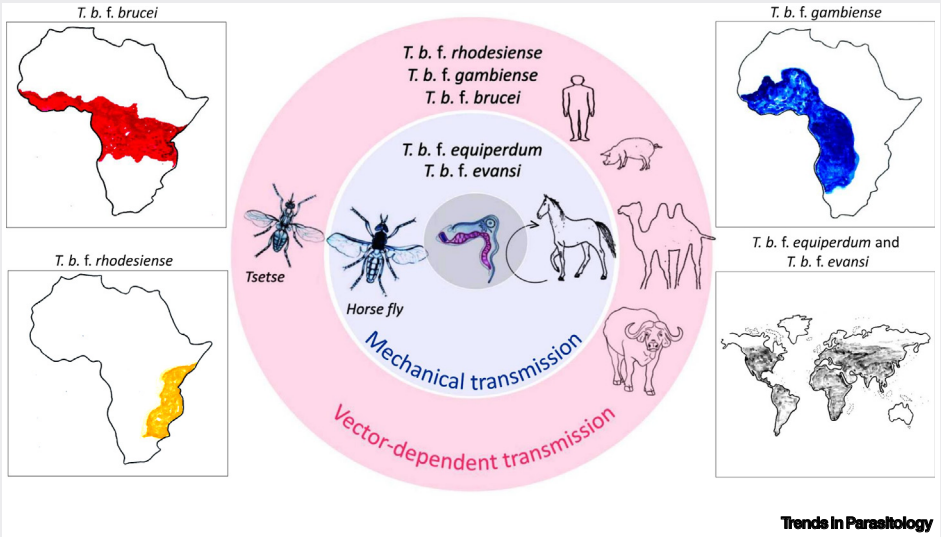


Figure I. Life cycles and geographic distribution of *Trypanosoma brucei* subspecies/ecotypes. A simplified scheme depicting the transmission of *T. b.* f. *brucei*, *T. b.* f. *gambiense*, and *T. b.* f. *rhodesiense* via tsetse flies to humans and other mammals (outer circle) and the mechanical transmission of *T. b.* f. *equiperdum* and *T. b.* f. *evansi* via horseflies to camels and other mammals. The arrow indicates direct sexual transmission.

The distinct character of animal trypanosomes was noticed early because flagellates parasitizing wildlife are potently lysed in human blood [29]. However, in the absence of significant morphological differences and with nuclear genomic data revealing extremely limited differences, they were reclassified to the currently accepted subspecies rank (*T. brucei*, *T. brucei gambiense*, and *T. brucei rhodesiense*) [30]. Indeed, although these subspecies differ in the distinct adaptation mechanisms tailored to their hosts, for which the molecular details are partially known, there is no evidence for the erection of genetic exchange barriers [7].

This picture is further complicated by *T. brucei* f. *evansi* and *T. b.* f. *equiperdum*, which were for more than one century considered as separate species (*Trypanosoma evansi* and *Trypanosoma equiperdum*) causing two distinct lethal pathologies, mainly in ungulates and known as **surra** and **dourine**, respectively [28]. A curious parallel was drawn between leukemia in mammals and *T. b.* f. *evansi* and *T. b.* f. *equiperdum*, where the loss of capacity to produce the stumpy form is associated with uncontrolled proliferation [31]. In the absence of clear differences from *T. b.* f. *brucei* in their nuclear genome [32], deletions or complete loss of mitochondrial DNA (= kDNA) renders *T. b.* f. *evansi* and *T. b.* f. *equiperdum* as *petite* mutants [6]. Why, when, and how this host specialization developed is unclear. Possible reasons are discussed in Box 2.

## Limits to the concept of five *T. brucei* (sub)species

The concept of each *T. brucei* (sub)species being responsible for a specific disease is both logical and vital for clinicians and veterinarians for appropriate diagnosis and treatment. However, expanding sequence data and increased understanding of molecular processes repeatedly evolved to avoid lysis and survive kDNA deletion are incompatible with the concept of five separate monophyletic (sub)species and, ultimately, restrictive. Moreover, 'gray zone' cases, such as combinations of characteristics discussed earlier, can break (sub)species boundaries (Box 2). Similarly, occasional difficulties in labeling disease as gambiense/rhodesiense HAT, or surra/dourine in animals, extend this challenge to the clinic. Veterinarians have treated the latter

two diseases strictly separately, yet this decision is increasingly being questioned [33]. As both *T. b.* f. *evansi* and *T. b.* f. *equiperdum* constitute *petite* mutants of *T. brucei* [6], it is highly unlikely that they cause truly distinct diseases but rather constitute a single ailment with a symptom spectrum depending on factors such as host species, immune status, genotype, general health, and route of infection. The critical differences between (sub)species have been selected via reduced availability of tsetse vectors, decreasing transmission frequency, and emergence of new hosts. Importantly, none of these (sub)species constitutes a monophyletic branch but rather radiates from a genetically diverse *T. b. brucei* population (see later and Figure 2).

*T. b.* f. *rhodensiense* is distinguished by the presence of the **serum resistance-associated (SRA)** gene, yet the gene seems to be occasionally exchanged between *T. b.* f. *rhodesiense* and *T. b.* f. *brucei* [34], especially in East Africa [35]. Genes similar to *T. b.* f. *gambiense*-specific glycoprotein (TgsGP) were identified in some strains of *T. b.* f. *brucei* and *T. b.* f. *rhodesiense* [36]. Furthermore, allegedly *T. b.* f. *evansi*-specific **variant surface glycoproteins** (**VSGs**) [37,38]

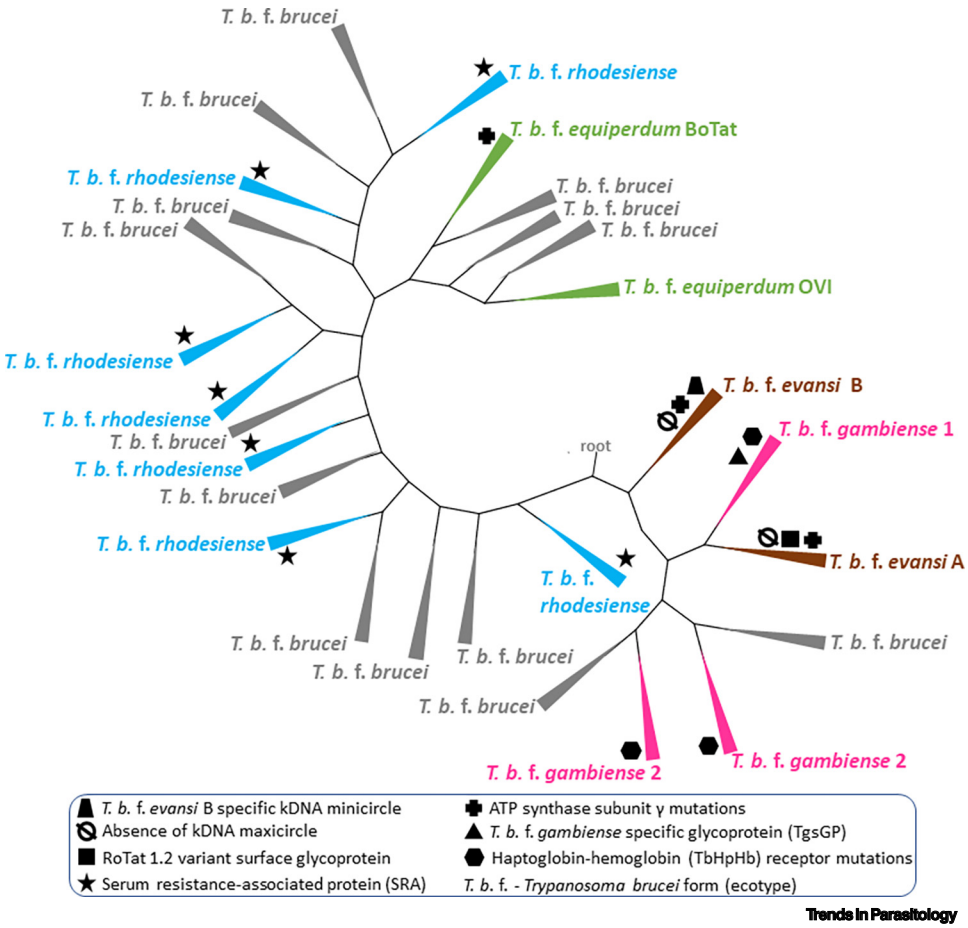


Figure 2. *Trypanosoma brucei* subspecies/ecotypes are not monophyletic. A schematic cladogram demonstrating multiple cases of emergence of *T. brucei* f. *brucei* (dark gray triangles), *T. b.* f. *evansi* (brown), *T. b.* f. *equiperdum* (green), *T. b.* f. *rhodesiense* (blue), and *T. b.* f. *gambiense* (magenta) from a genetic pool of *T. brucei*. The topology is based on published phylogenetic and clustering analyses [33,39,52]. Genetic markers traditionally used to distinguish trypanosome subspecies/ecotypes are shown with black-colored symbols. The tree rooting is arbitrary. Abbreviations: kDNA, kinetoplast DNA; SRA, serum resistance-associated protein; TbHpHb receptor, haptoglobin-hemoglobin receptor; TgsGP, *T. b.* f. *gambiense*-specific glycoprotein.

have recently been identified in several strains of *T. b.* f. *brucei* and *T. b.* f. *gambiense* group 2 [39]. Hence, ambiguous distinctions between *T. b.* f. *brucei*, *T. b.* f. *gambiense*, and *T. b.* f. *rhodensiense* are tenuous at best and depend essentially on the evolution of VSGs. Both SRA and TgsGP proteins are derived from VSGs, as are several other surface proteins, including the invariant surface glycoproteins and the transferrin receptor (Box 3). We propose that stability of the VSG-fold in the face of primary structure evolution has provided considerable evolutionary flexibility to these parasites, including the capacity to evolve receptors and resistance mechanisms. The rather impressive efficiency of this mechanism is exemplified by recent identification, through selective *in vitro* culturing, of a VSG variant ($VSG^{sur}$) that is capable of providing resistance to the trypanocide suramin [40]. $VSG^{sur}$ possesses a number of unusual features, including a β-sheet domain, but is part of a VSG subfamily, indicating the ready presence of this diverse structure [40].

*T. b.* f. *equiperdum* and *T. b.* f. *evansi* can obviously be segregated based on specific kDNA features [41] (Box 2), but this is also problematic [42]. Currently, *T. b.* f. *equiperdum* is differentiated from *T. b.* f. *evansi* based on retention of kDNA maxicircles [43]. However, elimination of the procyclic stage from the life cycles of both *T. b.* f. *equiperdum* and *T. b.* f. *evansi* permitted gradual kDNA loss, initially with deletions in the maxicircle followed by losses of sequence heterogeneity of kDNA minicircles, terminating with gradual elimination and eventual complete loss

## Box 3. A flexible protein framework for evolvability

The VSG-fold (pfam PF00913 and PF13206) is essentially a small bundle of α-helices with intervening loops prominently displayed at the face of the molecule exposed to the environment (Figure I). Variability in these loops is a major contributor to antigenic differences between VSGs [102]. The flexibility of the fold has been known for a considerable time due to structural studies indicating that very similar folds are maintained even between VSGs with low sequence identity [103]. More recently, as additional structures have emerged, the full utility of the VSG-fold has become apparent and is shared between several receptors, the SRA protein and others, based on structural predictions. Moreover, quaternary structure is variable as VSGs occur as dimers and trimers, depending on the variant. We suggest that the VSG-fold likely emerged from an invariant surface protein, possibly an ancestral invariant surface glycoprotein or receptor, and that has come to be adopted for many distinct functions. The stability of the VSG-fold in the face of sequence variance is likely a selective advantage powering evolvability and, in the presence of a large VSG gene repertoire as in extant African trypanosomes, provides a driver for potential very rapid adaptation.

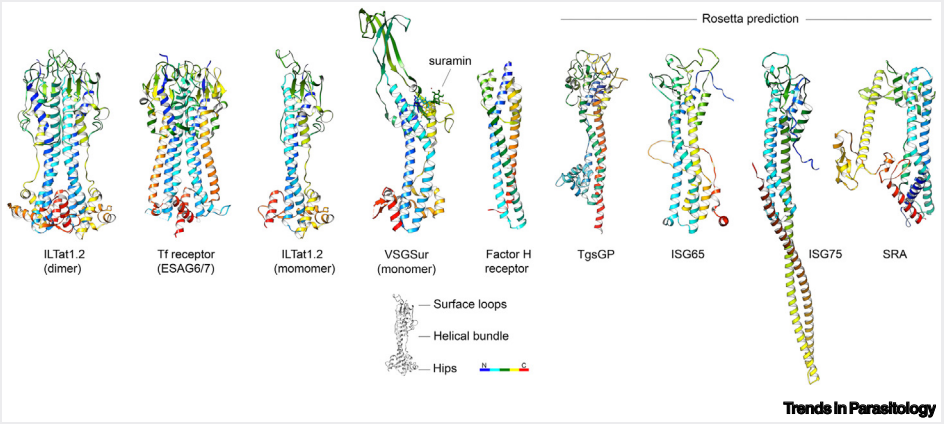


Figure I. Structural predictions for various VSG-related surface glycoproteins. Experimental structure data were obtained from the Protein Data Bank (https://www.rcsb.org), and modeled structures used Rosetta with default setting and the first model taken in each instance (https://robetta.bakerlab.org). All structures were rendered using ChimeraX (https://www.cgl.ucsf.edu/chimerax) and rainbow colored from the N to C termini as indicated. All structures are shown at similar resolution. The extended C-terminal α-helix of ISG75 folded back on itself is likely an artifact.

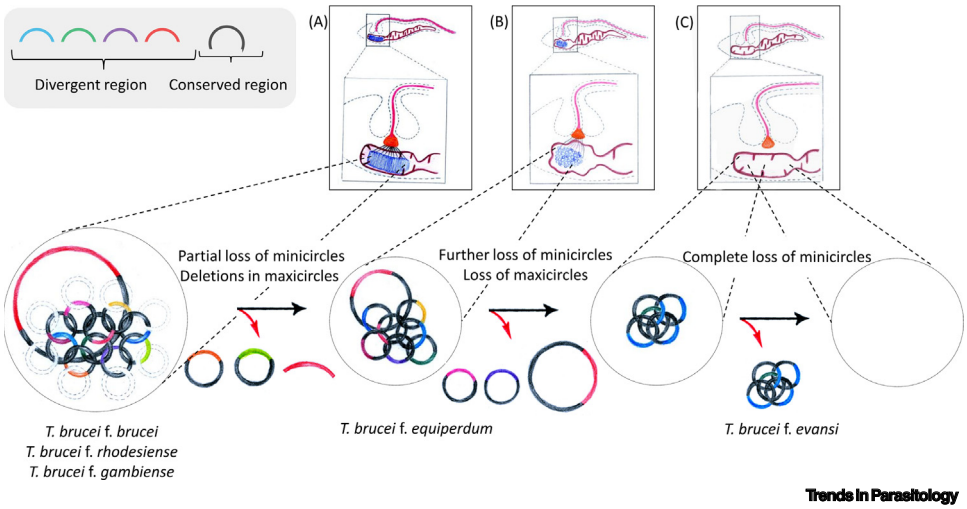


Figure 3. Distinguishing features in the kinetoplast DNA (kDNA) of *Trypanosoma brucei* subspecies/ecotypes. (A) The disk-shaped kDNA network of *T. brucei* f. *brucei*. Note the complete maxicircle and high number and sequence diversity of orderly catenated minicircles. (B) The irregular kDNA network of *T. b.* f. *equiperdum*. Note the truncated maxicircle and decreased number and diversity of disorderly catenated minicircles. (C) The highly reduced or eliminated kDNA of *T. b.* f. *evansi*. Note the absence of maxicircles and the near homogeneity of minicircles, which are completely lost in some strains.

(Figure 3). This suggests that *T. b.* f. *equiperdum* and *T. b.* f. *evansi* represent snapshots in the process of loss of the kDNA rather than distinct endpoints.

## Experimental generation of *T. brucei* (sub)species/ecotypes

Dyskinetoplastic (partial kDNA loss) or akinetoplastic forms (total kDNA loss) of *T. b.* f. *brucei* can be chemically induced in the laboratory [44]. At the time of these studies, it was unknown that these cell lines are analogous to naturally occurring *T. b.* f. *equiperdum* or *T. b.* f. *evansi*. More recently, a similar phenotype has been achieved by inactivating genes involved in kDNA replication. Indeed, ablating topoisomerase II generates akinetoplastic trypanosomes [45], essentially identical to *T. b.* f. *evansi*. Inhibition of another kDNA replication machinery component, PIF2-type helicase, leads to selective elimination of kDNA maxicircles [46], generating essentially *T. b.* f. *equiperdum*. Again, at the time, the virtual identity among nuclear genomes of *T. b.* f. *equiperdum*, *T. b.* f. *evansi*, and *T. b.* f. *brucei* remained to be discovered [32,39,47], but it is now clear that if a single specific protein out of ~8700 in *T. b.* f. *brucei* [48] is disrupted, *T. b.* f. *equiperdum* or *T. b.* f. *evansi* come into existence. Finally, the dys- and akinetoplastic forms occur as a result of drug pressure with standard trypanocides [49], and the generation of dyskinetoplastic flagellates also represents a resistance mechanism [50].

Hence, if a critical gene is lost or rearranged in a wild strain of *T. b.* f. *brucei* that lowers overall fitness, the progeny is under strong selection for an altered life cycle, host range, pathogenicity, and/or geographic distribution, as compared with the original strain. Consequently, extending this argument to HAT disease-causing (sub)species, as even *T. b.* f. *evansi* can infect humans [51], is highly reasonable. Indeed, one parasite causing one disease (easily) becomes another parasite causing another disease.

## The end of monophyly

In regions of high human density and decreasing wild animal populations, infecting humans is a clear advantage to *T. b.* f. *brucei*, and, since neither *T. b.* f. *gambiense* nor *T. b.* f. *rhodesiense*

is monophyletic (Figure 2), this suggests multiple ingress events [39,52]. There are just a few routes by which *T. b.* f. *brucei* can succeed in this transition. Moreover, sequence data support the existence of hybrids between ecotypes [53–55]. *T. b.* f. *rhodesiense* polyphyly was explained as a consequence of recombination between *T. b.* f. *brucei* and *T. b.* f. *rhodesiense* [34], which frequently intermingle in phylogenetic reconstructions (Figure 2), and these minimal genetic differences increase the possibility of SRA gene exchange [56]. Consequently, it is difficult to resolve exact phylogenetic relationships between these ecotypes even with whole-genome sequencing [39,52], and hence it is highly likely that *T. b.* f. *rhodesiense* emerged multiple times from *T. b.* f. *brucei* [35,56,57], with both ecotypes likely originating in East Africa, where they are most heterogeneous [39,52]. Moreover, *T. b.* f. *gambiense* group 2, a genetically heterogeneous assemblage originating from West and Central Africa and not closely affiliated with *T. b.* f. *gambiense* group 1, is also polyphyletic (Figure 2), with no available specific genetic markers [58].

The strongly supported polyphyly of *T. b.* f. *evansi* and *T. b.* f. *equiperdum* provides additional evidence for highly complex relationships. Numerous studies based on just a handful of genes or whole genomes predict multiple independent origins of *T. b.* f. *evansi* and *T. b.* f. *equiperdum*, with a minimum of four instances [32,33,39,59]. Moreover, complete loss of kDNA is elegantly compensated for by a single amino acid change in the $F_1F_O$-ATPase γ-subunit [60], and identification of several different mutations is in agreement with the lack of monophyly of the dys- and akinetoplastic trypanosomes. Genome-based analyses point to East Africa as the region from which these ecotypes originate [32,39,59].

## Clear and speculative trade-offs

We propose that the antecedent *T. brucei* was exceptionally flexible, allowing descendants to assimilate new hosts. These events occurred repeatedly with a restricted set of outcomes and all arising from simple genetic changes representing trade-offs, the most striking of which is the arms race between *T. b.* f. *brucei* and primate apolipoprotein 1 (APOL1) (Figure 4) [61–63]. Specific APOL1 variants can prevent interaction with SRA and are more common among Africans than among the general population [64,65], but they are also associated with early-onset renal disease [66], schizophrenia, stroke, cancer, and other diseases, explaining the failure of such alleles to sweep the population [67]. This Red Queen race is a textbook example of dynamic host–parasite relationships. Such a mechanism is less clear for *T. b.* f. *gambiense*, which has significantly lower pathogenicity than *T. b.* f. *rhodesiense.* Several explanations have been offered, including mutations within the HpHb receptor leading to decreased trypanolytic factor (TLF) accumulation [68,69]. *T. b.* f. *gambiense* also has abrogated heme import [70], representing another possible candidate behind attenuated pathogenicity.

With this dexterity in mind, we propose another scenario, albeit speculative. Due to low parasitemia in mammalian blood, short life, and the low probability of being taken up by tsetse, the stumpy form represents a bottleneck for transmission. When, for eons, high numbers of tsetse were around, this state was tolerable, but now the parasite likely faces a decreasing distribution and density of its insect vector. Hence, trypanosomes may attempt to overcome this limitation, as reflected by the so-called transmission paradox, in which they keep circulating even when the parasitemia level is extremely low [71]. While the presence of flagellates in human skin may be a solution to this problem [72], what if the transmission capacity of slender bloodstream stage [73] has been relatively newly acquired in order to compensate for the unsustainable transmission via the stumpy form, although the latter remains more efficient in infecting flies [74]? Alternatively, reduced stumpy formation might favor higher parasitemias, thus promoting transmission. After all, the related *Trypanosoma congolense* and *Trypanosoma vivax* do not use the stumpy form in their life cycle [75,76]. The latter species not only lacks a form morphologically resembling stumpy bloodstream

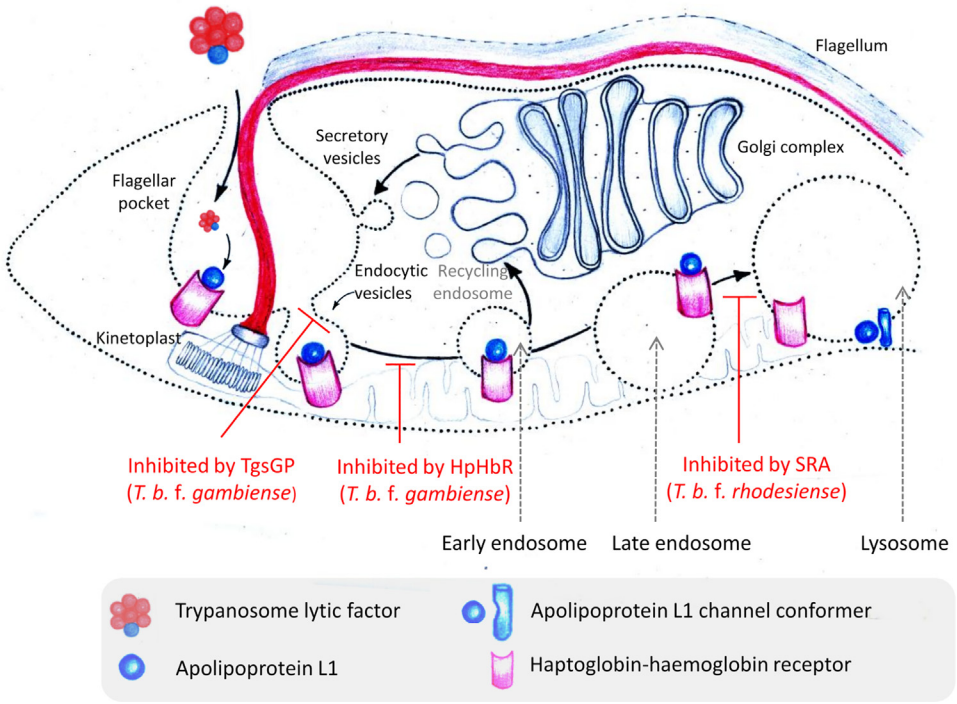


Figure 4. Distinguishing features within the endocytic pathway of *Trypanosoma brucei* subspecies/ecotypes. Schematic drawing of the trypanosome cell, depicting the endocytic pathway for trypanolytic factor (TLF). The endocytosis of the TLF–apolipoprotein 1 (APOL1) complex via the haptoglobin-hemoglobin receptor (HpHbR) results in expansion and eventual rupture of the lysosome, leading to cell death of *T. brucei* f. *brucei*. The mechanism of resistance of *T. b.* f. *gambiense* (group 1) is based on (partial) prevention of internalization of TLF–ApoL1 via mutated HpHbR. TLF–APOL1 is also prevented from being taken into the cell by *T. b.* f. *gambiense*-specific glycoprotein (TgsGP), but the mechanism remains unclear. In *T. b.* f. *rhodesiense*, delivery of APOL1 to the lysosome is prevented by the activity of the serum resistance-associated (SRA) protein. The likely locations of inhibitory steps are indicated in red.

*T. brucei* but also lacks identifiable homologs of protein associated with differentiation, the molecular marker of the stumpy form. However, it remains to be elucidated whether trypanosomes outside the *T. brucei* clade are able to differentiate into stages, which are functionally analogous to the stumpy form and yet are morphologically and physiologically different [76].

When camelids and other large African mammals moved to northern desertification areas, trypanosome infections would terminate with their death unless a tsetse-independent transmission to another host was available [41]. This exercised huge pressure to sustain transmission in the absence of a vector, which becomes possible in the presence of compensatory nuclear mutations enabling kDNA loss [60,77]. The latter is facilitated by lack of requirement for kDNA in the bloodstream stage [78], allowing *T. brucei* to leave Africa and spread around the world [79], although at the same time losing the capacity for genetic exchange that occurs only in the tsetse salivary glands [80]. Since asexuality reduces the efficacy by which selection acts, the differences among *T. b.* f. *equiperdum* and *T. b.* f. *evansi* may therefore be caused by differences in time since loss of meiosis. Their switch to monomorphism is potentially associated with loss of growth control in the absence of stumpy forms, leading to an increased parasitemia to improve the likelihood of transmission. In the long run, loss of meiosis comes with a cost as it appears that the dys- and akinetoplastic trypanosomes are slowly but steadily being replaced by newly emerging strains, although this hypothesis has yet to be tested.

## Concluding remarks

Available evidence convincingly argues that a (sub)species designation does not suit *T. brucei*. We propose that ecotypes provide a more accurate grouping (e.g., *T. brucei* form *gambiense*) and can be used equally well for strains isolated from nature (e.g., *T. b.* f. *brucei*) or generated *in laboratorio* (e.g., *T. b.* f. *evansi*). While there is evidence for emergence in nature of various ecotypes from the ancestral pool, it remains unclear how often this happens and how stable these ecotypes are, especially when they become asexual, as is the case of *T. b.* f. *evansi* and *T. b.* f. *equiperdum*. Based on recent data, it is plausible to consider that the pathogenicity of *T. b.* f. *gambiense* becomes decreased due to abrogated heme import with downstream lack of heme-carrying proteins. This would extend viability of its hosts, which may be necessary in light of its poor transmissibility via tsetse. Our attempt for a more holistic view on African trypanosomes may help propel understanding of the origins of parasitism and host range as more aspects of the biology of *T. brucei* continue to be uncovered (see Outstanding questions).

## Outstanding questions

What is the frequency for emergence of ecotypes within the ancestral pool?

How long can asexual strains sustain their transmission between vertebrate hosts without genetic recombination?

What is the extent of undersampling of natural diversity of *T. brucei*?

Is the lower pathogenicity of *T. b.* f. *gambiense* influenced by abrogated heme import?

Is the poor transmissibility of *T. b.* f. *gambiense* the result of inefficient differentiation into the stumpy form?

What are the molecular determinants of human serum resistance of the *T. b.* f. *gambiense* group 2?

### Acknowledgments

This work was supported by Czech Science Foundation grants 20-071856S and 21-09283S, ERC CZ (LL1601), and the ERD funds of the Czech Ministry of Education (16_019/0000759).

### Declaration of interests

The authors have no interests to declare.

### References

1. Pawlowski, J. *et al.* (2012) CBOL Protist Working Group: barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. *PLoS Biol.* 10, e1001419
2. Mayr, E. (1942) Systematics and the Origin of Species. Columbia University Press
3. Fišer, C. *et al.* (2018) Cryptic species as a window into the paradigm shift of the species concept. *Mol. Ecol.* 27, 613–635
4. Roux, C. *et al.* (2016) Shedding light on the grey zone of speciation along a continuum of genomic divergence. *PLoS Biol.* 14, e2000234
5. Hamilton, P.B. and Stevens, J.R. (2017) Classification and phylogeny of *Trypanosoma cruzi*. In *American Trypanosomiasis Chagas Disease: One Hundred Years of Research* (2nd edn) (Telleria, J. and Tibayrenc, M., eds), pp. 321–344, Elsevier
6. Lai, D.H. *et al.* (2008) Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are *petite* mutants of *T. brucei*. *Proc. Natl. Acad. Sci. U. S. A.* 105, 1999–2004
7. Gibson, W. (2016) Kinetoplastea. In *Handbook of the Protists* (Archibald, J.M. *et al.*, eds), pp. 1–65, Springer
8. Kay, C. *et al.* (2022) Signatures of hybridization in *Trypanosoma brucei*. *PLoS Pathog.* 18, e1010300
9. Sazmand, A. *et al.* (2022) *Trypanosoma evansi*. *Trends Parasitol.* 38, 489–490
10. Jackson, A.P. *et al.* (2016) Kinetoplastid phylogenomics reveals the evolutionary innovations associated with the origin of parasitism. *Curr. Biol.* 26, 161–172
11. Votýpka, J. *et al.* (2021) How monoxenous trypanosomatids revealed hidden feeding habits of their tsetse fly hosts. *Folia Parasitol. (Praha)* 68, 019
12. Lukeš, J. *et al.* (2018) Trypanosomatids are much more than just trypanosomes: clues from the expanded family tree. *Trends Parasitol.* 34, 466–480
13. Frolov, A.O. *et al.* (2021) Development of monoxenous trypanosomatids and phytomonads in insects. *Trends Parasitol.* 37, 538–551
14. Poinar Jr., G. and Poinar, G. Jr. and Poinar, R. (2004) *Paleoleishmania proterus* n. gen., n. sp., (Trypanosomatidae: Kinetoplastida) from Cretaceous Burmese amber. *Protist* 155, 305–310
15. Li, S.-J. *et al.* (2020) Novel organization of mitochondrial minicircles and guide RNAs in the zoonotic pathogen *Trypanosoma lewisi*. *Nucleic Acids Res.* 48, 9747–9761
16. Poinar Jr., G.Poinar, G. Jr. (2007) Early Cretaceous trypanosomatids associated with fossils and fly larvae in Burmese amber. *Mem. Inst. Oswaldo Cruz* 102, 635–637
17. Butenko, A. *et al.* (2021) Reductionist pathways for parasitism in euglenozoans? Expanded datasets provide new insights. *Trends Parasitol.* 37, 100–116
18. Kay, C. *et al.* (2020) Mitochondrial DNAs provide insight into trypanosome phylogeny and molecular evolution. *BMC Evol. Biol.* 20, 161
19. Grimaldi, D.A. (1992) Vicariance biogeography, geographic extinctions, and the North American Oligocene tsetse flies. In *Extinction and Phylogeny* (Novacek, M.J. and Wheeler, Q.D., eds), pp. 178–204, Columbia University Press
20. Wedmann, S. (2000) Die Insekten der oberoligozänen Fossillagerstätte Enspel Westerwald, Deutschland) - Systematik, Biostratonomie und Paläoökologie. *Mainzer Naturwiss. Arch. Beih.* 23, 1–154
21. Grimaldi, D.A. and Engel, M.S. (2005) Evolution of the Insects. Cambridge University Press
22. Maslov, D.A. *et al.* (2019) Recent advances in trypanosomatid research: genome organization, expression, metabolism, taxonomy and evolution. *Parasitology* 146, 1–27
23. Kostygov, A.Y. *et al.* (2021) Euglenozoa: taxonomy, diversity and ecology, symbioses and viruses. *Open Biol.* 11, 200407
24. Wyatt, K.B. *et al.* (2008) Historical mammal extinction on Christmas Island (Indian Ocean) correlates with introduced infectious disease. *PLoS One* 3, e3602
25. Horn, D. (2014) Antigenic variation in African trypanosomes. *Mol. Biochem. Parasitol.* 195, 123–129
26. Quintana, J.F. and Field, M. (2021) Evolution, function and roles in drug sensitivity of trypanosome aquaglyceroporins. *Parasitology* 148, 1137–1142
27. Weir, W. *et al.* (2016) Population genomics reveals the origin and asexual evolution of human infective trypanosomes. *eLife* 5, e11473
28. Hoare, C.A. (1972) The Trypanosomes of Mammals. Blackwell Scientific

29. Tomlinson, S. and Raper, J. (1996) The lysis of *Trypanosoma brucei* by human serum. *Nat. Biotechnol.* 14, 717–721
30. Welburn, S.C. *et al.* (2001) Sleeping sickness: a tale of two diseases. *Trends Parasitol.* 17, 19–24
31. Lun, Z.R. *et al.* (2015) Cancer in the parasitic protozoans *Trypanosoma brucei* and *Toxoplasma gondii*. *Proc. Natl. Acad. Sci. U. S. A.* 112, 8835–8842
32. Carnes, J. *et al.* (2015) Genome and phylogenetic analyses of *Trypanosoma evansi* reveal extensive similarity to *T. brucei* and multiple independent origins for dyskinetoplasty. *PLoS Negl. Trop. Dis.* 9, e3404
33. Oldrieve, G. *et al.* (2021) Monomorphic *Trypanozoon*: towards reconciling phylogeny and pathologies. *Microb. Genom.* 7, 000632
34. Gibson, W. *et al.* (2002) The human serum resistance associated gene is ubiquitous and conserved in *Trypanosoma brucei rhodesiense* throughout East Africa. *Infect. Genet. Evol.* 1, 207–214
35. Balmer, O. *et al.* (2011) Phylogeography and taxonomy of *Trypanosoma brucei*. *PLoS Negl. Trop. Dis.* 5, e961
36. Gibson, W. *et al.* (2010) Conserved sequence of the TgsGP gene in Group 1 *Trypanosoma brucei gambiense*. *Infect. Genet. Evol.* 10, 453–458
37. Verloo, D. *et al.* (2001) General expression of RoTat 1.2 variable antigen type in *Trypanosoma evansi* isolates from different origin. *Vet. Parasitol.* 97, 183–189
38. Njiru, Z.K. *et al.* (2010) Loop-mediated isothermal amplification (LAMP) test for detection of *Trypanosoma evansi* strain B. *Exp. Parasitol.* 125, 196–201
39. Cuypers, B. *et al.* (2017) Genome-wide SNP analysis reveals distinct origins of *Trypanosoma evansi* and *Trypanosoma equiperdum*. *Genome Biol. Evol.* 9, 1990–1997
40. Zeelen, J. *et al.* (2021) Structure of trypanosome coat protein VSGsur and function in suramin resistance. *Nat. Microbiol.* 6, 392–400
41. Schnaufer, A. *et al.* (2002) Natural and induced dyskinetoplastic trypanosomatids: how to live without mitochondrial DNA. *Int. J. Parasitol.* 32, 1071–1084
42. Büscher, P. *et al.* (2019) Equine trypanosomosis: enigmas and diagnostic challenges. *Parasit. Vectors* 12, 234
43. Li, F.-J. *et al.* (2007) PCR approach for the detection of *Trypanosoma brucei* and *T. equiperdum* and their differentiation from *T. evansi* based on maxicircle kinetoplast DNA. *Mol. Cell. Probes* 21, 1–7
44. Stuart, K. and Gelvin, S.R. (1980) Kinetoplast DNA of normal and mutant *Trypanosoma brucei*. *Am. J. Trop. Med. Hyg.* 29, 1075–1081
45. Wang, Z. and Englund, P.T. (2001) RNA interference of a trypanosome topoisomerase II causes progressive loss of mitochondrial DNA. *EMBO J.* 20, 4674–4683
46. Liu, B. *et al.* (2009) Trypanosomes have six mitochondrial DNA helicases with one controlling kinetoplast maxicircle replication. *Mol. Cell* 35, 490–501
47. Desquesnes, M. *et al.* (2013) *Trypanosoma evansi* and surra: a review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. *Biomed. Res. Int.* 2013, 321237
48. Warrenfeltz, S. *et al.* (2018) EuPathDB: the eukaryotic pathogen genomics database resource. *Methods Mol. Biol.* 1757, 69–113
49. Shapiro, T.A. and Englund, P.T. (1990) Selective cleavage of kinetoplast DNA minicircles promoted by antitrypanosomal drugs. *Proc. Natl. Acad. Sci. U. S. A.* 87, 950–954
50. Eze, A.A. *et al.* (2016) Reduced mitochondrial membrane potential is a late adaptation of *Trypanosoma brucei* to isometamidium preceded by mutations in the γ subunit of the $F_1F_o$-ATPase. *PLoS Negl. Trop. Dis.* 10, e0004791
51. Vanhollebeke, B. *et al.* (2006) Human *Trypanosoma evansi* infection linked to a lack of apolipoprotein L-1. *N. Engl. J. Med.* 355, 2752–2756
52. Richardson, J.B. *et al.* (2017) Genomic analyses of African *Trypanozoon* strains to assess evolutionary relationships and identify markers for strain identification. *PLoS Negl. Trop. Dis.* 11, e0005949
53. Capewell, P. *et al.* (2013) The TgsGP gene is essential for resistance to human serum in *Trypanosoma brucei gambiense*. *PLoS Pathog.* 9, e1003686
54. Duffy, C.W. *et al.* (2014) Population genetics of *Trypanosoma brucei rhodesiense*: clonality and diversity within and between foci. *PLoS Negl. Trop. Dis.* 7, e0002526
55. Echodu, R. *et al.* (2015) Genetic diversity and population structure of *Trypanosoma brucei* in Uganda: implications for the epidemiology of sleeping sickness and nagana. *PLoS Negl. Trop. Dis.* 19, e0003353
56. Sistrom, M. *et al.* (2016) *De novo* genome assembly shows genome wide similarity between *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*. *PLoS One* 11, e0147660
57. Gibson, W.C. and Mizen, V.H. (1997) Heritability of the trait for human infectivity in genetic crosses of *Trypanosoma brucei* ssp. *Trans. R. Soc. Trop. Med. Hyg.* 91, 236–237
58. Jamonneau, V. *et al.* (2019) *Trypanosoma brucei gambiense* group 2: the unusual suspect. *Trends Parasitol.* 35, 983–995
59. Kamidi, C.M. *et al.* (2017) Multiple evolutionary origins of *Trypanosoma evansi* in Kenya. *PLoS Negl. Trop. Dis.* 11, e0005895
60. Dean, S. *et al.* (2013) Single point mutations in ATP synthase compensate for mitochondrial genome loss in trypanosomes. *Proc. Natl. Acad. Sci. U. S. A.* 110, 14741–14746
61. Pays, E. *et al.* (2014) The molecular arms race between African trypanosomes and humans. *Nat. Rev. Microbiol.* 12, 575–584
62. Peréz-Morga, D. *et al.* (2005) Apolipoprotein L-I promotes trypanosome lysis by forming pores in lysosomal membranes. *Science* 309, 469–472
63. Oli, M.W. *et al.* (2006) Serum resistance-associated protein blocks lysosomal targeting of trypanosome lytic factor in *Trypanosoma brucei*. *Eukaryot. Cell* 5, 132–139
64. Lecordier, L. *et al.* (2009) C-terminal mutants of apolipoprotein L-I efficiently kill both *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*. *PLoS Pathog.* 5, e1000685
65. Thomson, R. *et al.* (2009) Hydrodynamic gene delivery of baboon trypanosome lytic factor eliminates both animal and human-infective African trypanosomes. *Proc. Natl. Acad. Sci. U. S. A.* 106, 19509–19514
66. Genovese, G. *et al.* (2010) Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 329, 841–845
67. Friedman, D.J. and Pollak, M.R. (2020) APOL1 and kidney disease: from genetics to biology. *Annu. Rev. Physiol.* 82, 323–342
68. Symula, R.E. *et al.* (2012) *Trypanosoma brucei gambiense* group 1 is distinguished by a unique amino acid substitution in the HpHb receptor implicated in human serum resistance. *PLoS Negl. Trop. Dis.* 6, e1728
69. Higgins, M.K. *et al.* (2013) Structure of the trypanosome haptoglobin-hemoglobin receptor and implications for nutrient uptake and innate immunity. *Proc. Natl. Acad. Sci. U. S. A.* 110, 1905–1910
70. Horáková, E. *et al.* (2022) Heme-deficient metabolism and impaired cellular differentiation as an evolutionary trade-off for human infectivity in *Trypanosoma brucei gambiense*. *bioRxiv* Published online May 13, 2022. https://doi.org/10.1101/2022.05.12.491725
71. Capewell, P. *et al.* (2019) Resolving the apparent transmission paradox of African sleeping sickness. *PLoS Biol.* 17, e3000105
72. Capewell, P. *et al.* (2016) The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. *eLife* 5, e17716
73. Schuster, S. *et al.* (2021) Unexpected plasticity in the life cycle of *Trypanosoma brucei*. *eLife* 10, e66028
74. Matthews, K.R. and Larcombe, S. (2022) Comment on 'Unexpected plasticity in the life cycle of *Trypanosoma brucei*. *eLife* 11, e74985
75. Rotureau, B. and Van Den Abbeele, J. (2013) Through the dark continent: African trypanosome development in the tsetse fly. *Front. Cell. Infect. Microbiol.* 4, 53
76. Szöőr, B. *et al.* (2020) A leap into the unknown - early events in African trypanosome transmission. *Trends Parasitol.* 36, 266–278
77. Jensen, R.E. *et al.* (2008) What happens when *Trypanosoma brucei* leaves Africa. *Trends Parasitol.* 24, 428–431
78. Speijer, D. (2006) Is kinetoplastid pan-editing the result of an evolutionary balancing act? *IUBMB Life* 58, 91–96
79. Lun, Z.-R. *et al.* (2010) *Trypanosoma brucei*: two steps to spread out from Africa. *Trends Parasitol.* 26, 434–437

80. Peacock, L. *et al.* (2011) Identification of the meiotic life cycle stage of *Trypanosoma brucei* in the tsetse fly. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3671–3676
81. Lowry, D.B. (2012) Ecotypes and the controversy over stages in the formation of new species. *Biol. J. Linn. Soc.* 106, 241–257
82. Finlay, B.J. (2004) Protist taxonomy: an ecological perspective. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 359, 599–610
83. James, M.E. *et al.* (2021) Highly replicated evolution of parapatric ecotypes. *Mol. Biol. Evol.* 38, 4805–4821
84. Yaro, M. *et al.* (2016) Combatting African animal trypanosomiasis (AAT) in livestock: the potential role of trypanotolerance. *Vet. Parasitol.* 225, 43–52
85. Raper, J. *et al.* (1999) Characterization of a novel trypanosome lytic factor from human serum. *Infect. Immun.* 67, 1910–1916
86. Franco, J.R. *et al.* (2014) Epidemiology of human African trypanosomiasis. *Clin. Epidemiol.* 6, 257–275
87. Heisch, R.B. *et al.* (1958) The isolation of *Trypanosoma rhodesiense* from a bushbuck. *Br. Med. J.* 2, 1203–1204
88. Fèvre, E.M. *et al.* (2001) The origins of a new *Trypanosoma brucei rhodesiense* sleeping sickness outbreak in eastern Uganda. *Lancet* 358, 625–628
89. Xong, H.V. *et al.* (1998) A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell* 95, 839–846
90. Capewell, P. *et al.* (2015) A co-evolutionary arms race: trypanosomes shaping the human genome, humans shaping the trypanosome genome. *Parasitology* 142, S108–S119
91. Büscher, P. *et al.* (2018) Do cryptic reservoirs threaten gambiense-sleeping sickness elimination? *Trends Parasitol.* 34, 197–207
92. Lindner, A.K. and Priotto, G. (2010) The unknown risk of vertical transmission in sleeping sickness – a literature review. *PLoS Negl. Trop. Dis.* 4, e783
93. Truc, P. *et al.* (1997) Confirmation of two distinct classes of zymodemes of *Trypanosoma brucei* infecting man and wild mammals in Côte d'Ivoire: suspected difference in pathogenicity. *Ann. Trop. Med. Parasitol.* 91, 951–956
94. Kieft, R. *et al.* (2010) Mechanism of *Trypanosoma brucei gambiense* (group 1) resistance to human trypanosome lytic factor. *Proc. Natl. Acad. Sci. U. S. A.* 107, 16137–16141
95. Umeakuana, P.U. *et al.* (2019) Identification of *Trypanosoma brucei gambiense* in naturally infected dogs in Nigeria. *Parasit. Vectors* 12, 420
96. Vourchakbé, J. *et al.* (2020) Molecular identification of *Trypanosoma brucei gambiense* in naturally infected pigs, dogs and small ruminants confirms domestic animals as potential reservoirs for sleeping sickness in Chad. *Parasite* 27, 63
97. DeJesus, E. *et al.* (2013) A single amino acid substitution in the group 1 *Trypanosoma brucei gambiense* haptoglobin-hemoglobin receptor abolishes TLF-1 binding. *PLoS Pathog.* 9, e1003317
98. Berberof, M. *et al.* (2001) A receptor-like flagellar pocket glycoprotein specific to *Trypanosoma brucei gambiense*. *Mol. Biochem. Parasitol.* 113, 127–138
99. Claes, F. *et al.* (2005) *Trypanosoma equiperdum*: master of disguise or historical mistake? *Trends Parasitol.* 21, 316–321
100. Paris, Z. *et al.* (2011) Futile import of tRNAs and proteins into the mitochondrion of *Trypanosoma brucei evansi*. *Mol. Biochem. Parasitol.* 176, 116–120
101. Gahura, O. *et al.* (2021) Redesigned and reversed: architectural and functional oddities of the trypanosomal ATP synthase. *Parasitology* 148, 1151–1160
102. Field, M.C. and Boothroyd, J.C. (1996) Sequence divergence in a family of variant surface glycoprotein genes from trypanosomes: coding region hypervariability and downstream recombinogenic repeats. *J. Mol. Evol.* 42, 500–511
103. Blum, M.L. *et al.* (1993) A structural motif in the variant surface glycoprotein of *Trypanosoma brucei*. *Nature* 362, 603–609