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## 16           **Abstract**

17           Sexual recombination between pathogenic microbes has the potential to mobilise  
18 genes for harmful traits into new genetic backgrounds creating new pathogen strains. Since  
19 1986 we have known that genetic exchange can occur in trypanosomes, but we are only  
20 now starting to unravel details of the process. In *Trypanosoma brucei* genetic exchange  
21 occurs in the tsetse vector, but is not an obligatory part of the life cycle. The process  
22 involves meiosis and production of haploid gametes, and thus appears to be true sexual  
23 reproduction. This review looks at the experimental evidence concerning genetic exchange  
24 and identifies current gaps in our knowledge.

25

## 26   **1. Introduction**

27           Trypanosomes are protozoan parasites with a single flagellum that are commonly  
28 found in the blood of vertebrates, typically appearing as elongated, writhing organisms  
29 among the red blood cells in a wet blood smear. Though some trypanosomes show tissue-  
30 tropism or have intracellular stages, it is these blood-dwelling parasites that are transmitted  
31 from one vertebrate to another by blood-sucking arthropods or leeches. The drastic change  
32 from the environment of the vertebrate bloodstream to the invertebrate gut must be  
33 successfully accomplished within seconds, and this transition usually initiates a complex  
34 cycle of differentiation and development within the invertebrate host before infective  
35 trypanosomes are ready for transfer back to another vertebrate.

36           Of the hundreds of trypanosome species described, few are known to be pathogenic  
37 to their vertebrate hosts, and only two cause human disease:

- 38       • *Trypanosoma cruzi* is the parasite responsible for Chagas disease in Latin America  
39       and is transmitted by blood-sucking triatomine bugs. Infective parasites are excreted  
40       in bug faeces and gain entry into the vertebrate host via contamination of abraded  
41       skin or mucosal surfaces such as the conjunctiva of the eye. A number of domestic  
42       (e.g. cats, dogs) and wild animals (e.g. opossums) have been implicated as reservoir  
43       hosts, allowing the disease to circulate in domestic or sylvatic transmission cycles  
44       where suitable triatomine vectors are present.
- 45       • *T. brucei* is the causative agent of sleeping sickness or human African  
46       trypanosomiasis (HAT) and is transmitted by the bite of blood-sucking tsetse flies,  
47       large dipteran flies found mainly in tropical Africa. Besides humans, *T. brucei* infects  
48       a wide range of mammals, both wild and domesticated, that serve as food sources  
49       for tsetse; some of these animals can act as reservoir hosts of HAT, if the parasites  
50       they harbour are infective to humans. However, only some *T. brucei* strains are  
51       human-infective and these are conventionally recognised as two subspecies: *T. b.*  
52       *rhodesiense* in East Africa and *T. b. gambiense* in West and Central Africa. *T. b.*  
53       *gambiense* is further divided by both phenotype and genotype into two groups; the  
54       majority of isolates from patients belong to type 1.

55       Trypanosomes are kinetoplastid flagellates, characterised by the unique  
56       conformation of the mitochondrial DNA, which is packaged into an organelle called the  
57       kinetoplast. Kinetoplastids belong to the eukaryote supergroup Excavata, which is  
58       considered to be an early diverging branch of the eukaryote tree [1, 2]. Although biologists  
59       now believe that sex and meiosis were present in basal eukaryotes, evidence to support this  
60       contention has been lacking with respect to the excavate group. Some form of genetic

61 exchange has been experimentally demonstrated in a few representative genera: the  
62 kinetoplastids, *Trypanosoma* [3, 4], *Leishmania* [5] and *Crithidia* [6], and the diplomonad  
63 *Giardia* [7]; in addition, genetic recombination in *Trichomonas vaginalis* is suggested by  
64 population genetics analysis [8]. While genes associated with the mechanics of meiotic  
65 division have been identified in several excavate genera by phylogenomic analysis [9, 10],  
66 experimental confirmation of function has been carried out only in *Giardia* [7] and  
67 *Trypanosoma brucei* [11].

68           Why is it important to find out more about the mechanisms of genetic  
69 recombination used by the excavates? This will increase understanding of the evolution of  
70 sex in eukaryotes, because of the assumed early divergence of this group and its basal  
71 position in eukaryote trees [1]. Furthermore, as several important human and animal  
72 parasites are found among the Excavata, it is imperative to find out if and how virulence  
73 genes can be transferred between different pathogen strains and whether new pathogen  
74 strains are generated by genetic exchange. For example, two of the six recognised genetic  
75 lineages (or discrete typing units, DTUs) of *T. cruzi* are hybrids that have combined genetic  
76 material from other DTUs; these hybrid DTUs occur with high prevalence in patients with  
77 Chagas disease in southern countries of South America such as Bolivia, Paraguay, Chile and  
78 Argentina [12]. Regarding human African trypanosomiasis the virulence gene, *SRA*, is  
79 responsible for human infectivity in *T. b. rhodesiense* [13]. In the laboratory transfer of this  
80 single gene can convert a strain of *T. b. brucei* to human infectivity [13] and evidence from  
81 the field suggests that this has occurred through genetic recombination between *T. b.*  
82 *rhodesiense* and *T. b. brucei* in East Africa [14]. These two examples serve to demonstrate

83 how genetic recombination between pathogen strains can have profound epidemiological  
84 consequences and hence is of more than academic interest.

85

## 86 **2. Genetic exchange in trypanosomes**

87 Genetic exchange has been studied in depth in *Trypanosoma brucei* and *T. cruzi* by  
88 performing experimental crosses in the laboratory. Results to date suggest that the process  
89 is quite different in the two species. *T. brucei* mates in its tsetse fly vector rather than the  
90 mammalian host [3], whereas *T. cruzi* appears to mate in the mammalian host rather than  
91 the insect vector, since hybrids appeared in cultures of mammalian cells infected with two  
92 different trypanosome strains [4]. *T. cruzi* hybrids appear to result from fusion of parental  
93 trypanosomes with subsequent random loss of DNA [4]. While early experiments suggested  
94 that *T. brucei* hybrids were also produced by fusion, because hybrid progeny had raised DNA  
95 contents [15, 16], subsequent results contributed to the present consensus that Mendelian  
96 inheritance and diploid progeny are the norm [17-24]. To date only a single *T. cruzi* cross has  
97 resulted in production of hybrids [4], whereas many successful *T. brucei* crosses have been  
98 carried out (Table 1), and consequently more is known about genetic exchange in *T. brucei*,  
99 which is therefore the focus of the rest of this review.

100 That said, analysis of genetic exchange in *T. brucei* is not without challenges. In  
101 contrast to other parasitic protists such as *Plasmodium*, where sexual reproduction in the  
102 mosquito vector is an obligatory part of the transmission cycle, genetic exchange in *T. brucei*  
103 appears to be a non-essential event in the trypanosome life cycle. As mating takes place in  
104 the tsetse fly among life cycle stages that are not amenable to *in vitro* culture, experimental  
105 crosses require access to specialist facilities for tsetse fly transmission. Tsetse are relatively

106 refractory to trypanosome infection [25], with an extensive arsenal of immune defences  
107 that counter each stage of the trypanosome's developmental cycle in the insect [26-28].  
108 This severely restricts the number of infected flies that are produced, and on top of this,  
109 genetic exchange can, of course, only occur in flies infected with not just one, but two *T.*  
110 *brucei* strains, further reducing the likelihood of finding flies containing hybrids.

111         The development of approaches to overcome these obstacles has been crucial to  
112 progress on elucidating the mechanism of genetic exchange in *T. brucei*. For example,  
113 methods to enhance trypanosome infection through inhibition of tsetse immune defences  
114 [29-32] have greatly increased the numbers of infected flies available for analysis, while  
115 techniques to facilitate the identification of hybrids have diminished effort wasted on  
116 analysis of parental genotypes. In the first *T. brucei* crosses, hybrids were found by isolating  
117 trypanosome clones at random, a labour-intensive and time-consuming "needle in a  
118 haystack" approach [3, 18, 33]. With the advent of techniques to genetically engineer  
119 trypanosomes in the 1990's, it became possible first to select hybrids by double drug  
120 resistance [22, 34], and subsequently to identify trypanosome hybrids directly inside the  
121 tsetse fly by the use of fluorescent proteins to visualize the living cells [35-37]. Using  
122 parental lines distinguishable by fluorescence had the additional advantage that visual  
123 inspection could detect co-infected flies. This overturned the belief that genetic exchange  
124 was an infrequent event in the *T. brucei* life cycle, because hybrids were almost invariably  
125 found in tsetse flies with a mixed infection of the two parental trypanosomes in the salivary  
126 glands [37].

127         In addition to these advances, progress in understanding the developmental cycle of  
128 *T. brucei* in the tsetse fly, particularly the role of the foregut migratory stages, has been

129 crucial to interpretation [38-40]. The various developmental stages of *T. brucei* are shown in  
130 Fig. 1. While it has taken many years of research effort to put all these individual pieces in  
131 place, research is now able to move forward rapidly.

132

### 133 **3. Mating in *Trypanosoma brucei***

134 The first experimental cross of *T. brucei* established that mating took place during  
135 the trypanosome's developmental cycle in the tsetse fly [3], but definitive answers to the  
136 questions "where" and "when" were not forthcoming until crosses with genetically modified  
137 trypanosomes were carried out.

138 During the life cycle of *T. brucei* in the fly, trypanosomes first differentiate and  
139 multiply as procyclics in the midgut before migrating via the foregut to the salivary glands,  
140 where the infective metacyclic forms are produced [38, 39]. Comparison of trypanosome  
141 populations from the midgut and salivary glands of flies with a mixed infection of parental  
142 lines with different antibiotic-resistance genes showed that hybrids were only recovered  
143 from salivary glands not midguts [22, 34, 41]. The occurrence of hybrids solely in the salivary  
144 glands was confirmed by analysis of a cross where one of the parental strains had the gene  
145 for green fluorescent protein (GFP) under control of the bacterial Tet repressor, such that  
146 segregation of the GFP and Tet repressor genes produced fluorescent hybrids [35].  
147 Furthermore this experiment indicated that genetic exchange happened at or before the  
148 attached epimastigote stage in the salivary glands, as these life cycle stages, as well as  
149 metacyclics, were observed to be fluorescent [35].



150           In crosses with red and green fluorescent trypanosomes, no yellow fluorescent  
151 hybrids were observed among trypanosomes obtained from the midgut or foregut via  
152 examination of regurgitated material from salivating flies [37], demonstrating that mating  
153 takes place only after the migratory trypanosomes have reached the salivary glands as  
154 epimastigotes. The earliest this happened was 13 days after the infective feed when the first  
155 yellow hybrids were detected in the salivary glands [37]. Previous experiments have shown  
156 that mating continues through the duration of the infection [33], perhaps dependent on the  
157 arrival of the second parent in the salivary glands [41]. Meiotic stages have been detected  
158 from 14 to 38 days after infection [42], showing that production of mating stages is not  
159 synchronous or limited to a particular phase of establishment of infection in the salivary  
160 glands. Thus, although a mixed infection is a prerequisite for production of hybrids, both  
161 trypanosomes do not necessarily have to be picked up during the same feed from a single  
162 animal. Mixed infections of two or more genotypes were found among 9.5% of laboratory  
163 isolates of *T. brucei* from vertebrates [43], suggesting that the prevalence of multiple strain  
164 infections in nature may be quite high.

165           In summary, the where and when questions have been answered: mating takes  
166 place in the salivary glands as soon as trypanosomes arrive there; this can be as early as day  
167 13 after flies take the infective feed, but hybrid production can continue for weeks  
168 afterwards, possibly for the lifespan of the fly.

169

#### 170 **4. Mechanism of genetic exchange**

171           Evidence that the mechanism of genetic exchange involves meiosis was deduced  
172 indirectly from comparison of parental and progeny genotypes, which showed that

173 inheritance of alleles largely obeyed Mendelian rules [17]. The frequent observation of  
174 triploid hybrids, potentially explicable as errors in fusion of haploid and diploid nuclei, also  
175 suggested the presence of haploid nuclei at some stage during genetic exchange [22, 44].  
176 The discovery that trypanosome genomes contain genes encoding meiosis-specific proteins  
177 [9] suggested a more direct experimental approach: to test for gene expression.  
178 Accordingly, four meiosis-associated proteins (SPO11, MND1, DMC1, HOP1) were tagged  
179 with yellow fluorescent protein (YFP) to examine timing and place of expression in the fly  
180 [11]. Three of the four proteins were expressed in the nucleus of a dividing epimastigote  
181 stage found attached or free in the salivary glands [11]. These dividing epimastigotes were  
182 atypical, lacking the characteristic long posterior protrusion seen in attached epimastigotes  
183 [45, 46] and having the nucleus in a posterior rather than central position in the cell (Fig. 2).  
184 This putative meiotic stage was found in the largest numbers early in establishment of the  
185 salivary gland infection (around 20 days after the infective feed), but continued to be found  
186 up to 38 days (when the experiment terminated) [11, 42]. The meiotic stage was observed  
187 in single infections of *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* types 1 and 2 [42],  
188 indicating that meiosis is not triggered by the presence of a mixed trypanosome infection in  
189 the salivary glands, but is a normal part of the developmental cycle. In an experimental  
190 cross, it was observed that hybrid trypanosomes were seldom found to co-express a YFP-  
191 tagged meiosis-specific protein together with cytoplasmic RFP obtained from the other  
192 parental trypanosome, indicating that meiosis takes place before cell fusion [11].

193           The discovery of a putative meiotic stage led to a search for haploid gametes,  
194 targeting the period of maximal production of meiotic stages around day 20 following the  
195 infective feed [42]. Measurement of DNA contents of salivary gland stages revealed a

196 population of haploid cells. These cells had a peculiar morphology with a long free flagellum  
197 and pear-shaped body (Fig. 3), reminiscent of the promastigote cell morphology that is  
198 characteristic of other trypanosomatids such as *Leishmania*; the haploid cells were therefore  
199 referred to as promastigote-like [42]. These cells were present in relatively small numbers  
200 inside the lumen of the salivary gland, and were more easily found during the early phase of  
201 salivary gland establishment before epimastigotes and metacyclics became numerous.  
202 When salivary gland derived, red and green fluorescent trypanosomes of mating-compatible  
203 strains were mixed *in vitro*, the promastigote-like cells were observed to interact by  
204 intertwining their flagella in behaviour suggestive of the interaction of gametes prior to  
205 fusion, and yellow fluorescent hybrid cells appeared within 30 minutes of mixing [42]. In  
206 contrast, mixtures of red and green fluorescent trypanosomes of a single strain rarely  
207 produced yellow fluorescent hybrid cells, but the promastigote-like cells were still observed  
208 to interact via their intertwined flagella [47]. This suggests that fusion depends on the  
209 expression of additional factors that allow non-self gamete recognition (see below). The fate  
210 of the haploid gametes in single infections is unknown, but presumably those that do not  
211 fuse eventually die. Intermediate stages between the meiosis 1 dividing epimastigotes and  
212 the putative haploid gametes have yet to be described.

213         The mechanics of DNA exchange also await elucidation. In the simplest model, the  
214 haploid nuclei would combine after fusion of two promastigote-like cells, but there is as yet  
215 no proof of this. Early experiments concluded that inheritance of kinetoplast DNA (kDNA)  
216 was uniparental, because analysis of the maxicircles of hybrid progeny clones showed  
217 identity to one or other of the parental genotypes [18, 19, 48], but subsequent analysis of  
218 the minicircle component, which consists of about 5000 intercalated 1kb circular DNA

219 molecules [49], showed that hybrid progeny clones had a mixture of minicircles derived  
220 from the two parents [50, 51]. Therefore, contrary to initial ideas, kDNA is indeed  
221 exchanged during mating, and this was confirmed by PCR-based analysis of maxicircles of  
222 hybrid clones [37, 52]. In theory, random partitioning of the small number of maxicircles  
223 relative to minicircles (estimated ratio of 50 maxicircles to 5000 minicircles per kinetoplast)  
224 would lead to uniformity of the maxicircle component after several generations without  
225 affecting the heterogeneity of the minicircles [53], but there are other explanations  
226 consistent with the experimental observations [49]. The fact that kDNA is exchanged implies  
227 fusion of mitochondria, since the kDNA resides within the mitochondrial membrane, and  
228 this in turn requires fusion of cell membranes. To date, kDNA exchange is the key piece of  
229 evidence supporting the idea that cell fusion occurs during trypanosome mating rather than  
230 just exchange of nuclei [50, 51].

231

## 232 **5. Mating compatibility**

233         The factors that allow mating between different strains of *T. brucei* are not yet  
234 understood. It has proved possible to cross different subspecies in the lab, except for *T. b.*  
235 *gambiense* type 1 (Table 1). This is in line with the consensus from population genetic  
236 analyses that *T. b. gambiense* type 1 is genetically homogeneous and reproduces clonally  
237 [54, 55], whereas the other *T. brucei* subspecies, including *T. b. gambiense* type 2, are  
238 genetically heterogeneous [14, 56, 57]. But note that *T. b. gambiense* type 1 expresses  
239 meiosis-specific genes in common with the other *T. brucei* subspecies [42], and so it remains  
240 a possibility that, given the right circumstances of tsetse fly host and compatible mating  
241 partner, this trypanosome too might be capable of genetic recombination. Despite the fact

242 that *T. b. gambiense* type 2 combines human infectivity with the fly transmissibility and  
243 virulence of *T. b. brucei*, there is no evidence to support the idea that this trypanosome is a  
244 hybrid between *T. b. gambiense* type 1 and *T. b. brucei* [14, 58]. However, *T. b. gambiense*  
245 type 2 itself probably undergoes genetic recombination with *T. b. brucei* in nature. The  
246 similarity of *T. b. gambiense* type 2 to West African isolates of *T. b. brucei*, together with the  
247 heterogeneity of the few isolates that have been genotyped, are both suggestive of genetic  
248 exchange with *T. b. brucei*, and this idea is backed up by several successful laboratory  
249 crosses with *T. b. brucei* and *T. b. rhodesiense* (Table 1).

250           Whether *T. brucei* has a system of mating types or sexes that govern mating  
251 compatibility has yet to be established. Three different *T. brucei* strains were shown to cross  
252 in all pairwise combinations [20], indicating flexibility in mating type determination.  
253 However, as noted above, intraclonal crosses are far less successful than out crosses of  
254 different *T. brucei* strains [41, 59, 60], supporting the hypothesis that trypanosomes have  
255 some means of distinguishing self and non-self. This appears to act at the level of the  
256 gamete, because red and green fluorescent gametes of the same trypanosome strain failed  
257 to fuse even though they displayed the cell-cell interactions with intertwining flagella typical  
258 of compatible parental trypanosomes [47]. While F1 and F2 crosses, as well as back crosses  
259 of F1 or F2 progeny with parental trypanosomes, produced hybrids with varying levels of  
260 success, systematic analysis failed to elucidate any pattern of mating indicative of mating  
261 types [47].

262           It has been assumed that mating in *T. brucei* is a non-obligatory event during the life  
263 cycle, but the finding that production of meiotic forms and gametes is a normal part of the  
264 trypanosome's development in the salivary glands throws this assumption into doubt.

265 However, it has long been established that *T. brucei* clones can be transmitted through  
266 tsetse with no evidence of recombination [61], suggesting that the sexual cycle is simply by-  
267 passed.

268

## 269 **6. Transfer of virulence**

270 Analysis of experimental crosses could help to elucidate the genetic basis of key  
271 phenotypic characters, such as drug resistance or human infectivity, but up to now  
272 identification of such genes has relied on molecular genetic approaches [13, 62-65]. These  
273 approaches were very successful in discovering the genetic basis of human infectivity in the  
274 pathogens *T. b. rhodesiense* and *T. b. gambiense* type 1 [66]. While a single gene, *SRA*, is  
275 responsible for human infectivity in *T. b. rhodesiense* [13], three different loci (TgsGP,  
276 HpHbR, cysteine protease [63]) contribute to the ability of *T. b. gambiense* type 1 to survive  
277 in human blood [66]. Crosses of *T. b. gambiense* type 2 with non-human-infective *T. b.*  
278 *brucei* have produced potentially human infective (as judged by resistance to lysis by human  
279 serum) hybrid progeny, allowing linkage analysis with microsatellite markers [23, 24], but  
280 this has not yet led to identification of a particular gene or genes associated with human  
281 infectivity in *T. b. gambiense* type 2.

282 Several crosses of *T. b. rhodesiense* with *T. b. brucei* have yielded hybrid progeny,  
283 making it possible to examine the inheritance of human infectivity, both at the phenotype  
284 and genotype levels. Some hybrid clones inherit the human infective phenotype, manifested  
285 in their ability to resist lysis by human serum [67, 68] and these progeny clones have  
286 generally inherited one or more copies of the *SRA* gene [68]. The *SRA* protein interacts  
287 directly with the trypanolytic protein contained in human serum, Apolipoprotein L1,

288 preventing the formation of pores in the lysosomal membrane [69], and thus rendering the  
289 trypanosome resistant to lysis by human serum. As noted earlier, there is abundant  
290 population genetics evidence of gene flow between *T. b. rhodesiense* and *T. b. brucei* in  
291 nature [14, 70, 71].

292

## 293 **7. Conclusions**

294 While we have come a long way in understanding the process of genetic exchange in  
295 *Trypanosoma brucei* since the first experimental cross in 1986 [3], important details still  
296 remain to be worked out. For example, we now know where and when genetic exchange  
297 takes place and that it is true sexual reproduction involving a meiotic division and  
298 production of haploid gametes, but details of the second meiotic division, nuclear and  
299 kinetoplast DNA exchange and zygote formation are current gaps in our knowledge.  
300 Nevertheless, the epidemiological importance of genetic exchange in the generation of new  
301 strains of the human pathogens *T. b. rhodesiense*, and also *T. b. gambiense* type 2, are clear.

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477 **Legends to Figures**

478 **Figure 1** Developmental stages of *Trypanosoma brucei*. The classic life cycle of *T. brucei* [46]  
479 has been augmented with foregut migratory stages [38, 39], meiotic dividers [11] and  
480 gametes [42].

481 **Figure 2** Meiotic stage of *Trypanosoma brucei rhodesiense*. Dividing epimastigote recovered  
482 from the salivary glands. Panel A, phase contrast image. Panel B, YFP fluorescence showing  
483 nuclear expression of YFP-tagged DMC1. Panel C, DAPI stain showing nucleus and two  
484 smaller kinetoplasts. Panel D, merge. Scale bar 10  $\mu\text{m}$ .

485 **Figure 3** Promastigote-like gametes of *Trypanosoma brucei brucei*. Two different  
486 trypanosomes recovered from tsetse salivary glands are shown in rows A and B. The  
487 trypanosome in row A has one nucleus and two kinetoplasts, while that in row B has a single  
488 nucleus and kinetoplast. Left, phase contrast image; right, DAPI image. Scale bar 5  $\mu\text{m}$ .

489

490 **Tables**

## 491 Table 1

492 Experimental crosses of *Trypanosoma brucei* ssp. that produced hybrid progeny

Parents <sup>a</sup>	Hybrid selection	References
<i>Tbb</i> STIB 247 x <i>Tbg</i> 2 STIB 386	None	[3, 15, 16, 19, 48]
<i>Tbb</i> STIB 247 x <i>Tbb</i> TREU 927/4	None	[20]
<i>Tbb</i> TREU 927/4 x <i>Tbg</i> 2 STIB 386	None	[20]
<i>Tbb</i> STIB 247 x <i>Tbb</i> STIB 777	None	[21]
<i>Tbb</i> TSW 196 x <i>Tbr</i> 058	None	[18, 44, 50]
<i>Tbb</i> TSW 196 x <i>Tbb</i> J10	None	[72]
<i>Tbb</i> KP2N x <i>Tbr</i> 058H <sup>b</sup>	Double drug resistance	[22, 34, 41]
<i>Tbr</i> 058H x <i>Tbb</i> P20 (F1 hybrid)	Double drug resistance	[73]
<i>Tbb</i> STIB 826 x <i>Tbb</i> STIB 829	None	[74]
<i>Tbr</i> 058H x <i>Tbg</i> 2 TH2N	Double drug resistance	[41]
<i>Tbb</i> KP2N x <i>Tbg</i> 2 TH2H	Double drug resistance	[41]
<i>Tbb</i> KP2N x <i>Tbg</i> 2 TH2 Tet GFP <sup>b</sup>	GFP fluorescence	[35]
<i>Tbb</i> J10 RFP x <i>Tbb</i> 1738 GFP	GFP/RFP dual fluorescence	[37]
F1, F2 and back crosses from J10 RFP x 1738 GFP	GFP/RFP dual fluorescence	[47]
<i>Tbb</i> 1738 RFP x <i>Tbb</i> 1738 GFP <sup>c</sup>	GFP/RFP dual fluorescence	[59]
<i>Tbb</i> 427 var 3 RFP x <i>Tbb</i> 1738 GFP	GFP/RFP dual fluorescence	[75]



<i>Tbr</i> 058 GFP x <i>Tbb</i> J10 RFP	GFP/RFP dual fluorescence	[68]
<i>Tbr</i> 058 GFP x <i>Tbb</i> 1738 RFP	GFP/RFP dual fluorescence	[68]
<i>Tbr</i> 058 GFP x <i>Tbb</i> 427 var 3 RFP	GFP/RFP dual fluorescence	[68]
<i>Tbr</i> LUMP 1198 GFP x <i>Tbb</i> 1738 RFP	GFP/RFP dual fluorescence	[68]
<i>Tbr</i> TOR11 GFP x <i>Tbb</i> J10 RFP	GFP/RFP dual fluorescence	[68]
<i>Tbr</i> TOR11 GFP x <i>Tbb</i> 1738 RFP	GFP/RFP dual fluorescence	[68]

493

494 <sup>a</sup> Trypanosome origins: STIB 247, hartebeest, Tanzania, 1971; STIB 386, human, Côte  
495 d'Ivoire, 1978; TREU 927/4, tsetse, Kenya, 1970; STIB 777, tsetse, Uganda, 1971; TSW 196,  
496 pig, Côte d'Ivoire, 1978; 058 (058H), human, Zambia, 1974; J10, hyena, Zambia, 1973; KP2  
497 (KP2N), tsetse, Côte d'Ivoire, 1982; STIB 826, 829, tsetse, Uganda, 1990; TH2 (TH2N, TH2H),  
498 human, Côte d'Ivoire, 1978; 1738, sheep, Kenya, 1970; 427 var 3, sheep, Uganda, 1960;  
499 TOR11, human, Uganda, 1988; LUMP 1198, human, Uganda, 1986.

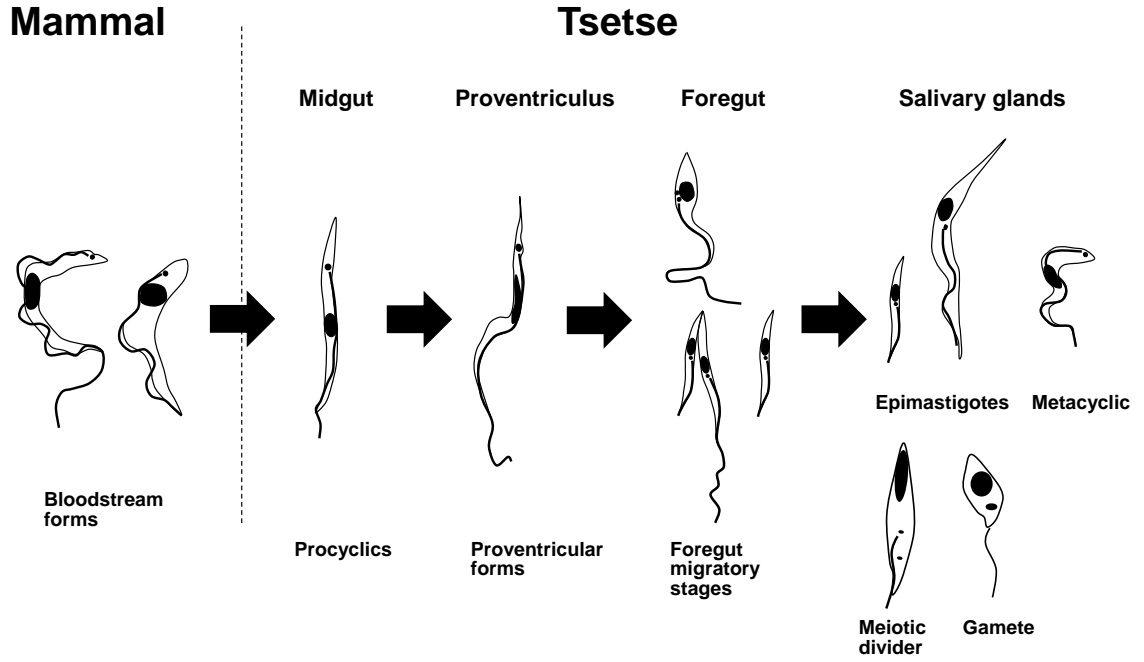
500 <sup>b</sup> Abbreviations: H, hygromycin resistant; N, Geneticin resistant; Tet, Tet operator; GFP,  
501 green fluorescent protein; RFP, red fluorescent protein.

502 <sup>c</sup> This was an intracloonal cross and hence produced recombinant rather than hybrid progeny.

503

504 **Figures**

505 **Figure 1**

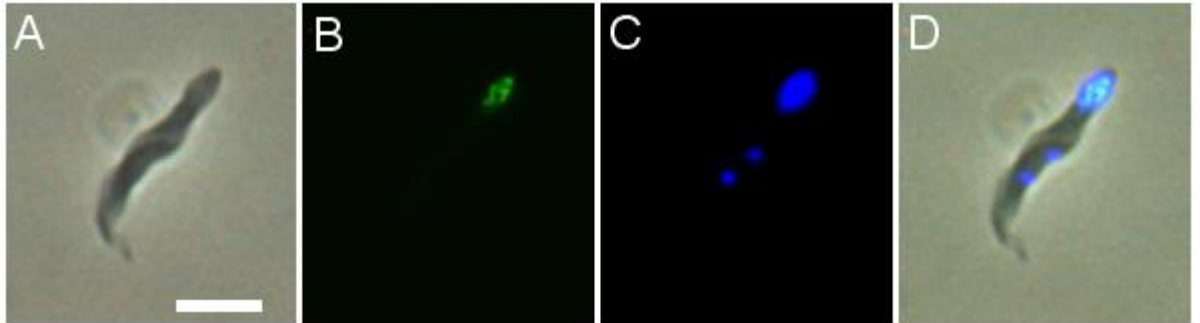


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508 **Figure 2**

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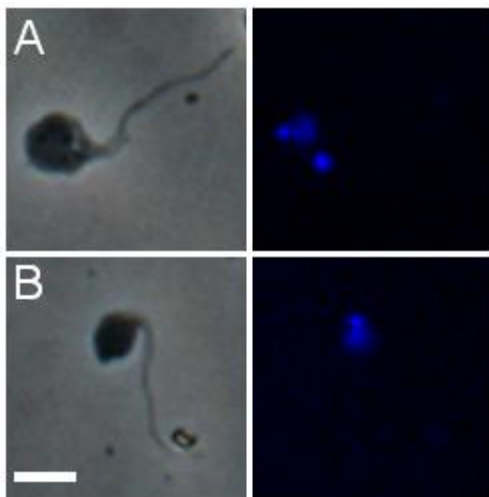
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513 **Figure 3**

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