Update

Research Focus

Visualizing trypanosome sex

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Genetic exchange in *Trypanosoma brucei* is now well characterized. It is a key tool that has enabled an understanding of important parasite genetic traits and underpinned the *Trypanosoma brucei* genome project. However, a key aspect that has eluded us is the point in the trypanosome life cycle where genetic exchange occurs. Research using green and red fluorescent trypanosomes for visualizing genetic crosses has now identified this stage.

Trypanosome genetics

The interactions between trypanosomes and their hosts are complex. They involve both parasite and host contributions [1] and must be dependent on genes from both. Of considerable importance, in terms of human and animal disease, are genes that influence aspects such as host range, virulence, drug resistance, host immunity to the parasite and transmissibility. These aspects, alongside others, determine the overall epidemiology of the disease [2,3]. The bases of some such genetic traits have been established, such as the single SRA gene that confers human infectivity on *Trypanosoma brucei rhodesiense*, the agent of East African human sleeping sickness [4,5]. However, in many cases, these phenotypic effects are probably controlled by more complex genetic mechanisms. Genetics (host, parasite and vector) is, therefore, key to the understanding of host–parasite interactions and epidemiology.

That trypanosomes undergo genetic exchange was predicted nearly 30 years ago [6,7] and demonstrated shortly after [8]. Much evidence points to the fact that genetic exchange takes place in the tsetse-fly vector, as previously reviewed (see Ref. [9]). Genetic crosses in trypanosomes are technically difficult to carry out because they involve mixing strains through tsetse (Figure 1). This is confounded by the fact that genetic exchange is not obligatory [9] and, therefore, progeny trypanosomes are not detected in all experimentally infected tsetse [10]. Furthermore, they are extremely laborious when it comes to analysing progeny, and experiments can often result in insufficient progeny for powerful definitive genetic analyses. Despite this, more than ten genetic crosses have been carried out [11] and considerable progress has been made in elucidating the process of genetic exchange in *Trypanosoma brucei*. Genetic crosses have been used first to predict [12] and later to prove [11] that trypanosomes have Mendelian genetics that involve meiosis. However, a haploid stage has not yet been observed [13,14]. The appearance of triploid progeny [15,16] has complicated the interpretation of the mechanism, although these might simply be generated by nondisjunction. Proof of meiosis [11] and the presence of meiosis-specific genes [17] support a classical mechanism of genetic exchange in *T. brucei*. More recently, genetic exchange has been reported in *Trypanosoma cruzi* [18], albeit using a different mechanism, which indicates that this process might be general among trypanosomatids.

Two key areas of research have emerged: the role of genetic exchange in nature and the mechanism of genetic exchange in the vector. In nature, the role of genetic exchange has been controversial with three levels of activity detected: clonality (no genetic exchange), panmixis (random mating) and epidemic population structuring (frequent genetic exchange followed by the epidemic expansion of some genotypes) [9,19–21]. These different types of genetic exchange seem to vary between different foci for sleeping sickness. Key questions concerning the process of genetic exchange have centred around the mechanism and the point in the life cycle at which it occurs. These two questions, of course, are interlinked because it is difficult to establish a mechanism without knowing where and when it occurs.

When do trypanosomes undergo genetic exchange?

Two approaches have been employed to address this question. First, the use of classical genetics, by the analysis of progeny, to detect pre- and post-genetic stages. This approach has narrowed the timing of the genetic exchange to a stage before the development of the metacyclic [22]. The second approach has been to use visualization techniques to try to observe directly the point at which genetic exchange occurs. An initial approach, which employed trypanosomes that express a green fluorescent protein (GFP) reporter gene, indicated that visualization of trypanosome crosses could be achieved [23,24].

The subject article of this Research Focus by Gibson *et al.* [25] demonstrates that this is, indeed, possible. Gibson *et al.* generated parental trypanosome clones, which were transfected with either a gene encoding GFP or a gene encoding red fluorescent protein (RFP). Trypanosomes that express both genes (i.e. progeny trypanosomes) are visualized by a yellow fluorescence. Thus, any structure within the tsetse fly that is infected with both parents and progeny can be easily identified as containing red, green and yellow trypanosomes. Figure 2 shows a salivary gland infected with parental types (red and green) and progeny (yellow). The article [25] additionally shows video clips of parents and offspring visualized within the salivary gland. This demonstrates, for the first time, a technique that can be used both to visualize the location of mating within the tsetse fly and to determine precisely the devel-

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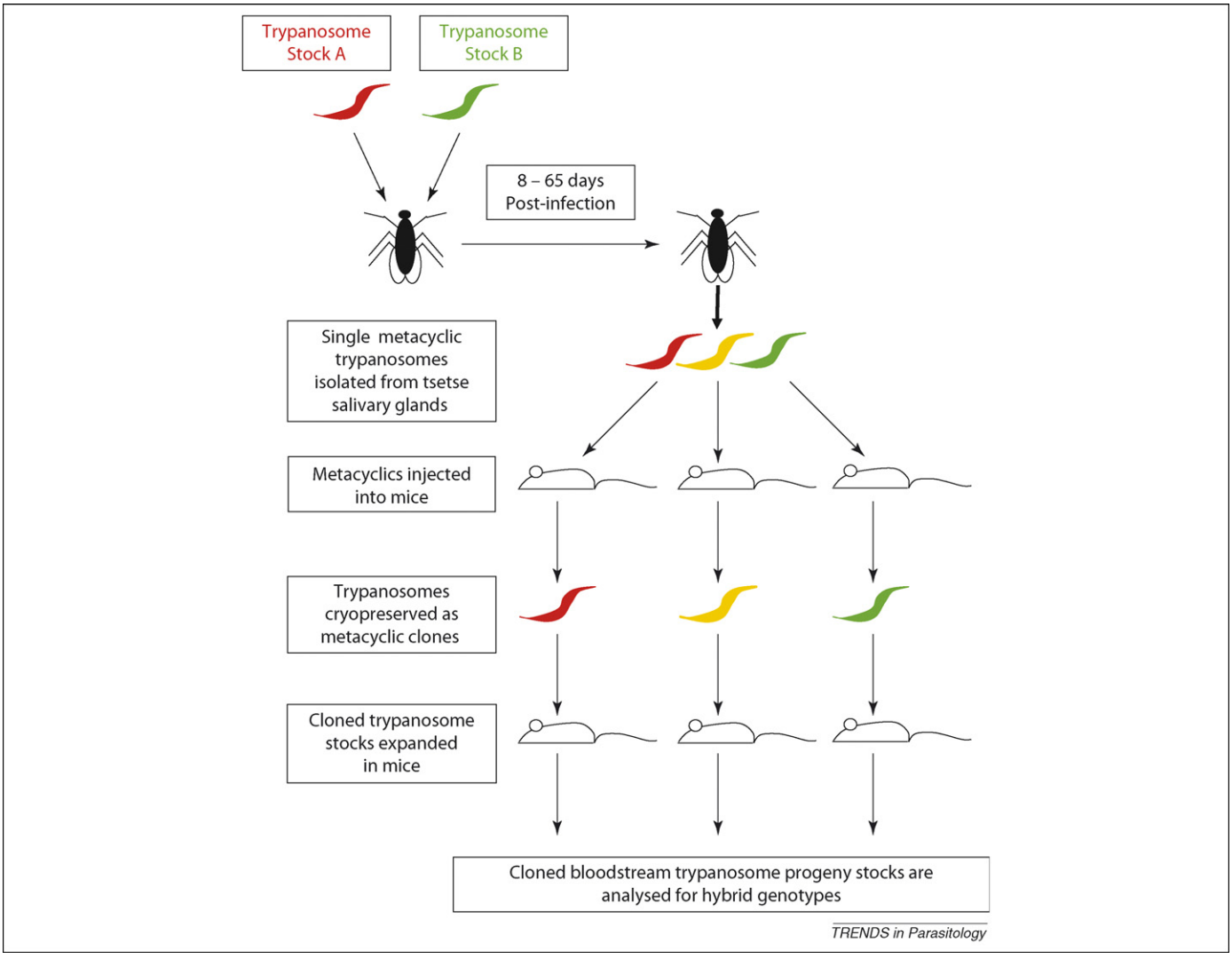

Figure 1. Experimental genetic crosses in *Trypanosoma brucei*. This schematic shows the procedure for carrying out genetic crosses in *Trypanosoma brucei*. Two parental stocks are mixed by feeding tsetse flies with infected blood that contains both parents. Tsetse are maintained and, at varying time points between eight and 65 days, are dissected to isolate single metacyclic trypanosomes. These single trypanosomes are further expanded by growth in mice and then cryopreserved. Progeny clones are then resurrected by expansion in mice, followed by DNA extraction for analysis by a variety of genetic techniques.

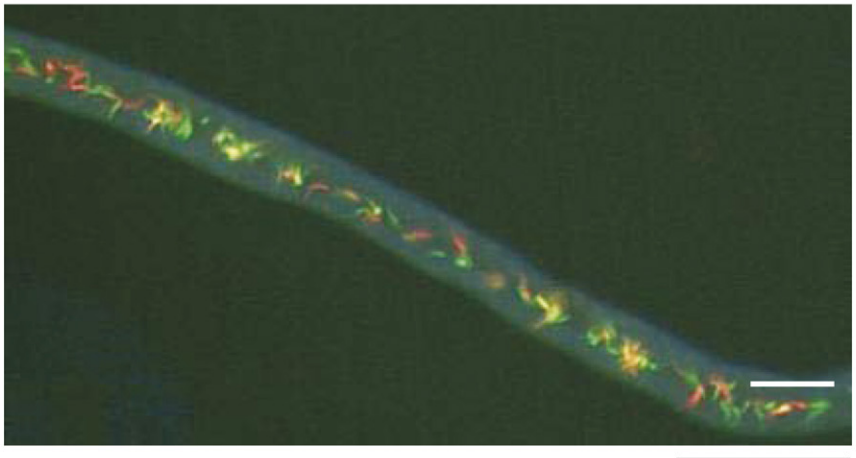

Figure 2. A dissected salivary gland that contains a mixed infection of red, green and yellow trypanosomes. A dissected salivary gland taken from a tsetse that is 27 days postinfection with two parental *T. brucei* stocks. The parental trypanosomes (red and green) and their hybrid progeny (yellow) can be observed. Reproduced, with permission, from Ref. [25]. Scale bar = 100 μm.

opmental timing of the first appearance of progeny trypanosomes. In addition, this technique is also valuable for investigating the distribution of the parental types and gives an insight into the possible reasons for the relatively low frequency of mating. An analysis of the distribution of red and green trypanosomes in mixed infected flies (as defined by presence of both colours in the midgut) showed that only 37% had mixtures in one or both salivary glands – the remaining having either only red or only green [25]. Furthermore, differences were often observed in the distribution between the left and right salivary glands in a single fly [25]. Because mating occurs in the salivary gland, these data offer an explanation as to why mating does not always occur – if the gland is infected with only a single parent, there is simply not an opportunity available.

Gibson *et al*. [25] investigated the location of mating by examining trypanosomes within various tsetse structures. In the midgut, despite the presence of red and green trypanosomes, yellow progeny were never found. Furthermore, they did not find yellow progeny in samples that were taken from the route between the proventriculus and the salivary glands – this ruled out several stages, including proventricular trypomastigotes and unattached epimastigotes, as the mating stages. The absence of yellow trypanosomes in salivary glands that contained only one parental type, even when both salivary glands were infected with different parents, supported the view that mating did not occur in transit to the salivary glands but must occur on arrival there. Finally, in movie clips taken from the salivary glands [25], yellow trypanosomes were visualized as attached forms, thus demonstrating that the earliest stage of mating was the attached epimastigote. The article [25] reports the first demonstration of the exact location of mating and supports other genetic data that implicates the attached epimastigote as the mating stage [22].

Space does not permit a detailed discussion of the other results presented by Gibson *et al*. [25]. However, notable molecular and genetic analyses were undertaken in this study to substantiate the validity of the observations made and the conclusions drawn from this visualization method. Thus, the method offers a robust and convenient way to examine genetic crosses.

An interesting observation made is that, although salivary glands that contained only one parental type never contained yellow progeny, the majority of flies with mixed infected glands did carry yellow trypanosomes [25]. This indicates that if both parents are present in the salivary gland simultaneously in time and space, mating occurs with high frequency. So, although mating might not be obligatory, it can be frequent under the correct circumstances.

Implications

The contribution of Ref. [25] brings us very close to knowing where trypanosome sex takes place – the attached epimastigote. What remains to be done? To date, it has not been possible to identify any haploid gametes that would be predicted to exist – however transiently that might be. Coloured fluorescent trypanosomes might offer an opportunity to delve, in more detail, into the events immediately before mating. Does it bring us closer to an understanding of the frequency and contribution of genetic exchange in nature? Possibly. It is clear that genetic exchange is crucially dependent on the co-existence of different parental (i.e. genetic) types within a single salivary gland of individual tsetse. Many factors might determine whether this will actually occur in nature – the trypanosome growth rate, the trypanosome maturation rate within the tsetse, intra- and inter-specific competition between trypanosome types, the timing of ingestion of parental types, the range of parental types in the sleeping-sickness focus and the possible existence of mating types. To address some of these issues, it might be possible to create fluorescent-coloured transgenic trypanosomes that derive from natural isolates that exhibit specific biological phenotypes, such as growth rate, and examine the behaviour of those transgenics during mating. However, it will be important to be aware that such biological properties might be modified by the introduction of transgenic constructs. However, if carefully controlled, this might offer approaches to gain an understanding of the factors that control the frequency of genetic exchange in nature. An understanding of the factors that affect the probability of the co-infection of the salivary gland by naturally occurring genetic types (trypanosome strains) might lead us closer to an understanding of the role of trypanosome genetics in the origins, generation and dynamics of sleeping-sickness epidemics.

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Research Focus

What happens when *Trypanosoma brucei* leaves Africa

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Julius Lukeš and co-workers evaluated the evolutionary origin of *Trypanosoma equiperdum* and *Trypanosoma evansi*, parasites that cause horse and camel diseases. Although similar to *T. brucei*, the sleeping-sickness parasite, these trypanosomes do not cycle through the tsetse fly and have been able to spread beyond Africa. Transmission occurs sexually, or via blood-sucking flies or vampire bats. They concluded that these parasites, which resemble yeast petite mutants, are *T. brucei* sub-species, which have evolved recently through changes in mitochondrial DNA.

Trypanosoma brucei and kinetoplast DNA

Trypanosoma brucei undergoes a complex life cycle within its mammalian host and tsetse-fly vector. Two life-cycle stages, the mammal-infective bloodstream form (BSF) and the procyclic form (PCF, which normally resides in the insect midgut), are easily cultured in the laboratory and are the subjects of this commentary. The single mitochondrion of *T. brucei* contains an amazing genome, kinetoplast DNA (kDNA), which is a network of interlocked DNA rings [1]. The rings include dozens of maxicircles and thousands of minicircles. Maxicircles encode ribosomal RNA (rRNA) and a handful of proteins, which are mostly subunits of respiratory complexes, but they cannot be translated until they are edited by the insertion or deletion of uridylates at specific internal sites [2,3]. Small guide RNA templates, which are mostly encoded by minicircles, determine editing specificity. Because most *T. brucei* maxicircle transcripts are edited, some extensively, many guide RNAs are needed and, therefore, the kDNA network carries a startling array of minicircle sequence classes. Because most minicircles encode essential guide RNAs, trypanosome viability depends upon preserving the minicircle repertoire. Mathematical modeling predicts that the random segregation of *T. brucei* minicircle progeny would lead to the rapid loss of essential minicircles, but this does not happen [4,5]. This is probably because the network structure and its complex replication mechanism have evolved to preserve the minicircle repertoire [6,7]. However, the replication mechanism must not provide precise segregation of minicircle progeny because there is considerable drift in minicircle copy number during two years of laboratory culture [5]. Another factor that contributes to the preservation of the minicircle repertoire is trypanosome mating, which occurs only in the tsetse vector and results in mixing of the parental minicircles [8]; thus, mating could rescue minicircles that are in danger of being lost [4].

Lai *et al.* [9] have extensively discussed the evolutionary origins of *Trypanosoma equiperdum* and *Trypanosoma evansi*. In this commentary, we do not address this controversial and complex subject (for an alternative view, see Ref. [10]). Instead, we focus on the molecular changes that have altered the biology of these trypanosomes and occurred during their departure from Africa.

T. equiperdum and T. evansi

It has been known for many years that *T. equiperdum* and *T. evansi* differ markedly from *T. brucei* in their kDNA structure, and Lai *et al.* [9] have extended these conclusions

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