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Trypanosoma brucei: two steps to spread out from Africa

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***Trypanosoma brucei equiperdum* and *Trypanosoma brucei evansi* are typically considered separate species, although a recent study suggested that these organisms can be classified as subspecies of *Trypanosoma brucei*, which we also favor. Here we present a scenario that attempts to explain the continuing evolution of the dyskinetoplastic and akinetoplastic strains, as a consequence of loss of selective pressure(s) leading to the loss of kinetoplast DNA.**

The biology of kinetoplast DNA in *Trypanozoon* species

Trypanosoma brucei is a kinetoplastid flagellate responsible for human sleeping sickness (trypanosomiasis), a devastating tropical disease in Africa. *Trypanosoma brucei equiperdum* and *Trypanosoma brucei evansi* are agents of dourine and surra, respectively, diseases of horses, camels and water buffaloes, which account for huge economic losses in Africa and Asia. Both trypanosomes resemble the mutants of *T. brucei* [1], but because they cause different pathology than *T. brucei*, we find it appropriate to consider them as subspecies, and they will be named as such herein. Genome sequencing will hopefully result in solving the species/subspecies status of these organisms, an endeavor that is under way. A distinctive feature of kinetoplastid flagellates is the presence of their eponymous kinetoplast [2]. The kinetoplast DNA (kDNA) consists of a huge network of usually interlocked circular DNA molecules of two types: maxicircles and minicircles. The maxicircles encode classical mitochondrial (mt) genes, with transcripts that are rendered translatable upon RNA editing, a process which involves uridine insertions and/or deletions. Minicircles encode small RNA molecules named guide (g)RNAs that are necessary for decoding encrypted maxicircle transcripts [3–6].

The kDNA of *T. b. brucei* is one of the largest mt genomes known, its faithful replication being ensured by a sophisticated machinery [5,7,8]. It includes dozens of maxicircles, each about 23 kilobases (kb) and thousands of minicircles, each about 1 kb in size. Surprisingly, a partial and even full loss of kDNA, known as dyskinetoplastidy (Dk) and akinetoplastidy (Ak), respectively, be induced in the laboratory [9].

The complex life cycle of *T. b. brucei* involves a bloodstream stage (BS) in a vertebrate host, where its proliferation relies on glycolysis, with a functionally downregulated mitochondrion, as well as a procyclic stage (PS) parasitizing the tsetse fly vector, which depends on a fully active organelle [10]. The total or partial loss of kDNA locks the trypanosome in the BS, because maxicircle-encoded proteins are essential for the survival of the PS [11], eliminating the insect stages from its life cycle (see Table 1). This is what has happened in *T. b. evansi*, which lost its maxicircle kDNA and is transmitted mechanistically from the proboscis of blood-feeding insects [1,12,13]. This, in turn, has made it possible for the parasite to leave the African tsetse distribution range and spread beyond it to other continents [14]. *T. b. evansi* is a mammalian pathogen that enjoys a broad range of hosts and wide geographic distribution in Africa, Asia and South America [15–17].

Another trypanosome without a PS or even an insect vector is *T. b. equiperdum*, morphologically indistinguishable from *T. b. evansi*, which is transmitted during coitus between equines (Table 1). The maxicircles of some but not all *T. b. equiperdum* strains contain large deletions in the gene-rich region [1,13,18]. Deletions in the maxicircle were also found in an acriflavine-induced Dk strain of *T. b. brucei* in the laboratory [9,19] and, interestingly, recently also in several isolates of *Trypanosoma cruzi*, the causative agent of Chagas disease [20].

Nonetheless, most *T. b. equiperdum* strains examined thus far have maxicircles of a similar size ($\sim$ 24 kb) as *T. b. brucei*, seemingly including all genes, with the Pasteur [9] and STIB842 strains [1] belonging to this category. Similarly, Hajduk and Vickerman [21] did not find any changes in the maxicircles of *T. brucei* that had lost the ability to infect the tsetse. The question is why these strains, with full-size maxicircles as well as minicircles in their kinetoplasts, fail to develop in the insect vector. This might be due to the fact that the minicircles of *T. b. equiperdum* are highly homogeneous (sometimes referred to as microheterogeneous, Table 1) in DNA sequence, a property that they share with *T. b. evansi* [1,9,22]. Indeed, it seems that *T. b. evansi* and *T. b. equiperdum* are virtually indistinguishable in morphology, biochemistry and molecular biology, except for the absence of maxicircles in *T. b. evansi* [1,13,14,16,23–27].

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Table 1. Characteristics of wild type T. brucei brucei, T. brucei equiperdum and T. brucei evansi

Characteristics	*T. b. brucei*	*T. b. equiperdum*	*T. b. evansi*	Ref.
kDNA maxi	full size maxi	full size or partially deleted maxi	no maxi	[1,13,15,16,18,24,26,27]
kDNA mini	heterogeneous, hundreds of types	homogenous/ microheterogeneous[a], few types	homogenous/ microheterogeneous or no mini, few types or none	[1,13,15,16,22,24–26,29,30,33]
Bloodstream stage	polymorphic, slender [b], intermediate [c] and stumpy forms [d]	monomorphic, slender form only	monomorphic, slender form only	[1,15,24]
In vitro at 27 °C	procyclic forms	cannot be cultured	cannot be cultured	[1,15,24]

[a]microheterogeneous = heterogeneity that occurs only on a very small scale; here is used to describe that the species of minicircles or gRNA are reduced from hundreds to a handful.
[b]slender form is the only dividing bloodstream form.
[c]intermediate form initiates irreversible differentiation into stumpy form.
[d]stumpy form are predestined for development in the tsetse midgut.Abbreviations: kDNA, kinetoplast DNA; maxi, maxicircles; mini, minicircles.

T. b. brucei encodes a full set of minicircle sequence classes with a potential to produce about 750 different transcripts, with approximately 300 putative gRNAs confirmed to date (http://rna.bmb.uga.edu/kiss/) [28]. By contrast, the (largely) homogeneous minicircle population of *T. b. equiperdum* and *T. b. evansi* means that the number of gRNA genes is reduced [29,30], and this loss will make these parasites incompetent for the PS in the tsetse [1,9]. For example, the BS of the STIB842 strain of *T. b. equiperdum*, which has full-size maxicircles that include all the same genes as *T. b. brucei*, but whose minicircles are highly homogeneous, is expected to be unable to transform into the PS [1].

How did African *T. brucei* spread out of Africa?

We purport that the evolution of *T. b. equiperdum* and *T. b. evansi* might have occurred in two stages. Initially, there would have been a loss of minicircle heterogeneity in the *T. b. brucei* BS due to asymmetric division of kinetoplast DNA [31]. The cause of this might have resulted from a mutation in enzyme(s) involved in kDNA replication and maintenance, such as mt topoisomerase II [7,31] or alternative RNA editing protein 1 (AEP-1) [32]. A possible consequence is that these parasites eventually would completely lose their ability to differentiate within the insect vector (no PS) and be limited to mechanical transmission (BS only and monomorphic) among their mammalian hosts, which would in turn result over many generations in the gradual or total loss of the maxicircles, owing to lack of selective pressure favoring their preservation (Figure 1). This sequence of events would result in the sequential evolution of *T. b. equiperdum* and *T. b. evansi*. This is consistent with the early observation that Dk individuals were observed in *T. b. brucei* strains that were long passaged in laboratory animals [15]. The complete loss of maxicircles and minicircles has also been observed in *T. b. evansi* infecting domestic and wild animals in Brazil and elsewhere in South America [33]. Consistent with these results is the observation of an increasing percentage of Dk cells found in *T. b. evansi* and *T. b. equiperdum* strains after long-term passage in the mouse or *in vitro* cultivation [15,34,35]. A similar phenomenon has been observed in another kinetoplastid flagellate, *Leishmania tarentolae*, where the editing potential gradually reduced as the length of laboratory culture was prolonged to 55 years [36,37]. It deserves notice that the appearance of Dk individuals has not been observed in wild type strains of *T. b. brucei* (or other trypanosome species) that retain the ability to infect their insect vectors, except in cases of drug treatments [9,15,19].

However, an alternative scenario for trypanosomes spreading out of Africa also needs consideration. Because *T. b. brucei* is transmitted by the tabanid flies with a surprising efficiency [38], the spreading outside of the tsetse belt might predate all mutations preventing tsetse-mediated transmission. The set of mutations

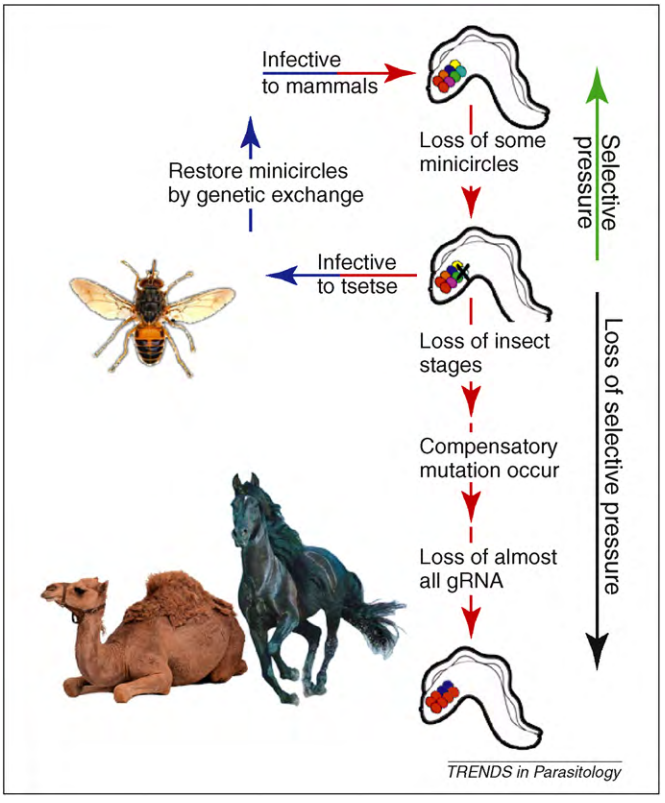

Figure 1. Suggested evolutionary pathway from *T. b. brucei* to *T. b. equiperdum* and *T. b. evansi*. Minicircle and gRNA diversity is lost during the BS of the parasite. If the infectivity to the tsetse remains after a decrease of gRNA diversity, selection pressure will favor the restoration of gRNA diversity by recombination in the tsetse vector. Once the infectivity is lost, the loss of selective pressure will probably favor further reduction of gRNA diversity (*T. b. equiperdum*) and eventually the complete loss of the kDNA maxicircles (*T. b. evansi*). The events that happened in the tsetse vector or mammal are outlined in blue and red, respectively (estimated frequencies are indicated by the size of arrows). Minicircle diversity of trypanosomes is shown as circles in variable colors.

described in *T. b. equiperdum* might thus be a consequence rather than the cause of elimination of the PS from their life cycle.

Although most mt genes are transcribed, and the transcripts are processed and edited in the BS of *T. b. brucei*, only a few proteins are translated, with the extreme possibility of ATPase subunit 6 and AEP-1 being the only proteins actually produced in the organelle of the BS [32,39,40]. However, the number of available gRNAs is the same as in the PS, where a set of mt proteins is produced. This disproportion might result in the loss of gRNAs, the risk for which increases as the parasites persist longer in the BS, considering that the BS only needs 20 to 30 gRNAs for editing of A6 and AEP-1 [32], whereas the PS is estimated to require at least 200 essential gRNAs [41]. One possible outcome is that, because of the loss of essential gRNAs, the parasites will no longer be able to infect the tsetse. Thus, the evolution of *T. b. equiperdum* strains and *T. b. evansi* strains might be initiated. Without infection of the tsetse, the process is irreversible because sexual and/or genetic recombination between minicircles that would restore the diversity of gRNAs can only occur in the insect vector [42–44]. In the absence of selective pressure for expression of the mt genes, which is the case in the BS and during cultivation *in vitro*, the loss of gRNA diversity seems to occur quite rapidly because of large-scale deletions and asymmetrical division of the kDNA network [1,45].

In the tsetse, selection will favor the recombination between minicircles and the ensuing increased diversity of the gRNAs because this will promote an effective expression of the mRNA genes. Thus, selection pressure in *T. b. brucei* might occur in two alternative ways (Figure 1). The prevailing type of pressure goes towards restoring the diversity of minicircles by genetic recombination. This would preserve the infectivity and other attributes of *T. b. brucei*. An alternative form of selection will occur in parasites that persist in the BS, so that the amount of kDNA will gradually decrease, and eventually a complete loss of the maxi- and minicircles might occur. The gradual loss of gRNA diversity (which occurs owing to the absence of selection conserving their diversity because this is not needed in the BS) might reach a point of no return, once the parasite is no longer capable of infecting the tsetse. Indeed, Lai *et al*. have shown that strains of *T. b. equiperdum* and *T. b. evansi* evolve repeatedly from different *T. b. brucei* lineages [1]. We suggest that the evolution of *T. b. equiperdum* and *T. b. evansi* from *T. b. brucei* is a gradual and continuing process. This view is supported by the presence, among investigated strains, of several intermediate states leading from a fully functional kDNA to its complete loss. If there was no steady flow of trypanosomes from Africa, and continuing evolution of *T. b. equiperdum* and *T. b. evansi*, only their terminal Ak state would probably be encountered.

Mutations can occur, however, that restore the infectivity of trypanosomes that have been transmitted directly between mammalian hosts and thus persisted for a prolonged period only in the BS. Even mutations in the nuclear-encoded subunits of ATPase have been found in the Ak and Dk strains of *T. b. brucei, T. b. equiperdum* and *T. b. evansi* that compensate for the loss of the only maxicircle-encoded subunit A6 [1,40]. These observations, conversely, do not exclude the possibility that in some strains the compensatory mutations might have occurred before the loss of the gRNAs needed only in the PS [21,46]. The model we are proposing assumes that the loss of the insect stages can occur rapidly and, indeed, Hajduk and Vickerman [21] recorded it during 77 passages of the parasite in mice.

Much was recently learned about differences concerning kDNA among *T. b. brucei*, *T. b. equiperdum* and *T. b. evansi*, as well as about the rather unexpected emergence of the latter two from *T. b. brucei*. Yet there has been little progress in understanding of the substantial differences in the pathologies they cause. This lack of understanding is a result of the surprising lack of newly isolated strains of *T. b. equiperdum* [26] and difficulties with experiments in animals. It is worth noting that *T. b. brucei* and *T. b. evansi* kill a horse significantly faster than *T. b. equiperdum* (two weeks to four months, versus several months to two years) [15] and that *T. b. equiperdum* has a predilection for immune privileged zones, such as testes, with only rare occurrence in blood. Moreover, whereas surra caused by *T. b. evansi* can be treated with available drugs, dourine, for which *T. b. equiperdum* is responsible, is usually considered incurable [47].

Concluding remarks

The available data support a scenario, in which *T. b. equiperdum* and *T. b. evansi* emerge repeatedly and relatively frequently from *T. b. brucei*. The hypothesis advanced here about the origin of both pathogens of ungulates will soon be tested by analyses of their entire nuclear genomes. Hopefully, these analyses will aid in discovering ways for limiting the enormous economic damage associated with the diseases they cause.

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