Sexual Reproduction Maintains Mitochondrial Genome Fitness in Trypanosomatid Parasites

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Clonal strains/subspecies have arisen multiple times independently from a *Trypanosoma brucei brucei* (*Tbb*) common ancestor

Background and Introduction

Mitochondrial DNA in kinetoplastids (kinetoplast DNA or kDNA) is unique in structure and gene content. kDNA comprises of maxicircles and minicircles, both necessary for gene expression (Fig. 1). During cell division, imperfect replication and segregation of kDNA result in fluctuation in the minicircle populations. Sexual reproduction reshuffles minicircles among insect-transmissible isolates and rescues underrepresented minicircles (ref1).

However, some strains have evolved from obligatory tsetse transmission to direct transmission between mammals and become tsetse independent, such as *T. b. equiperdum* (*Tbeq*) and *T. b. evansi* (*Tbev*) (Fig. 2). A causative agent of chronic HAT, *T. b. gambiense* type 1 *(Tbg 1),* has also abandoned sexual reproduction, although still transmitted by tsetse (ref 2). The lack of sexual reproduction and recombination appears to have led to substantial reduction of minicircle diversity.

To examine the impact of different lifecycles on kDNA complexity , kDNA assembly was performed on 262 *T. brucei* isolates from various geographic areas and representing all subspecies using KOMICS (ref 2).

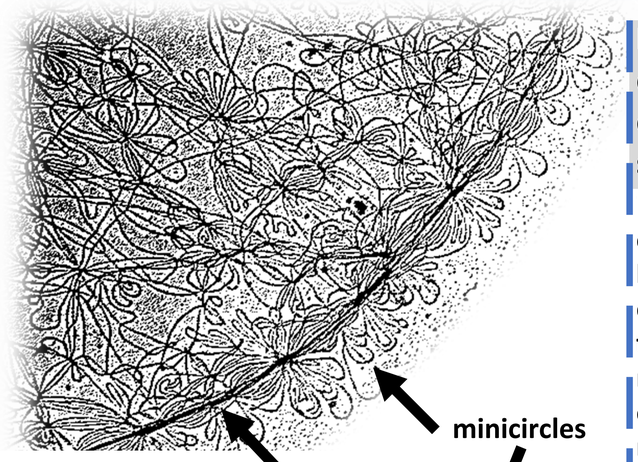

Fig. 1. Kinetoplast DNA is composed of maxi- and minicircles, linked like chain mail armour in a disk-shaped structure.

Tbb kDNA contains 20-50 identical copies of ~23-kb maxicircles and 5-10k highly heterogeneous 1-kb minicircles. Minicircles encode gRNAs required for post-transcriptional editing of several maxicircle-encoded mRNAs. The only edited mRNAs that are required in the mammalian bloodstream stage encode subunits A6 and RPS12 of the F_1F_o-ATPase and the mitoribosome, respectively (image from ref 1).

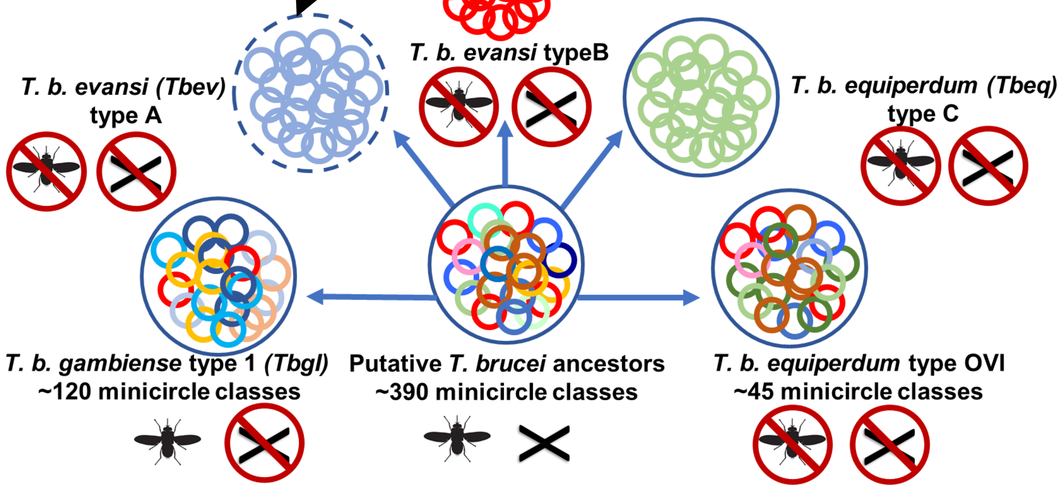

Fig. 2. kDNA network of clonal *T. brucei ssp:* Absence of sexual reproduction leads to various degrees of kDNA reduction in the asexual subspecies.

The kDNA of three groups of *Tbev* and *Tbeq* each contains a single minicircle class diagnostic of each group (type A, B, C). *Tbeq* type OVI and *Tbgl* have streamlined editing capacity, while the former can potentially edit A6 and RPS12 required in mammalian bloodstream, and the latter can usually edit all mRNAs required in the tsetse fly vector.

gRNA coverage of mRNAs essential in mammalian bloodstream is nearly complete in minicircle-reduced strains

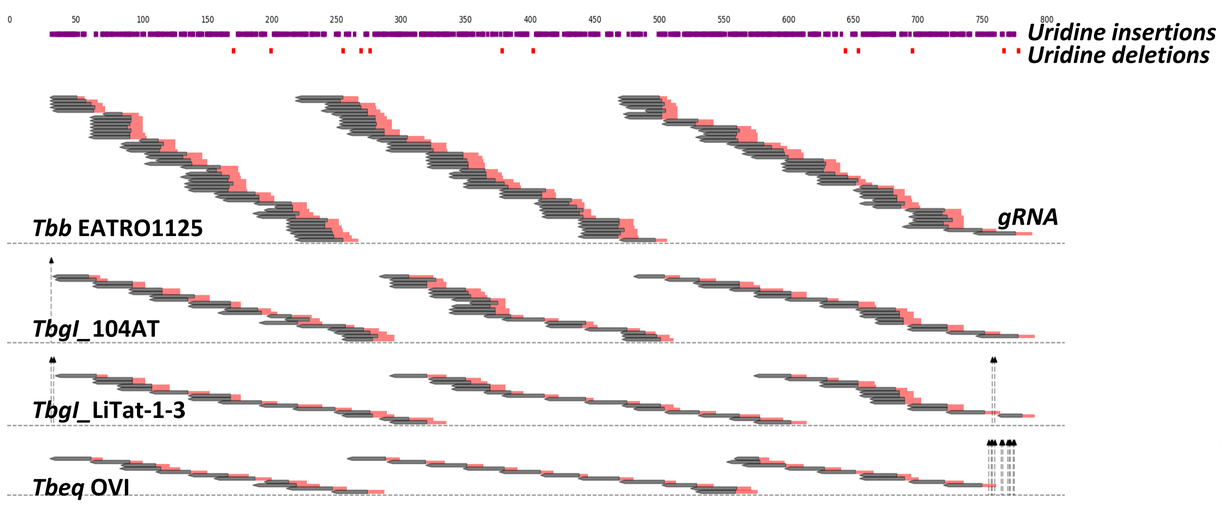

Fig. 3. Streamlined editing capacity in clonal *T. brucei ssp* indicates cumulative and irreversible loss of minicircle diversity

The pleomorphic *Tbb* strain EATRO1125 has highly redundant gRNA coverage of the mRNA A6 editing sites. A very similar scenario is observed for the RPS12 mRNA (not shown). (gRNAs: blue, anchors: yellow, U-deletions: red, U-insertions: purple)

References

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*Tbg*I has a highly conserved and less complex minicircle population

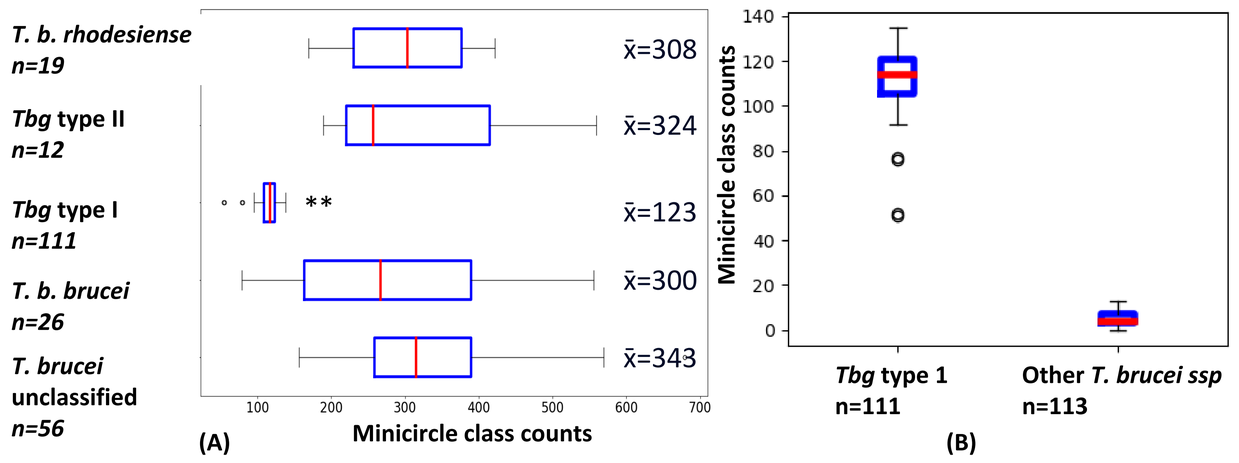

Fig. 4. The conservation and uniqueness support the hypothesis that *TbgI* is derived from a single progenitor that emerged within the last 10,000 years (ref 2).

(A) The mean network complexity for *TbgI* is 123 classes per network. significantly lower than other *T. brucei* subspecies that include sexual reproduction in their lifecycles (<= 300 unique minicircle per network). (B) From 224 tsetse-transmissible isolates we assembled 5668 distinct minicircle classes. Collectively, *TbgI* contributes 195 classes. Most of these minicircles are shared widely among *TbgI* isolates and underrepresented in other *T. brucei* isolates.

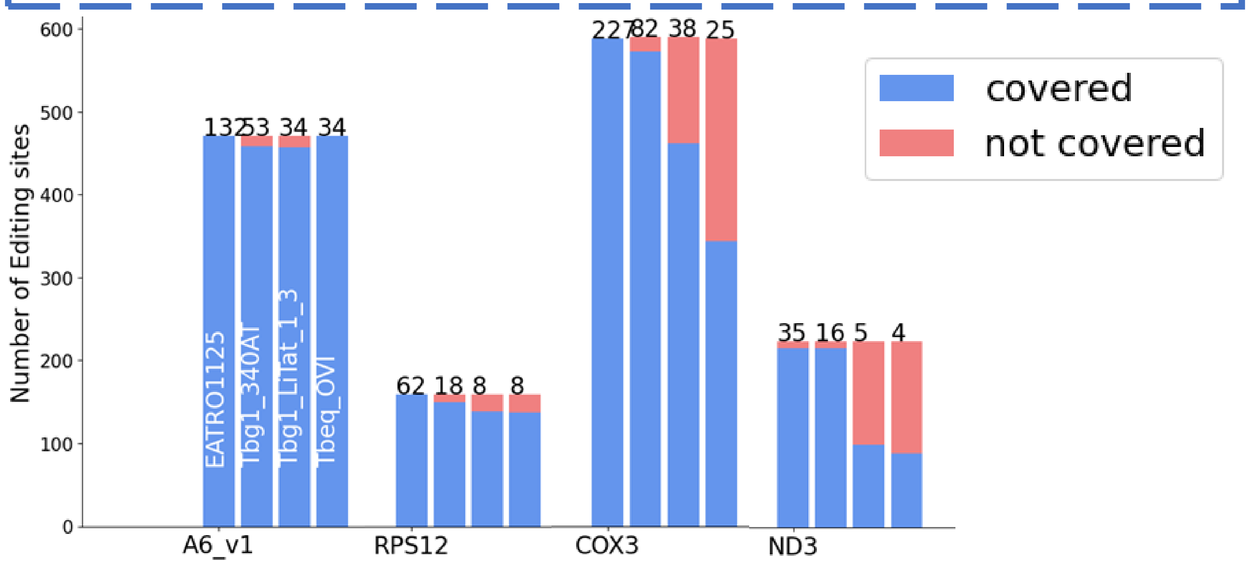

Fig. 5. Surprisingly, gRNA coverage for A6 and RPS12 mRNAs in *Tbeq* type OVI strains is complete or nearly complete. Coverage for insect-stage specific COX3 and ND3 mRNAs is incomplete in *Tbeq* OVI and *TbgI* isolate LiTat-1-3.

This suggests that: (i) contrary to long-standing assumptions, *Tbeq* OVI is either still kDNA-dependent or has become independent only relatively recently. (ii) Some *Tbg*1 strains can no longer survive in or be transmitted by tsetse flies. LiTat-1-3 has undergone prolonged culturing. EATRO1125 and 340AT: tsetse-transmissible *Tbb* and *Tbg1*.

Only a fraction of *Tbg*1 cells within each clonal population still retain the ability to survive in the tsetse vector

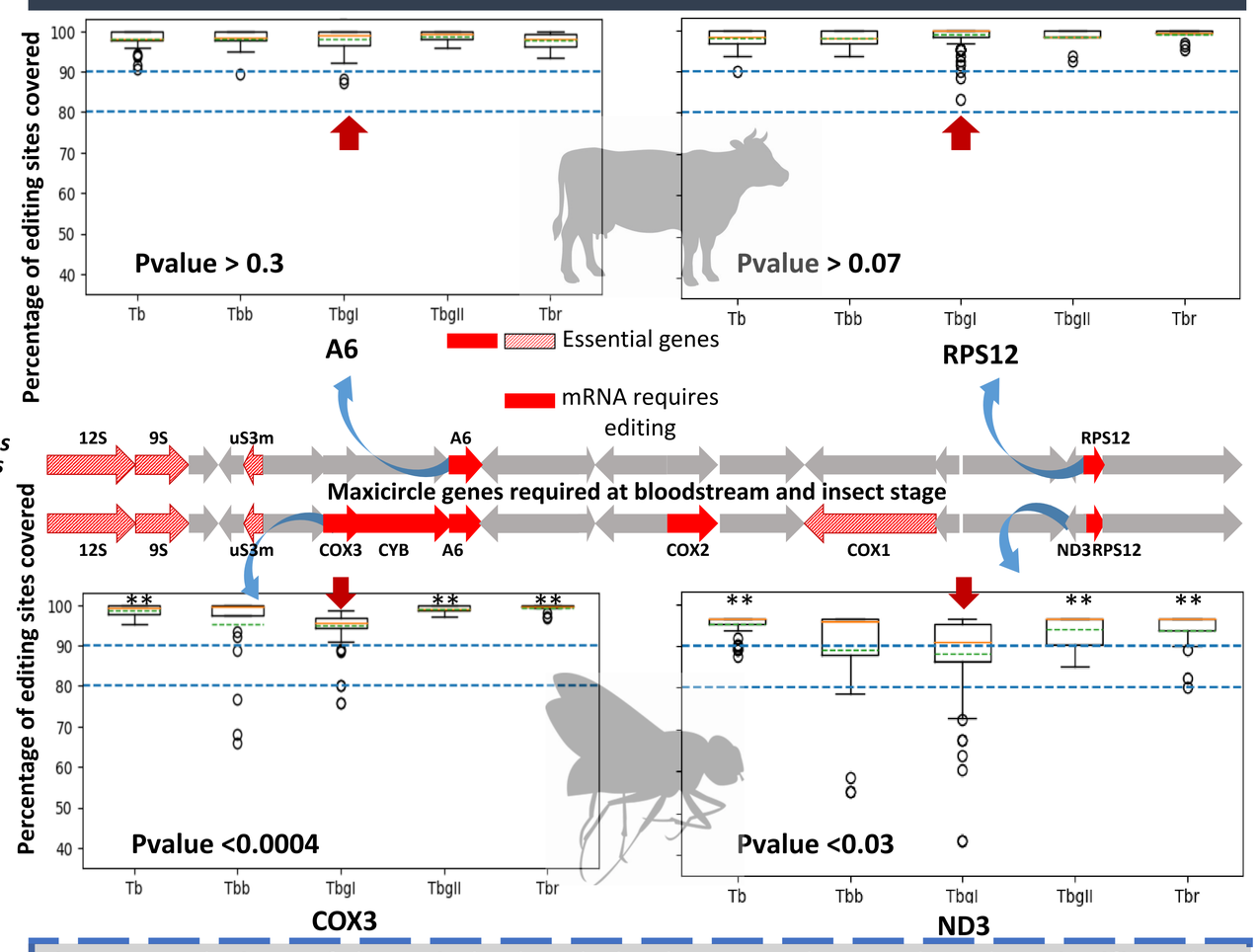

Fig. 6. Populations of the strictly clonal *TbgI* have nearly complete, but significantly lower, editing sites coverage for insect-stage specific gene mRNAs compared to other *T. brucei* subspecies capable of sexual reproduction

gRNA coverages for A6 and RPS12 do no differ significantly between subspecies. In contrast, coverage for COX3 and ND3 mRNAs is significantly lower, suggesting relative loss of the corresponding minicircles within the population. This may explain the variable tsetse infectivity reported for *Tbg*1 field isolates (ref 4).

Acknowledgements

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