Liaisons dangereuses: sexual recombination among pathogenic trypanosomes

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Abstract

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

1. Introduction

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

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

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

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

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

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

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

2. Genetic exchange in trypanosomes

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

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

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

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

In addition to these advances, progress in understanding the developmental cycle of *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].

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

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

4. Mechanism of genetic exchange

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

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

The discovery of a putative meiotic stage led to a search for haploid gametes, targeting the period of maximal production of meiotic stages around day 20 following the 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

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

5. Mating compatibility

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

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

7. Conclusions

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

References

[1] He D, Fiz-Palacios O, Fu C-J, Fehling J, Tsai C-C, Baldauf SL. An alternative root for the eukaryote tree of life. Curr. Biol. 2014;24:465-70.

[2] Adl SM, Simpson AGB, Lane CE, Lukes J, Bass D, Bowser SS, et al. The revised classification of Eukaryotes. J Euk Micro 2012;59:429-93.

[3] Jenni L, Marti S, Schweizer J, Betschart B, Lepage RWF, Wells JM, et al. Hybrid formation between African trypanosomes during cyclical transmission. Nature 1986;322:173-5.

[4] Gaunt MW, Yeo M, Frame IA, Stothard JR, Carrasco HJ, Taylor MC, et al. Mechanism of genetic exchange in American trypanosomes. Nature 2003;421:936-9.

311 [5] Akopyants NS, Kimblin N, Secundino N, Patrick R, Peters N, Lawyer P, et al. Demonstration of
312 genetic exchange during cyclical development of *Leishmania* in the sand fly vector. Science
313 2009;324:265-8.
314 [6] Schmid-Hempel R, Salathe R, Tognazzo M, Schmid-Hempel P. Genetic exchange and emergence
315 of novel strains in directly transmitted trypanosomatids. Infect. Genet. Evol. 2011;11:564-71.
316 [7] Poxleitner MK, Carpenter ML, Mancuso JJ, Wang CR, Dawson SC, Cande WZ. Evidence for
317 karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*.
318 Science 2008;319:1530-3.
319 [8] Conrad MD, Gorman AW, Schillinger JA, Fiori PL, Arroyo R, Malla N, et al. Extensive genetic
320 diversity, unique population structure and evidence of genetic exchange in the sexually transmitted
321 parasite *Trichomonas vaginalis*. PLoS NTD 2012;6.
322 [9] Ramesh MA, Malik SB, Logsdon JM. A phylogenomic inventory of meiotic genes: Evidence for sex
323 in *Giardia* and an early eukaryotic origin of meiosis. Curr. Biol. 2005;15:185-91.
324 [10] Malik SB, Pightling AW, Stefaniak LM, Schurko AM, Logsdon JM. An expanded inventory of
325 conserved meiotic genes provides evidence for sex in *Trichomonas vaginalis*. PLoS One 2008;3.
326 [11] Peacock L, Ferris V, Sharma R, Sunter J, Bailey M, Carrington M, et al. Identification of the
327 meiotic life cycle stage of *Trypanosoma brucei* in the tsetse fly. Proc. Natl. Acad. Sci. U S A
328 2011;108:3671-6.
329 [12] Miles MA, Llewellyn MS, Lewis MD, Yeo M, Baleela R, Fitzpatrick S, et al. The molecular
330 epidemiology and phylogeography of *Trypanosoma cruzi* and parallel research on *Leishmania*:
331 looking back and to the future. Parasitology 2009;136:1509-28.
332 [13] Xong VH, Vanhamme L, Chamekh M, Chimfwembe CE, Van den Abbeele J, Pays A, et al. A VSG
333 expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*.
334 Cell 1998;95:839-46.
335 [14] Balmer O, Beadell JS, Gibson W, Caccone A. Phylogeography and taxonomy of *Trypanosoma*
336 *brucei*. PLoS NTD 2011;5:e961.

337 [15] Wells JM, Prospero TD, Jenni L, Le Page RWF. DNA contents and molecular karyotypes of hybrid
338 *Trypanosoma brucei*. Mol. Biochem. Parasitol. 1987;24:103-16.
339 [16] Paindavoine P, Zampetti-Bosseler F, Pays E, Schweizer J, Guyaux M, Jenni L, et al. Trypanosome
340 hybrids generated in tsetse flies by nuclear fusion. EMBO J 1986;5:3631-6.
341 [17] MacLeod A, Tweedie A, McLellan S, Taylor S, Cooper A, Sweeney L, et al. Allelic segregation and
342 independent assortment in *Trypanosoma brucei* crosses: Proof that the genetic system is Mendelian
343 and involves meiosis. Mol. Biochem. Parasitol. 2005;143:12-9.
344 [18] Gibson WC. Analysis of a genetic cross between *Trypanosoma brucei rhodesiense* and *T. b.*
345 *brucei*. Parasitology 1989;99:391-402.
346 [19] Sternberg J, Turner CMR, Wells JM, Ranford-Cartwright LC, Lepage RWF, Tait A. Gene exchange
347 in African trypanosomes: frequency and allelic segregation. Mol. Biochem. Parasitol. 1989;34:269-
348 80.
349 [20] Turner CMR, Sternberg J, Buchanan N, Smith E, Hide G, Tait A. Evidence that the mechanism of
350 gene exchange in *Trypanosoma brucei* involves meiosis and syngamy. Parasitology 1990;101:377-86.
351 [21] Schweizer J, Pospichal H, Hide G, Buchanan N, Tait A, Jenni L. Analysis of a new genetic cross
352 between 2 East African *Trypanosoma brucei* clones. Parasitology 1994;109:83-93.
353 [22] Gibson W, Bailey M. Genetic exchange in *Trypanosoma brucei*: evidence for meiosis from
354 analysis of a cross between drug resistant transformants. Mol. Biochem. Parasitol. 1994;64:241-52.
355 [23] MacLeod A, Tweedie A, McLellan S, Taylor S, Hall N, Berriman M, et al. The genetic map and
356 comparative analysis with the physical map of *Trypanosoma brucei*. Nucl Acids Res 2005;33:6688-93.
357 [24] Cooper A, Tait A, Sweeney L, Tweedie A, Morrison L, Turner CMR, et al. Genetic analysis of the
358 human infective trypanosome, *Trypanosoma brucei gambiense*: chromosomal segregation, crossing
359 over and the construction of a genetic map. Genome Biology 2008;9.
360 [25] Maudlin I. Transmission of African Trypanosomiasis: Interactions among tsetse immune system,
361 symbionts and parasites. Adv. Dis. Vector Res. 1991;7:117-48.

362 [26] Nayduch D, Aksoy S. Refractoriness in tsetse flies (Diptera : Glossinidae) may be a matter of
363 timing. Journal of Medical Entomology 2007;44:660-5.
364 [27] Hu CY, Aksoy S. Innate immune responses regulate trypanosome parasite infection of the tsetse
365 fly *Glossina morsitans morsitans*. Mol Micro 2006;60:1194-204.
366 [28] Lehane MJ, Aksoy S, Levashina E. Immune responses and parasite transmission in blood-feeding
367 insects. Trends Parasitol 2004;20:433-9.
368 [29] Macleod ET, Maudlin I, Darby AC, Welburn SC. Antioxidants promote establishment of
369 trypanosome infections in tsetse. Parasitology 2007;134:827-31.
370 [30] Maudlin I, Welburn SC. The role of lectins and trypanosome genotype in the maturation of
371 midgut infections in *Glossina morsitans*. Trop Med Parasitol 1988;39:56-8.
372 [31] Welburn SC, Maudlin I, Molyneux DH. Midgut lectin activity and sugar specificity in teneral and
373 fed tsetse. Medical and Veterinary Entomology 1994;8:81-7.
374 [32] Peacock L, Ferris V, Bailey M, Gibson W. Multiple effects of the lectin-inhibitory sugars D-
375 glucosamine and N-acetyl-glucosamine on tsetse-trypanosome interactions. Parasitology
376 2006;132:651-8.
377 [33] Schweizer J, Tait A, Jenni L. The timing and frequency of hybrid formation in African
378 trypanosomes during cyclical transmission. Parasitol. Res. 1988;75:98-101.
379 [34] Gibson W, Whittington H. Genetic exchange in *Trypanosoma brucei*: Selection of hybrid
380 trypanosomes by introduction of genes conferring drug resistance. Mol. Biochem. Parasitol.
381 1993;60:19-26.
382 [35] Bingle LEH, Eastlake JL, Bailey M, Gibson WC. A novel GFP approach for the analysis of genetic
383 exchange in trypanosomes allowing the in situ detection of mating events. Microbiology
384 2001;147:3231-40.
385 [36] Gibson W, Peacock L, Ferris V, Williams K, Bailey M. Analysis of a cross between green and red
386 fluorescent trypanosomes. Biochemical Society Transactions 2006;34:557-9.

387 [37] Gibson W, Peacock L, Ferris V, Williams K, Bailey M. The use of yellow fluorescent hybrids to
388 indicate mating in *Trypanosoma brucei*. Parasit. Vector 2008;1:4.
389 [38] Sharma R, Peacock L, Gluenz E, Gull K, Gibson W, Carrington M. Asymmetric cell division as a
390 route to reduction in cell length and change in cell morphology in trypanosomes. Protist
391 2008;159:137-51.
392 [39] Van den Abbeele J, Claes Y, Van Bockstaele D, Le Ray D, Coosemans M. *Trypanosoma brucei* spp.
393 development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and
394 proboscis. Parasitology 1999;118:469-78.
395 [40] Vickerman K, Tetley L, Hendry KAK, Turner CMR. Biology of African trypanosomes in the tsetse
396 fly. Biol Cell 1988;64:109-19.
397 [41] Gibson W, Winters K, Mizen G, Kearns J, Bailey M. Intraclonal mating in *Trypanosoma brucei* is
398 associated with out-crossing. Microbiology 1997;143:909-20.
399 [42] Peacock L, Bailey M, Carrington M, Gibson W. Meiosis and haploid gametes in the pathogen
400 *Trypanosoma brucei*. Curr. Biol. 2014;24:1-6.
401 [43] Balmer O, Caccone A. Multiple-strain infections of *Trypanosoma brucei* across Africa. Acta Trop.
402 2008;107:275-9.
403 [44] Gibson W, Garside L, Bailey M. Trisomy and chromosome size changes in hybrid trypanosomes
404 from a genetic cross between *Trypanosoma brucei rhodesiense* and *T. b. brucei*. Mol. Biochem.
405 Parasitol. 1992;52:189-200.
406 [45] Tetley L, Vickerman K. Differentiation in *Trypanosoma brucei*: host-parasite cell junctions and
407 their persistence during acquisition of the variable antigen coat. J Cell Sci 1985;74:1-19.
408 [46] Vickerman K. Developmental cycles and biology of pathogenic trypanosomes. Brit Med Bull
409 1985;41:105-14.
410 [47] Peacock L, Ferris V, Bailey M, Gibson W. Mating compatibility in the parasitic protist
411 *Trypanosoma brucei*. Parasit. Vector 2014;7:78.

412 [48] Sternberg J, Tait A, Haley S, Wells JM, Lepage RWF, Schweizer J, et al. Gene exchange in African
413 trypanosomes: characterisation of a new hybrid genotype. Mol. Biochem. Parasitol. 1988;27:191-
414 200.
415 [49] Shapiro TA, Englund PT. The structure and replication of kinetoplast DNA. Annu. Rev. Microbiol.
416 1995;49:117-43.
417 [50] Gibson W, Garside L. Kinetoplast DNA mini-circles are inherited from both parents in genetic
418 hybrids of *Trypanosoma brucei*. Mol. Biochem. Parasitol. 1990;42:45-54.
419 [51] Gibson W, Crow M, Kearns J. Kinetoplast DNA minicircles are inherited from both parents in
420 genetic crosses of *Trypanosoma brucei*. Parasitol. Res. 1997;83:483-8.
421 [52] Turner CMR, Hide G, Buchanan N, Tait A. *Trypanosoma brucei* - inheritance of kinetoplast DNA
422 maxicircles in a genetic cross and their segregation during vegetative growth. Expt. Parasitol.
423 1995;80:234-41.
424 [53] Birky CW. Relaxed cellular controls and organelle heredity. Science 1983;222:468-75.
425 [54] Koffi M, De Meeus T, Bucheton B, Solano P, Camara M, Kaba D, et al. Population genetics of
426 *Trypanosoma brucei gambiense*, the agent of sleeping sickness in Western Africa. Proc. Natl. Acad.
427 Sci. U S A 2009;106:209-14.
428 [55] Morrison LJ, Tait A, McCormack G, Sweeney L, Black A, Truc P, et al. *Trypanosoma brucei*
429 *gambiense* Type 1 populations from human patients are clonal and display geographical genetic
430 differentiation. Infect. Genet. Evol. 2008;8:847-54.
431 [56] Mehlitz D, Zillmann U, Scott CM, Godfrey DG. Epidemiological studies on the animal reservoir of
432 gambiense sleeping sickness. III. Characterisation of *Trypanozoon* stocks by isoenzymes and
433 sensitivity to human serum. Tropenmed Parasit 1982;33:113-8.
434 [57] Godfrey DG, Baker RD, Rickman LR, Mehlitz D. The distribution, relationships and identification
435 of enzymic variants within the subgenus *Trypanozoon*. Adv. Parasitol. 1990;29:1-74.

436 [58] Symula RE, Beadell JS, Sistrom M, Agbebakun K, Balmer O, Gibson W, et al. *Trypanosoma brucei*
437 *gambiense* Group 1 is distinguished by a unique amino acid substitution in the HpHb receptor
438 implicated in human serum resistance. PLoS NTD 2012;6.
439 [59] Peacock L, Ferris V, Bailey M, Gibson W. Intraclonal mating occurs during tsetse transmission of
440 *Trypanosoma brucei*. Parasit. Vector 2009;2:43.
441 [60] Tait A, Buchanan N, Hide G, Turner M. Self-fertilisation in *Trypanosoma brucei*. Mol. Biochem.
442 Parasitol. 1996;76:31-42.
443 [61] Tait A, Turner CMR, Le Page RFW, Wells JM. Genetic evidence that metacyclic forms of
444 *Trypanosoma brucei* are diploid. Mol. Biochem. Parasitol. 1989;37:247-56.
445 [62] Berberof M, Perez-Morga D, Pays E. A receptor-like flagellar pocket glycoprotein specific to
446 *Trypanosoma brucei gambiense.* Mol. Biochem. Parasitol. 2001;113:127-38.
447 [63] Uzureau P, Uzureau S, Lecordier L, Fontaine F, Tebabi P, Homble F, et al. Mechanism of
448 Trypanosoma brucei gambiense resistance to human serum. Nature 2013;501:430-+.
449 [64] Carter NS, Fairlamb AH. Arsenical-resistant trypanosomes lack an unusual adenosine
450 transporter. Nature 1993;361:173-6.
451 [65] Alsford S, Eckert S, Baker N, Glover L, Sanchez-Flores A, Leung KF, et al. High-throughput
452 decoding of antitrypanosomal drug efficacy and resistance. Nature 2012;482:232-U125.
453 [66] Pays E, Vanhollebeke B, Uzureau P, Lecordier L, Perez-Morga D. The molecular arms race
454 between African trypanosomes and humans. Nature Reviews Microbiology 2014;12:575-84.
455 [67] Gibson WC, Mizen VH. Heritability of the trait for human infectivity in genetic crosses of
456 *Trypanosoma brucei* ssp. Trans Roy Soc Trop Med Hyg 1997;91:236-7.
457 [68] Gibson W, Peacock L, Ferris V, Fischer K, Livingstone J, Thomas J, et al. Genetic recombination
458 between human and animal parasites creates novel strains of human pathogen. PLoS NTD 2015;
459 9:e0003665.

460 [69] Perez-Morga D, Vanhollebeke B, Paturiaux-Hanocq F, Nolan DP, Lins L, Homble F, et al.
461 Apolipoprotein L-1 promotes trypanosome lysis by forming pores in lysosomal membranes. Science
462 2005;309:469-72.
463 [70] Tait A, Barry JD, Wink R, Sanderson A, Crowe JS. Enzyme variation in *Trypanosoma brucei* spp. II.
464 Evidence for *T. b. rhodesiense* being a set of variants of *T. b. brucei*. Parasitology 1985;90:89-100.
465 [71] Gibson WC, Wellde BT. Characterisation of *Trypanozoon* stocks from the South Nyanza sleeping
466 sickness focus in Western Kenya. Trans Roy Soc Trop Med Hyg 1985;79:671-6.
467 [72] Gibson WC, Garside LH. Genetic exchange in *Trypanosoma brucei brucei*: variable location of
468 housekeeping genes in different trypanosome stocks. Mol. Biochem. Parasitol. 1991;45:77-90.
469 [73] Gibson W, Kanmogne G, Bailey M. A successful backcross in *Trypanosoma brucei*. Mol. Biochem.
470 Parasitol. 1995;69:101-10.
471 [74] Degen R, Pospichal H, Enyaru J, Jenni L. Sexual compatibility among *Trypanosoma brucei* isolates
472 from an epidemic area in southeastern Uganda. Parasitol. Res. 1995;81:253-7.
473 [75] Peacock L, Ferris V, Bailey M, Gibson W. Fly transmission and mating of *Trypanosoma brucei*
474 *brucei* strain 427. Mol. Biochem. Parasitol. 2008;160:100-6.
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Legends to Figures

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

Figure 2 Meiotic stage of *Trypanosoma brucei rhodesiense*. Dividing epimastigote recovered from the salivary glands. Panel A, phase contrast image. Panel B, YFP fluorescence showing nuclear expression of YFP-tagged DMC1. Panel C, DAPI stain showing nucleus and two smaller kinetoplasts. Panel D, merge. Scale bar 10 µm.

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

Tables

Table 1

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

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

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

493

494 [a] 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.

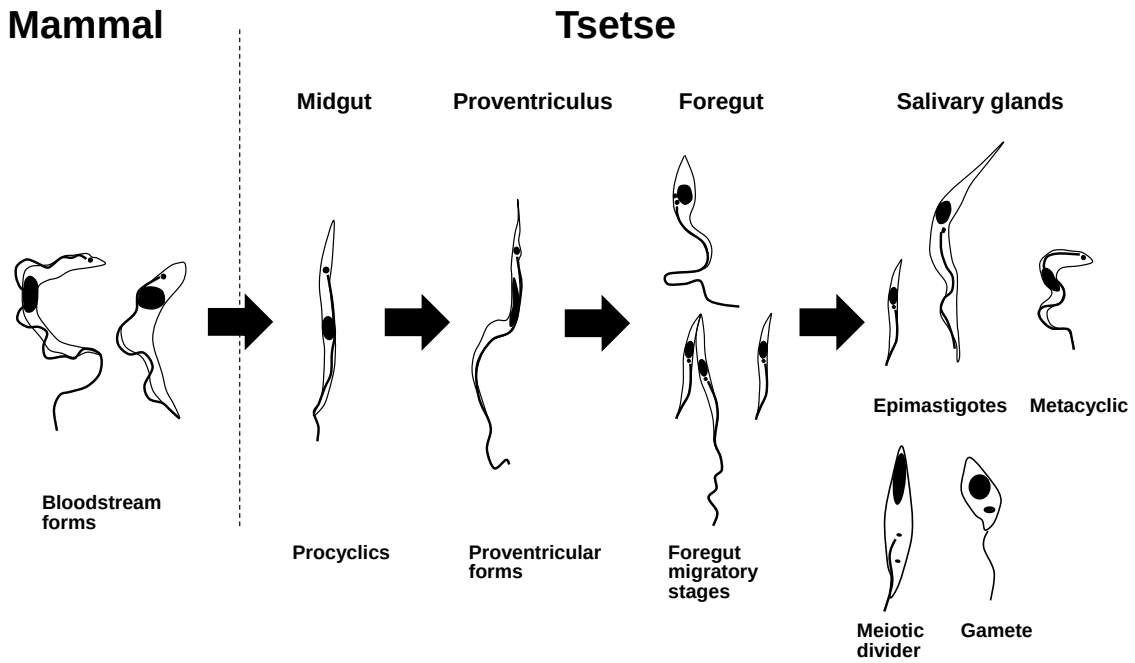
500 [b] Abbreviations: H, hygromycin resistant; N, Geneticin resistant; Tet, Tet operator; GFP,
501 green fluorescent protein; RFP, red fluorescent protein.

502 [c] This was an intraclonal cross and hence produced recombinant rather than hybrid progeny.

503

Figures

Figure 1



508 **Figure 2**

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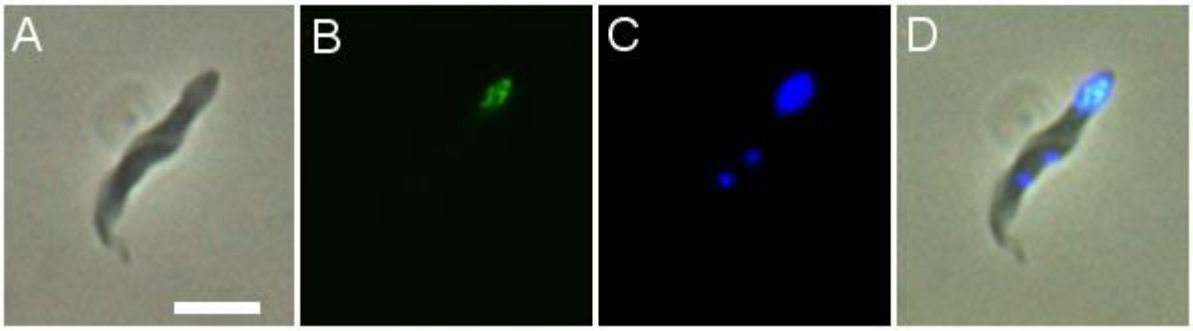

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513 **Figure 3**

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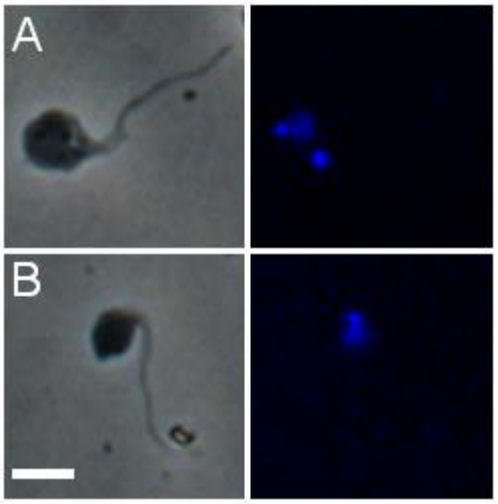

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