The System of Genetic Exchange in *Trypanosoma brucei* and Other Trypanosomatids

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Abstract

In this chapter, we discuss our current understanding of the systems of genetic exchange in trypanosomatids and the impact the recent genome projects have had on this area of research. We focus mainly on the details of *Trypanosoma brucei* as it is the most extensively studied of the "trityps", but will also refer to a recently discovered novel mechanism of genetic exchange in *T. cruzi* and the apparent rarity of genetic exchange in *Leishmania sp*.

The system of genetic exchange in *Trypanosoma brucei* has been known to exist since the late eighties when a genetic cross between different strains was carried out by co-transmission through the tsetse fly. We discuss the segregation of nuclear, chromosomal and kDNA markers and outline the two current models for the mechanism of genetic exchange. We also present how the completion of the genome project has allowed the identification of polymorphic micro and minisatellite markers distributed throughout the genome, which have been used to prove formally that meiosis, independent assortment and crossing over occur in this parasite, as would be predicted in a conventional Mendelian system. Such data have been used to construct the first genetic map of *T. brucei*, which opens up the use of genetic analysis, coupled with positional cloning and the genome sequence, as a tool to identify the genes involved in a range of traits relevant to the disease.

Introduction

The study of genetic exchange and the mechanisms of recombination are important areas of research as they define a basic biological feature of an organism that can be used both as a tool to define genes and their function as well as providing a basis for understanding variation in natural populations and the response to environmental change. The availability of genome sequences for an increasing number of organisms has facilitated genetic analysis both by providing genome wide polymorphic markers that can be used to identify genes under selection or associated with specific phenotypes in population based analyses and by allowing the identification of genes within marker intervals defined by crosses. In the kinetoplastid protozoa, reverse genetic techniques have been developed for more than a decade and used to determine the function of genes by what is mostly deletion analysis. While such approaches are powerful in terms of defining essential genes, one of the problems has been the identification of specific phenotypes. In contrast, classical and population

based genetic approaches start with a specific phenotype and then go on to identify the genes that determine the phenotype of interest largely accessing phenotypes determined by allelic variation. The possibility of using such genetic approaches with the kinetoplastids has been limited by the need to define the existence and nature of the genetic systems. A sexual cycle has been identified in many eukaryotic pathogens based on morphological and cytological observations, but with the kinetoplastids no evidence of this nature is available. In this chapter we review the evidence for the existence of systems of genetic exchange in the three species of kinetoplastids the genomes of which have been sequenced, discuss the nature of these processes and mechanisms of recombination and outline how the Mendelian system identified in *T. brucei* can be used to identify genes determining natural variation in a number of phenotypes.

Genetic exchange in *T. brucei*

The first suggestion that *Trypanosoma brucei* was a diploid sexual organism came from the analysis of the nature of isoenzyme variation in different trypanosome strains (Gibson *et al.*, 1980) and the frequency of alleles at different loci in populations (Tait, 1980, 1983). These data led to the conclusion that sexual recombination occurred, although it was not until 1986 that the first genetic cross between two genetically and phenotypically distinct *T. brucei* strains was demonstrated in the laboratory (Jenni *et al.*, 1986). The cross, which involved the co-transmission of two strains through tsetse flies, with subsequent cloning and characterization of the clones (Fig. 3.1), produced trypanosomes with hybrid, non-parental, genotypes and phenotypes, indicating that some form of genetic exchange had taken place. Since then, experimental crosses have been conducted between eight pairs of trypanosome strains, together with one backcross and two experiments in which the products of self-fertilization were detected (Sternberg *et al.*, 1988; Gibson, 1989; Turner *et al.*, 1990; Gibson and Garside, 1991; Gibson *et al.*, 1992; Gibson and Whittington, 1993; Gibson and Bailey, 1994; Schweizer *et al.*, 1994; Degan *et al.*, 1995; Tait *et al.*, 1996; Gibson *et al.*, 1997; MacLeod *et al.*, 2000) (summarized in Table 3.1). Since no chromosomal condensation has been observed in any life cycle stage and no gametes have been identified, the main approach to examining the key features of genetic exchange in *T. brucei* has been to analyse the products of mating with molecular markers. The *T. brucei* genome sequence (Berriman *et al.*, 2005) has provided the opportunity to develop markers across each of the 11 megabase chromosomes. This, together with the generation of more progeny clones, has opened up the possibility of using genetic analysis as a means of identifying loci that determine a range of phenotypes (Tait *et al.*, 2002; MacLeod *et al.*, 2005).

The role of genetic exchange in field populations has been the subject of debate, with interpretations of the population structure ranging from clonal through epidemic to panmictic. There is clear evidence for clonality in some populations of *T. b. rhodesiense* and *T. b. gambiense* (Tibayrenc *et al.*, 1990; MacLeod *et al.*, 2000) and epidemic population structures in other *T. b. brucei* populations (Maynard-Smith *et al.*, 1993; Hide *et al.*, 1994; MacLeod *et al.*, 2000). However, the debate has not been fully resolved and, as this topic has been reviewed relatively recently (Gibson and Stevens, 1999; Tait, 2000; MacLeod *et al.*, 2001; Tibayrenc and Ayala, 2002) and few new data have been published, it will

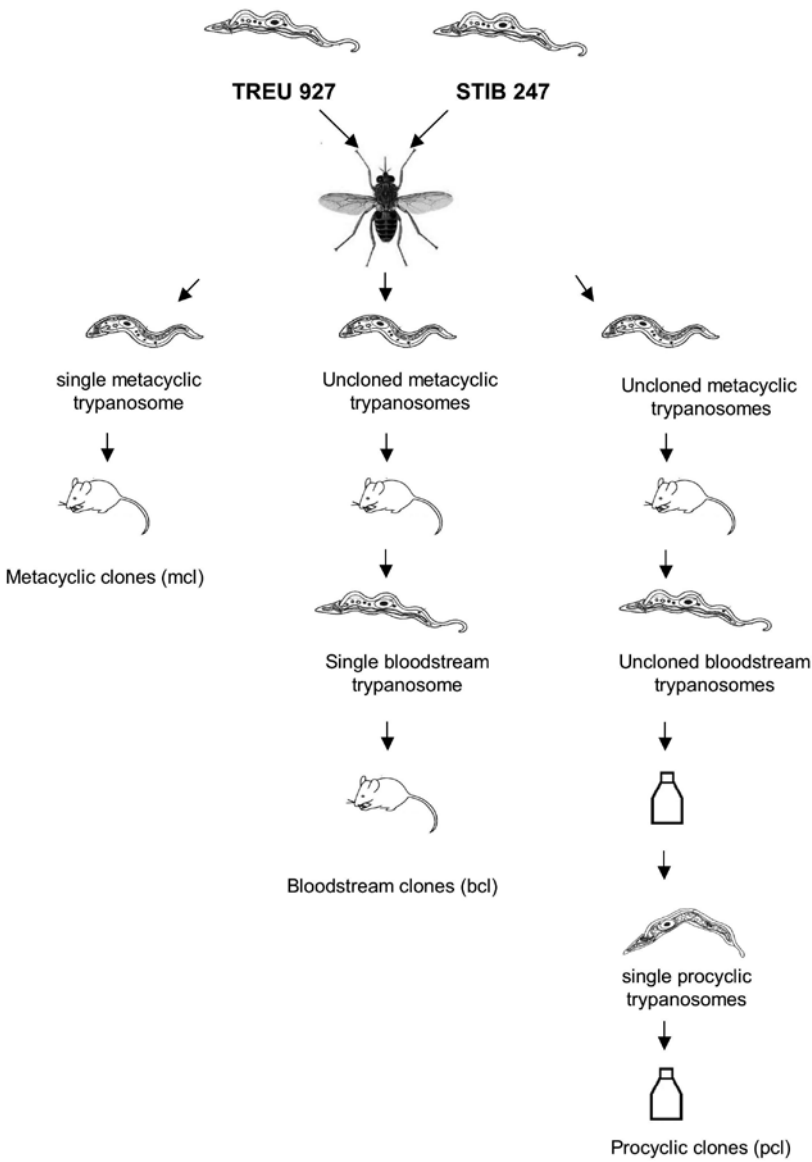

Figure 3.1 A schematic outline of how genetic crosses are conducted for *T. brucei*. There are three distinct methods of analysing the products of a cross: (1) Single non-dividing metacyclic trypanosomes are taken directly from the dissected salivary glands of a mixed-infected tsetse fly, and injected into a mouse where they differentiate to bloodstream forms and replicate. The resulting population is then analysed (for a novel hybrid genotype) using a series of genetic markers that differ between the two parental strains. (2) Either a mixed-infected tsetse fly is allowed to bite a mouse or part of the dissected salivary glands of the fly is injected into a mouse, thereby infecting the mouse with several thousand trypanosomes, which differentiate and multiply. Single trypanosomes are then isolated from the infected mouse blood and injected into separate mice where they multiply as clonal populations. Since there is a pre-amplification step in the first mouse before cloning, some of the resulting progeny bloodstream clones will be identical, having been derived from the same genetic exchange event. 3) As for (2) but the uncloned population is transformed into culture as procyclic forms and cloned.

Table 3.1 Summary of *T. brucei* genetic crosses performed to date

Parental clones for each cross						
Parent 1			Parent 2			
Clone name	Origin	Host	Clone name	Origin	Host	Reference
Crosses producing F1 progeny						
STIB 247	Tanzania	Hartebeast	STIB 386	Ivory Coast	Human	Jenni *et al.* (1986) Paindavoine *et al.* (1986) Wells *et al.* (1987) Sternberg *et al.* (1988) Sternberg *et al.* (1989) Turner *et al.* (1990) MacLeod *et al.* (2005)
STIB 247	Tanzania	Hartebeast	TREU 927/4	Kenya	Tsetse	Turner *et al.* (1990) MacLeod *et al.* (2005)
STIB 386	Ivory Coast	Human	TREU 927/4	Kenya	Tsetse	Turner *et al.* (1990)
STIB 247 LF	Tanzania	Hartebeast	STIB 777-A	Uganda	Tsetse	Schweizer *et al.* (1994)
058	Zambia	Human	196	Ivory Coast	Pig	Gibson (1989) Gibson *et al.* (1992)
196	Ivory Coast	Pig	J10	Zambia	Hyena	Gibson *et al.* (1991)
STIB 831Kcl1	Uganda	Tsetse	STIB831Kcl2	Uganda	Tsetse	Degen *et al.* (1995)
058H	Zambia	Human	KP2N	Ivory Coast	Tsetse	Gibson *et al.* (1993) Gibson *et al.* (1994)
TH2N	Ivory Coast	Human	058H	Zambia	Human	Gibson *et al.* (1997)
Backcross						
058H	Zambia	Human	P20 (hybrid of 058 × KP2)			Gibson *et al.* (1995)
Crosses generating self-fertilization products						
STIB 247	Tanzania	Hartebeast (in the presence of STIB 386)				Tait *et al.* (1996)
TH2N	West Africa	Human (in the presence of 058H)				Gibson *et al.* (1997)

STIB, Swiss Tropical Institute Basel; TREU, Trypanosome Research Edinburgh University.

not be covered here. Instead, the focus is on the laboratory genetics where there are novel data that address many of the key questions regarding the mechanism and consequences of genetic exchange.

Clones derived from mixed-infected tsetse flies have been examined using a range of genotypic and phenotypic markers, in particular by micro and minisatellites, isoenzymes and restriction fragment length polymorphisms in single copy genes. From these studies a number of the basic features of genetic exchange in *T. brucei* have been defined. It is clear that three distinct genotypes/phenotypes of trypanosomes can be identified from a cross: parental genotypes, indicating that the process of genetic exchange is non-obligatory (Sternberg *et al.*, 1989), hybrid genotypes (equivalent to F1s) (Gibson, 1989; Sternberg *et al.*, 1989) and the products of self-fertilization (Tait *et al.*, 1996; Gibson and Mizen, 1997). Molecular and, in particular, DNA content analysis of the progeny from the 10 genetic crosses performed to date indicate that the crosses can be divided into two categories: those that produce predominantly diploid hybrid progeny (Turner *et al.*, 1990; Hope *et al.*, 1999; MacLeod *et al.*, 2005) and those that produce a significant proportion of triploid hybrid progeny (Gibson and Garside, 1991; Gibson and Bailey 1994; Gibson *et al.*, 1995).

Mendelian inheritance in *T. brucei*

Molecular analysis of the progeny generated from the crosses listed in Table 3.1 indicated that they were equivalent to F1 hybrids as, at loci at which parents were homozygous but different from each other, the hybrids were heterozygous (Sternberg *et al.*, 1989; Turner *et al.*, 1990; MacLeod *et al.*, 2005). Analysis of these progeny at loci that were heterozygous in the parents showed segregation of alleles as would be predicted in a Mendelian system, leading to a model of mating involving meiosis and syngamy (see Fig. 3.4) (Tait *et al.*, 1988; Sternberg and Tait, 1990; Turner *et al.*, 1990; Gibson 1995). However, given the small number of available progeny clones from each cross and the possibility that some of the clones could be identical, having been derived from the same mating event (Fig. 3.1), it was not possible to prove Mendelian inheritance. Additionally, the crosses with triploid or trisomic progeny (reviewed by Gibson and Stevens, 1999) suggested that the system might not be Mendelian. To interpret these data, we consider in this chapter the two types of outcome separately. The progeny from pair-wise crosses between two strains of *T. b. brucei* and one strain of *T. b. gambiense* rarely (2/30), produced hybrids with raised DNA content which shows that triploidy is rare, at least for these particular crosses (Hope *et al.*, 1999). Recently, however, many more independent progeny were generated and a total of 79 independent progeny clones were isolated from a *T. b. brucei* × *T. b. brucei* (strains TREU 927 and STIB 247) and a *T. b. brucei* × *T. b. gambiense* Type 2 (strains STIB 247 and STIB 386) cross (MacLeod *et al.*, 2005). By using the genome sequence, a series of microsatellite markers distributed on each of the 11 megabase chromosomes were identified and screened against the parental isolates to identify those that were heterozygous and therefore informative for analysis of the progeny. Eleven markers were selected, each located on a separate chromosome and heterozygous for one of the parents in each cross, and used to genotype the progeny. The data showed that the progeny clones were diploid, that alleles segregate in the ratios predicted for a Mendelian system and that alleles at loci on different chromosomes segregate

independently (Fig. 3.2) (MacLeod *et al.*, 2005). These data provide statistically robust proof, using χ^2 analysis of segregation ratios, that the genetic system in *T. b. brucei* and *T. b. gambiense* (Type 2) is Mendelian and involves meiosis. The *T. b. brucei* parental strain common to both crosses proved to be homozygous at 94% of loci, but five heterozygous markers, each located on a different chromosome, also segregated in the predicted ratios, showing that this isolate also underwent meiosis (MacLeod *et al.*, 2005). These data provide the first formal proof that *T. brucei* has a Mendelian system of genetic exchange and is consistent with meiosis occurring to yield haploid gametes that fuse to produce diploid recombinant progeny, although other models cannot formally be excluded.

A genetic map

A further set of microsatellite markers (182 in total) have been identified that are heterozygous for the *T. b. brucei* genome strain TREU 927 and their segregation has been analysed in the 39 progeny from the cross with STIB 247, allowing generation of a genetic map of the genome strain (El-Sayed *et al.*, 2003; Hall *et al.*, 2003; MacLeod *et al.*, 2005). All 11 megabase chromosomes have been mapped and comparison with the physical map shows the order of the markers in the physical map correlates with that in the genetic map and that the average size of the recombination unit (cM) is 15.6 kb (MacLeod *et al.*, 2005) which is comparable to that found for *P. falciparum* (Su *et al.*, 1999). An example of the genetic map and its comparison to the

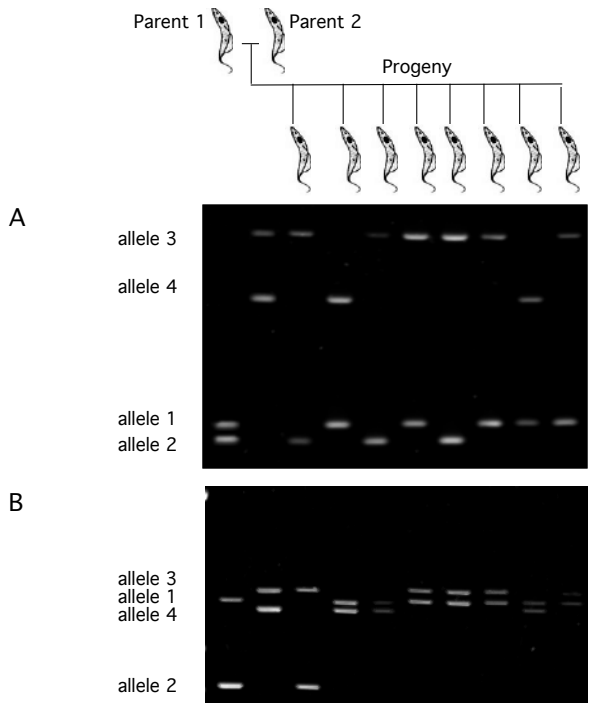

Figure 3.2 Allele segregation and independent assortment. In this illustration the two parents in tracks 1 and 2 were both heterozygous for markers shown in panels A and B. In each panel, the eight F1 hybrid progeny have inherited an allele from both parents in different combinations. A comparison of the 2 panels show independent assortment of alleles at these two unlinked loci.

physical map for chromosome 3 is shown in Fig. 3.3.

The generation of the *T. brucei* genetic map demonstrates that classical crossing over (recombination) occurs at meiosis and analysis of the progeny with this large set of markers allows haplotypes of each progeny clone to be generated, thus defining the two haplotypes of the parental strain as well as identifying recombinant haplotypes where both single and double crossovers have occurred (El-Sayed *et al.*, 2003; MacLeod *et al.*, 2005). The genetic map provides further evidence of a conventional system of genetic exchange.

The values for the size of the recombination unit vary by orders of magnitude between the lower and higher eukaryotes, with the value for *S. cerevisiae* being 3 kb/cM and that for humans 1 Mb/cM (Petes, 2001). The reasons for such variation are not totally clear but may reflect the number of available sites for the initiation of double strand breaks by the DNA topoisomerase II related protein, Spo11, and its associated complex of enzymes. One class of recombination site, in *S. cerevisiae*, is in promoter regions where specific transcription factors bind (de Massy, 2003) and so the variation between organisms may

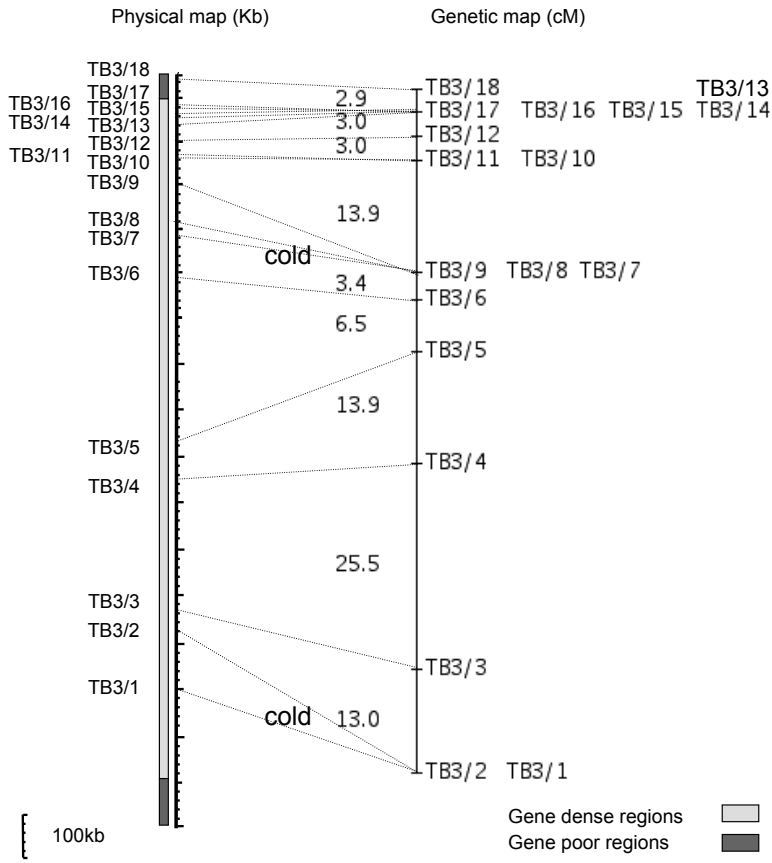

Figure 3.3 Comparison of the genetic and physical maps of *T. brucei* TREU 927, chromosome 3. Genetic map distances are Haldane corrected. Two potential cold spots for recombination are shown spanning markers TB3/1–3/2 and TB3/7–3/9 respectively.

reflect gene density. In *T. brucei*, however, this seems unlikely given the polycistronic nature of transcription and the consequent likely low density of putative promoter regions.

Hot and cold spots

The level of recombination varies across each chromosome in *T. brucei*, with "hot" and "cold" spots of recombination—regions where the size of the unit of recombination is significantly lower or higher than the genome average (El-Sayed *et al.*, 2003). Two potential cold spots are shown in Fig. 3.3 where, in each case, there is no recombination detected between markers ~110 kb apart. Hot spots of recombination have been identified in most organisms where recombination has been analysed in detail (Petes, 2001; de Massy, 2003; Mu *et al.*, 2005), but to date no specific sequence motif has been identified that is associated with high levels of recombination. A series of factors appear to be associated with both hotspots and sites of recombination, such as accessible regions of chromatin, regions of high GC content and promoters/transcription factor-binding domains, although none of these associations is absolute. Analysis of 12 hot spots in mice and humans, with recombination rates varying from 2 to >3,000 fold above the genome average, has shown that they are located in either introns or intergenic regions (Petes 2001). The most detailed and comprehensive analysis has been undertaken with *S. cerevisiae* (Gerton *et al.*, 2000), using an array-based approach that identified 177 hot spots distributed across the genome. These findings showed that there is a higher hot spot density on smaller chromosomes, no correlation with repetitive elements and a suppression of their occurrence at the centromeres and telomeres. This reduction of hot spots at centromeres is also observed in humans but recombination is higher at telomeres (Petes, 2001). Thus, the occurrence of such variation in recombination along the length of chromosomes is a common feature of eukaryotes shared by *T. brucei*. To identify any key features of the hot spots in *T. brucei* will require fine scale mapping of these regions in relation to the genome sequence as well as examination of the telomeres and subtelomeres in terms of levels of recombination. In this respect, Melville *et al.*, (1999) have mapped chromosome 1 in one F1 progeny clone and shown major size changes relative to the parental homologue in the subtelomeric region.

Cold spots are also a common feature of eukaryotic recombination and a total of 40 have been mapped in *S. cerevisiae* (Gerton *et al.*, 2000). Their distribution is not random and higher numbers are associated with the centromeres and telomeres. In *T. brucei*, cold spots have been identified on chromosomes 1 and 2 (El-Sayed *et al.*, 2003; Hall *et al.*, 2003) where no crossovers have been identified between markers separated by > 150 kb. In chromosome 1, the cold spot surrounds a strand switch region containing a degenerate 58 bp repeat and this region is conserved in *T. cruzi* chromosome 3, where fragmentation experiments show that this region is necessary for mitotic chromosome stability (Obado *et al.*, 2005). Unfortunately, there is a lack of conservation in centromere sequence motifs (see Chapter 1) and cold spots, while being more likely to occur around the centromere, are not an unequivocal marker for the centromere (Gerton *et al.*, 2000).

Chromosomal inheritance

The development of pulsed field gel electrophoresis (PFGE) has led to the characterization of the *T. brucei* karyotype, with three distinct chromosome classes being

identified: megabase chromosomes (1–5 Mb) (Berriman *et al.*, 2005), intermediate (200–700 kb) and mini (30–150 kb) (Melville *et al.*, 1998). The different classes of chromosomes are inherited in different ways.

There are 11 megabase chromosome pairs, which are diploid, contain housekeeping genes, range in size from 1 Mb to approximately 5 Mb based on PFGE (Melville *et al.*, 1998; Berriman *et al.*, 2005; Hertz-Fowler *et al.*, Chapter 1) and are inherited in a Mendelian fashion. Within a strain, homologous chromosomes can differ widely in size, while non-homologous chromosomes can be of the same size and co-migrate in the gel (Gottesdiener *et al.*, 1990; Melville *et al.*, 1998; Melville *et al.*, 1999). Although relatively few strains have been analysed, there is also a very significant size polymorphism of chromosomes between strains (Melville *et al.*, 1998; Melville *et al.*, 1999). The basis for homologue size polymorphism has not been fully elucidated but analysis of chromosome 1, using rare base cutting restriction enzymes, has shown that most of the size differences between homologues occur at the chromosome ends within the telomeric and subtelomeric regions (Melville *et al.*, 1999). The inheritance of chromosome 1 has been analysed in one cross, using PFGE and a chromosome-specific probe, and it was shown that each progeny clone inherited one homologue from each parent, a classic prediction of Mendelian inheritance at the chromosome level (Tait *et al.*, 2002). Analysis of the inheritance of the karyotype in a second cross using three progeny clones and a series of chromosome-specific gene probes suggested that the megabase chromosomes are inherited in a Mendelian fashion, but the generation of novel sized chromosomes in the progeny makes a definitive analysis difficult (Melville *et al.*, 1998, 1999). This high frequency of size change in the chromosomes at meiosis is likely to be the result of crossing over, and a detailed analysis of one homologue of chromosome 1 in one progeny clone has shown that the 10% change in chromosome size was due to the loss of subtelomeric sequences at one end of the chromosome (Melville *et al.*, 1999). Size changes in the megabase chromosomes have also been observed in other crosses (Gibson and Garside, 1991; Gibson *et al.*, 1992; Schweizer *et al.*, 1994; Degan *et al.*, 1995; Tait *et al.*, 1996; MacLeod *et al.*, 2000) and appear to be a general feature, providing evidence for chromosomal recombination (Gibson and Bailey, 1994).

The number and size of both intermediate and minichromosomes varies between strains. There are ~100 minichromosomes in the *T. brucei* genome (Wickstead *et al.*, 2004), and fewer (probably 1–10) intermediate chromosomes. Both classes act as a reservoir for variant surface glycoprotein (*VSG*) genes, which encode the parasite's surface coat (Van der Ploeg *et al.*, 1984). Both mini and intermediate chromosomes are probably essentially aneuploid (Gottesdiener *et al.*, 1990) but minichromosomes, at least, appear to be partitioned with fidelity at mitosis (Wickstead *et al.*, 2003), and segregate independently of the large chromosomes by association with the central intranuclear spindle (Ersfeld and Gull, 1997; Gull *et al.*, 1998). In contrast, the larger chromosomes are partitioned by kinetochore microtubules.

Analysis of the inheritance of intermediate chromosomes has shown that parental chromosomes appear to be randomly distributed among the progeny, as would be expected if the chromosomes were not segregating as homologues at meiosis (Sternberg *et al.*, 1987; Wells *et al.*, 1987; Le Page *et al.*, 1988; Gibson 1989).

Similarly, available data suggest that, at meiosis minichromosomes are inherited in a non-Mendelian manner. Wells *et al.* (1987) demonstrated that hybrid progeny clones inherited a full complement of minichromosomes from each parent. The hybrid clones analysed in these experiments all had elevated DNA content, although the inheritance of all minichromosomes from both parents accounted for only a small proportion of this. The hybrid clones examined were probably triploid for megabase chromosomes. A comparable analysis of mini-chromosome inheritance in diploid progeny clones has not yet been undertaken. Consequently, it is possible that the results of Wells *et al.* (1987) are aberrant and do not reflect the true pattern of inheritance of this class of chromosomes. It is important to resolve this issue, not only for our understanding of inheritance, but also to clarify the apparent paradox that minichromosomes segregate with fidelity at mitosis, but not meiosis.

Overall, the inheritance of chromosomes in genetic crosses has not been studied extensively and the available data suggest that a more detailed investigation is warranted in order to allow us to understand fully the basis of the size changes in the megabase chromosomes and the segregation patterns of the intermediate and minichromosomes.

Kinetoplast inheritance

The inheritance of the kinetoplast organelle has been examined by analysing the segregation of polymorphic regions within maxi- or minicircle DNA. Initial studies indicated that maxicircles were inherited uniparentally from either parent and minicircles were inherited biparentally (Gibson 1989; Sternberg *et al.*, 1989; Gibson and Garside,1990). Subsequent studies of hybrid clones during their early stages of bloodstream growth after fly transmission and close in time to meiosis indicated, however, that both maxi- and minicircles were inherited biparentally with subsequent stochastic segregation at each mitotic division (Turner *et al.*, 1995). This model predicts rapid fixation to a uniparental pattern for the maxicircles (but not for the minicircles due to their higher copy number), and could explain the results of earlier reports and the observation that maxicircles are genotypically homogeneous. The evidence for biparental inheritance is strengthened by the observation of two hybrid clones that appear to have identical nuclear genotypes and mixed minicircle genotypes but different maxicircle genotypes (Gibson and Garside 1990). The clones are believed to be the two daughter cells from the same mating event, suggesting that parental kinetoplasts were present in the same cell but then the maxicircles segregated in subsequent mitotic divisions. This has led Gibson and Stevens (1999) to propose a second model for the events following mating, based on the different mechanisms of maxi- and minicircle replication. In this model, the two kinetoplasts do not fuse and the maxicircles remain separate. The minicircles, when they detach from the network during replication, could detach from one parental network but reattach to the second parental network, so generating the mixed minicircle genotype of the progeny. At the present time there are no experimental data to distinguish between these two models. It would be informative to examine the structure of the kinetoplast in the progeny with mixed maxicircles described by Turner *et al.* (1995). At mitosis, the replication and partition of kinetoplasts are linked with the basal bodies and flagella. At meiosis the mitochondria must presumably fuse and this must lead to a cell with two flagella and two basal bodies. As

the progeny from crosses have a normal complement of these organelles, the initial fusion product of mating must dissociate nuclear replication from organelle replication and so divide to yield a cell with normal structural features.

Non-obligatory mating and barriers to mating

It is clear now that genetic exchange in *T. brucei* is non-obligatory. Nevertheless, most flies with a mature mixed infection will eventually produce hybrid trypanosomes (Sternberg *et al.*, 1989). By analysing the metacyclics produced by these flies over time, it is clear that, early in infection, the parental strains are transmitted without mating, but that, after approximately 25–50 days of infection, hybrid trypanosomes (as well as parentals) are observed, and continue to be produced for the life time of the fly (Schweizer *et al.*, 1988; Sternberg *et al.*, 1989; Turner *et al.*, 1990). The fact that eight pairs of strains have been crossed (Table 3.1) and progeny obtained suggests that there are limited genetic barriers; furthermore these crosses include some between subspecies as well as strains from geographically very distinct regions. Three strains have been successfully crossed in all pairwise combinations (Turner *et al.*, 1990), which, if there were a mating type system, would mean that at least one of the strains was heterozygous for alleles at a putative mating type locus. Such heterozygosity could account for self-fertilization within a strain.

Self-fertilization

Whilst the most common class of progeny in crosses are hybrids, another class of recombinant progeny from mixed strain transmissions has been identified as the product of self-fertilization (Tait *et al.*, 1996; Gibson *et al.*, 1997). Whether self-fertilization is commonplace or rare is difficult to discern, however, as the markers used in several of the crosses would not necessarily have been able to identify self-fertilization products. It is more interesting to note perhaps that such progeny appeared to occur only in the context of cross-fertilization. Single strain transmissions through flies have so far only ever produced clones that were identical to the original strain (Tait *et al.*, 1989) and the double drug selection of progeny from a cross between two drug-resistant transformants of the same strain did not generate the products of self-fertilization (Gibson *et al.*, 1997). This means that the sexual process is induced only when two strains interact with each other, suggesting that there is a system of strain recognition, perhaps pheromone-based, which can induce both self and cross-fertilization.

Stage of life cycle

It is clear from the results of the laboratory crosses that mating takes place at some stage during the trypanosome life cycle in the tsetse fly, i.e. at the procyclic, proventricular, epimastigote or metacyclic stages. Analysis of progeny vegetatively derived from single metacyclics has shown them to be hybrid. It is reasonable to assume that such metacyclics are the products of mating and that mating occurs at an earlier stage (Tait *et al.*, 1989). Although an analysis of DNA content of the metacyclics (Zampetti-Bosseler *et al.*, 1986) suggested that metacyclics are haploid gametes, this has subsequently been shown to be incorrect, based on re-evaluation of such physical measurements (Kooy *et al.*, 1989) and the genetic demonstration that heterozygous loci did not segregate in metacyclic clones derived from single strain transmissions (Tait *et al.*, 1989). The crossing of drug resistant strains of *T. brucei* and

the double drug selection of the progeny (Gibson and Whittington, 1993; Gibson *et al.*, 1997) has shown that hybrids can be isolated from the salivary glands, but not the midgut, of mixed-infected tsetse flies, indicating that the probable site of genetic exchange is the salivary glands. Using an innovative approach involving GFP tagging, the Gibson group showed that the products of mating occur only in the salivary glands although the precise stage in trypanosome development was not discernable (Bingle *et al.*, 2001). One report (Schweizer and Jenni, 1991) described the detection of hybrids in the midgut of tsetse flies using isoenzyme markers but, as no clones were isolated and demonstrated to be of the predicted hybrid phenotype, its significance is unclear. A detailed morphological analysis of the developmental stages in the tsetse fly (Van Den Abbeele *et al.*, 1999) has identified a series of novel stages in the foregut and proboscis of trypomastigote morphology prior to colonization of the salivary gland and the attachment and division of the conventional epimastigote stage. Mating could take place at any one of these stages. However the most robust data are from Bingle *et al.* (2001) and clearly implicate stages in the salivary gland, which indicates that either the migratory trypomastigote or epimastigote stage is likely to be involved in genetic exchange.

Triploid or trisomic progeny

DNA content analysis of progeny clones from a number of crosses has shown that progeny clones can have elevated levels, higher than either parental level and this has been observed in five of the ten crosses (Gibson and Stevens, 1999). The measures of DNA content, coupled with marker and karyotype analysis of these clones, are consistent with these products of mating being triploid or trisomic (Gibson *et al.*, 1992; Gibson and Bailey, 1994). The analysis of a number of progeny clones from two crosses shows that the proportion of such clones can vary, with 6/12 in a cross between *T. b. gambiense* and *T. b. rhodesiense* being triploid (Gibson and Mizen 1997) and 3/20 occurring in a cross between *T. b. brucei* and *T. b. gambiense* (Hope *et al.*, 1999). Importantly, these crosses also produced diploid progeny showing that meiosis is taking place. The trypanosome genetic crosses that produce triploid progeny are between subspecies (Gibson *et al.*, 1992; Gibson and Bailey 1994) that will have been genetically isolated for many generations and there is no evidence for inter subspecies mating in the field (MacLeod *et al.*, 2000). A further potential confounding issue is that drug selection was used to select for progeny in three of the five crosses producing progeny with elevated DNA content. The critical conclusion is that, in the crosses undertaken to date, the majority of progeny are diploid and in crosses between strains of the same subspecies no triploids have been reported. This raises the question as to how triploidy arises. In this context, it is well documented that meiosis is not a "perfect" process. In humans for example, a high proportion of conceptions result in early abortion due to trisomy or triploidy (Hassold, 1986). Thus, in *T. brucei*, the triploids may be aberrant but not lethal, given the ability of the parasite to tolerate variable levels of ploidy. While non-disjunction of one or more chromosomes would explain trisomy, the available evidence suggests that the progeny are triploid and probably the result of mating between a parental diploid that failed to undergo meiosis and the haploid product of meiosis from the other parent, as suggested by Gibson and Stevens (1999). In higher eukaryotes, non-disjunction at meiosis is considered to occur in many cases of trisomy. Extensive

marker analysis of field isolates over many years has not identified triploid trypanosomes, suggesting such progeny are rare in natural populations or are possibly less viable than diploid progeny.

Models of genetic exchange

The data on the segregation and independent assortment of alleles in crosses of *T. brucei* show that genetic exchange involves meiosis and syngamy (Turner *et al.*, 1990; MacLeod *et al.*, 2005) and within this context a number of possible models can be envisaged. The common mechanism of mating for diploid eukaryotes involves the formation of post meiotic haploid gametes followed by their fusion to generate diploid progeny. In lower eukaryotes such as *S. cerevisae*, under specific environmental conditions the haploid vegetative stages express alleles of the mating type locus and then fuse to form a diploid stage that undergoes meiosis to yield haploid recombinant products. In haploid protozoa, such as *P. falciparum*, morphologically distinct gametes are formed that fuse to yield a diploid zygote that subsequently undergoes meiosis (Walliker *et al.*, 1987) to yield haploid recombinant progeny. In diploid protozoa, such as the ciliates, no gametes are formed but contact between cells of different mating type induces the diploid micronuclei to undergo meiosis to yield a pair of haploid nuclei in each cell. The mating cells then exchange one nucleus each, followed by fusion to yield a diploid nucleus. Subsequent mitotic divisions yield the macro- and micronuclei. For *T. brucei*, three models of genetic exchange have been proposed: a diploid cell fusion/chromosome loss system (Paindavoine *et al.*, 1986), a haploid gamete system (Sternberg and Tait, 1990) and a diploid cell fusion/meiosis model (Gibson 1995).

The fusion/chromosome loss model (Fig. 3.4A) (Paindavoine *et al.*, 1986) proposes that mating occurs by the fusion of diploid nuclei to form a tetraploid nucleus followed by random chromosome loss to restore diploidy. This model explains the elevated DNA content found in some hybrids, but predicts that the DNA levels fall during vegetative growth, in a fashion similar to that proposed for *T. cruzi* (Gaunt *et al.*, 2003). However, the available evidence does not support this model. Wells *et al.* (1987) analysed the hybrids with raised DNA content and found no evidence for chromosomal loss. Furthermore, this model predicts non-Mendelian allelic segregation in the progeny which is refuted by recent evidence that Mendelian segregation occurs (MacLeod *et al.*, 2005): this model can be rejected. The classical Mendelian model (Fig. 3.4B) involving the generation of haploid gametes is supported by the demonstration that meiosis and crossing over occur, but to date there has been no direct demonstration of a haploid gametic stage. However, all the detailed cytofluorometric analyses of the stages in the salivary glands (Kooy *et al.*, 1989; Van Den Abbeele *et al.*, 1999) have been undertaken with single strain infections (rather than mixtures of strains), conditions in which mating does not appear to occur, and no haploid gametes are produced (Tait *et al.*, 1989). Thus, the critical experiments to test for the existence of a haploid stage have not been undertaken and so direct evidence for this model is not available. The third model (Fig. 3.4C), proposed by Gibson (1995), involves the fusion of diploid parental cells to form an intermediate polyploid cell but, crucially, this model does not involve the fusion of diploid nuclei, which would result in non-Mendelian inheritance. The intact diploid nuclei then undergo meiosis to

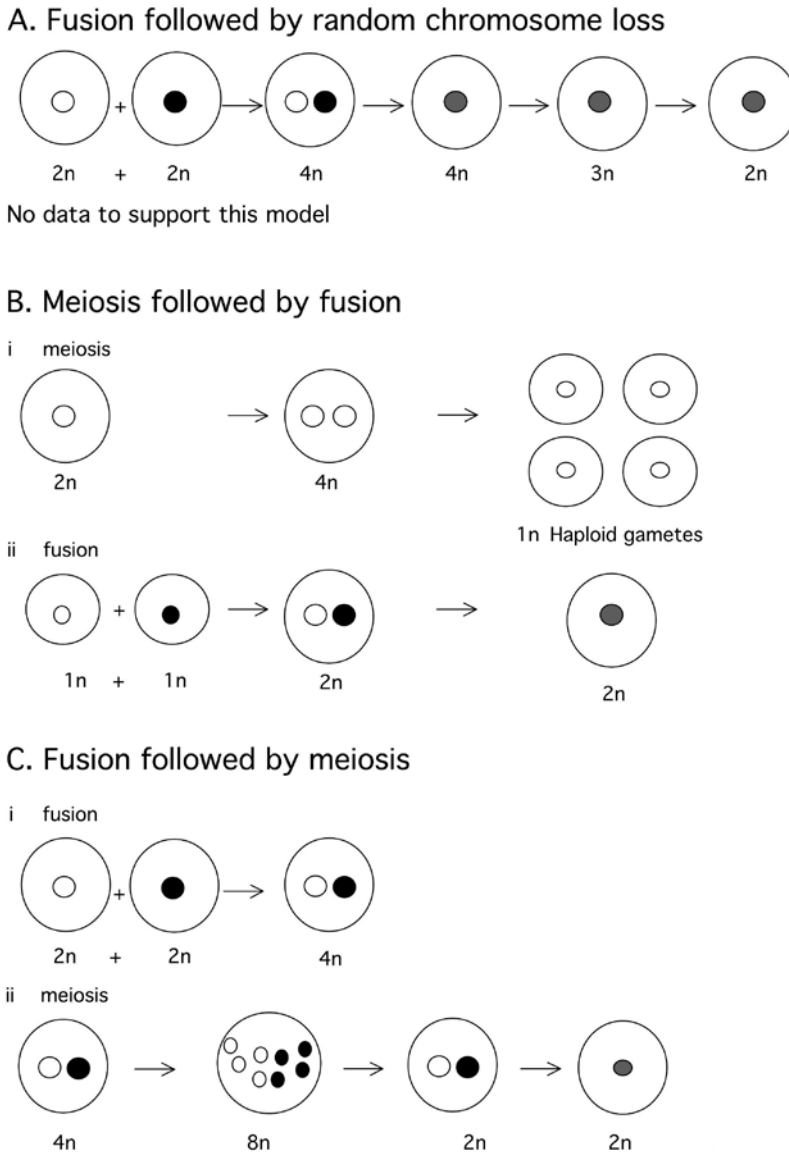

Figure 3.4 Models of mating. Model A, proposed by Paindavoine *et al.*, in 1986, suggested that genetic exchange involved fusion of two diploid cells (2n) to generate a 4n tetraploid cell, followed by random elimination of DNA to return to a diploid state. This model would predict non-Mendelian allele segregation, which has not been observed. Since this model does not fit the data (Turner *et al.*, 1990; Gibson 1995; MacLeod *et al.*, 2005), it can be disregarded. Model B, proposed by Sternberg and Tait (1990), suggests that genetic exchange involves (i) meiosis to generate haploid gametes (1n), which (ii) fuse with gametes from a second strain to produce diploid (2n) progeny. This model predicts Mendelian inheritance of megabase chromosomes. Model C, proposed by Gibson *et al.*, (1995) suggests (i) fusion of diploid (2n) cells to produce a 4n tetraploid cell. (ii) This is followed by meiosis to generate an 8n intermediate cell. Nuclei disintegrate to return the cell to a diploid (2n) state. This model also predicts Mendelian inheritance of megabase chromosomes.

yield eight haploid nuclei, four originating from one parent and four from the other. Upon fusion of a pair of haploid nuclei, the remaining nuclei disintegrate, resulting in diploid progeny. Depending on the parental origin of the two haploid nuclei the progeny could result in either cross- or self-fertilization products. This model is also consistent with the available genetic data but, for the reasons described for the haploid gametic model, no direct identification of a multinucleate stage has been obtained. At present there are no data that can formally distinguish between the models in Fig. 3.4B and C. Which is correct would be potentially resolved by detailed cell biological analysis of the life cycle stages in the salivary gland while mating is taking place. Clearly, there are still many unknowns that await further investigation.

The clear evidence for meiosis raises the question of how progeny with raised DNA content are generated in either of the proposed models. Karyotype analysis indicated that such progeny were trisomic for all chromosomes analysed and were probably triploid (Wells *et al.*, 1987; Gibson *et al.*, 1992; Hope *et al.*, 1999), with one parent contributing two homologues to the progeny and the other a single homologue. The simplest explanation is the failure of one parental strain or nucleus to undergo meiosis, but the ability to mate or fuse with a haploid gamete or nucleus from the other parent to produce viable triploid offspring.

A large number of proteins are required for meiosis; approximately 500 in *S. cerevisiae* (Chu *et al.*, 1998). Only a small proportion, however, are specific determinants of the meiotic process. Given the highly conserved nature of several elements of the meiotic machinery (Tzung *et al.*, 2001), it is hardly surprising that many, though by no means all, of the expected genes have been identified in *T. brucei* (El-Sayed *et al.*, 2005). Some of the genes presumed to be most important are discussed elsewhere in this book (Chapter 5) though, as that chapter highlights, there is a paucity of direct experimental evidence and a requirement for further studies.

Genetic exchange in *Leishmania* and *T. cruzi*

Laboratory crosses between strains of *Leishmania major* have been undertaken by mixed infection of sand flies and subsequent culture of amastigotes, but no evidence for genetic exchange was obtained (Panton *et al.*, 1991). Analysis of field populations with isoenzyme, RAPD (randomly amplified polymorphic DNA) and karyotype markers has led to controversy about the existence and role of genetic exchange (Gibson and Stevens, 1999; Victoir and Dujardin, 2002), although the levels of linkage disequilibrium generally support a clonal population structure (Tibayrenc and Ayala, 2002) with limited genetic exchange. There are a number of reports (see review by Gibson and Stevens, 1999) of hybrids between different species of both the Old and New World *Leishmania* parasites. The hybrids have been found where the species are sympatric and their existence suggests that genetic exchange can occur, although probably only rarely (Victoir and Dujardin, 2002). These findings create a paradox in which genetic exchange between strains within a species is unlikely but inter-species mating does occur. The *L. major* genome sequence (El-Sayed *et al.*, 2005) contains orthologues of genes that are uniquely expressed during meiosis in other organisms and are components of the double strand break-induced pathway of recombination (Roeder, 1997). One gene in this pathway, *MSH4*, has no orthologue in the *Leishmania* genome, but data from *S. cerevisiae* show that it is not essential for

meiosis (Ross-MacDonald, P. and Roeder, G.S. 1994). So, this parasite appears to be potentially recombination-competent but does not seem to undergo genetic exchange to a significant degree. We know nothing, however, about the expression of meiosis-specific genes or whether they are functional.

The population genetics of *T. cruzi* presents one of the paradigms of a clonal protozoan parasite (Tibayrenc and Ayala, 2002), where genetic exchange is largely absent except, perhaps, rarely in the past. Again, analysis of the genome sequence has identified a series of key gene orthologues in the recombination pathway (El-Sayed *et al.*, 2005), suggesting that the parasite is potentially recombination competent. Recently, a laboratory cross has provided evidence for genetic exchange between isolates. Two isolates were transfected with genes conferring resistance to different antibiotics, co-cultivated, followed by selection for double antibiotic resistance. No double resistant recombinants were obtained when mixtures of the two lines were passaged as epimastigote cultures, through the triatome vector or in genetically immunocompromised SCID mice, but they were obtained from trypomastigote cultures (Gaunt *et al.*, 2003). Double antibiotic resistant cloned lines were typed with a range of markers and shown to have inherited markers from both parental lines. Some markers showed the progeny to be triploid—a result consistent with fusion having occurred. Evidence for allele loss was obtained at heterozygous marker loci but probably the most surprising result was the identification of genes that were mosaics of the sequences of the two parental alleles, providing evidence for intragenic recombination (Gaunt *et al.*, 2003). The absence of evidence of allelic segregation and independent assortment of alleles at unlinked loci indicates that this system does not involve meiosis, despite the presence in the genome of gene orthologues associated with meiosis (El-Sayed *et al.*, 2005), but involves a mitotic recombination system. These results raise the obvious question of whether this process occurs in natural populations of the parasite. Sequence and microsatellite marker analysis of a series of field isolates from two *T. cruzi* lineages revealed both mosaic gene sequences and microsatellite loci with three alleles, thereby indicating that a similar recombination mechanism occurs in the field (Gaunt *et al.*, 2003). It is not clear at present what the mechanism for these intragenic recombination events might be and whether there is reciprocal exchange or gene conversion.

Future perspectives

The availability of genome sequences from several trypanosomatids provides a number of opportunities for genetic research. In *T. brucei*, three areas can be considered: population genetics and molecular epidemiology, the cell/molecular biology of the system of genetic exchange and the application of genetic and linkage analysis as a tool to identify genes that determine phenotypes of relevance to the disease and its transmission.

The availability of a panel of microsatellite markers (MacLeod *et al.*, 2005) across all the 11 megabase chromosomes will allow a range of outstanding questions to be addressed concerning the population genetics of all three subspecies and their evolutionary relationships. Such markers can be amplified from samples of blood or tsetse tissues spotted onto filters, as has been described for other organisms and potentially would overcome some of the limitations of previous analyses such as low sample size and the use of rodent amplification. Furthermore, with the availability

of genome sequence data from *T. vivax* and *T. congolense*, similar markers could be developed to address the population genetics of these important cattle pathogens. The identification of the stages involved in meiosis could be undertaken using antibodies raised to the expressed products of the orthologues of genes associated with this process that have been identified from the annotated genome sequence. Such studies could also address whether these genes are expressed and are potentially functional.

The genome sequence, coupled with the genetic analysis of variation in phenotype (Gibson *et al.*, 1997; Tait *et al.*, 2002) and the availability of a genetic map (MacLeod *et al.*, 2005), opens up the possibility of undertaking linkage analysis and positional cloning of genes determining such phenotypic variation. Linkage analysis, with the current marker coverage, is capable of defining loci determining specific phenotypes to a defined region on the genome sequence of ~100–150 kb. This allows candidate genes to be identified within the region and, in combination with reverse genetic techniques with loss or gain of phenotype, will allow gene function to be determined.

In terms of *Leishmania* and *T. cruzi*, the use of classical genetic analysis as a tool is clearly not yet an option, but the genome sequence information can be used to help determine whether the meiosis-related genes are expressed and whether they have a novel function or, in the case of *T. cruzi*, have a role in the system of laboratory genetic exchange. The development of genome-wide microsatellite markers in *Leishmania* species would have obvious applications for molecular epidemiological studies and also would provide very useful tools for characterizing inter-species hybrids, defining whether they are triploid or diploid, and determining whether they involve genetic exchange of all chromosome homologues.

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References

Berriman, M., Ghedin, E., Hertz-Fowler, C., Blandin, G., Renauld, H., Bartholomeu, D.C., Lennard, N.J., Caler, E., Hamlin, N.E., Haas, B., *et al.*, (2005). The genome of the African trypanosome *Trypanosoma brucei*. Science *309* (5733), 416–422.

Bingle, L.E., Eastlake, J.L., Bailey, M. and Gibson, W.C. (2001). A novel GFP approach for the analysis of genetic exchange in trypanosomes allowing the detection of mating events. Microbiology *147* (Pt 12), 3231–3240.

Chu, S., J. DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O. and Herskowitz, I. (1998). The transcriptional program of sporulation in budding yeast. Science *282* (5389), 699–705.

de Massy, B. (2003). Distribution of meiotic recombination sites. Trends Genet. *19* (9), 514–522.

Degan, R., Pospichal, H., Enyaru, J. and Jenni, L. (1995). Sexual compatibility among *Trypanosoma brucei* isolates from an epidemic area in southeastern Uganda. Parasitol. Res. *81*, 253–257.

El-Sayed, N.M., Ghedin, E., Song, J., MacLeod, A., Bringaud, F., Larkin, C., Wanless, D., Peterson, J., Hou, L., Taylor, S., *et al.*, (2003). The sequence and analysis of *Trypanosoma brucei* chromosome II. Nucleic Acids Res. *31* (16), 4856–4863.

El-Sayed, N.M., Myler, P J., Bartholomeu, D.C., Nilsson, D., Aggarwal, G., Tran, A.N., Ghedin, E., Worthey, E.A., Delcher, A.L., Blandin, G., *et al.*, (2005). The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. Science *309* (5733), 409–415.

El-Sayed, N.M., Myler, P.J., Blandin, G., Berriman, M., Crabtree, J., Aggarwal, G., Caler, E., Renauld, H., Worthey, E. A., Hertz-Fowler, C., Ghedin, E., *et al.*, (2005). Comparative genomics of trypanosomatid parasitic protozoa. Science *309* (5733), 404–409.

Ersfeld, K. and Gull, K. (1997). Partitioning of large and minichromosomes in *Trypanosoma brucei*. Science *276* (5312), 611–614.

Gaunt, M.W., Yeo, M., Frame, I.A., Stothard, J.R., Carrasco, H.J., Taylor, M.C., Mena, S.S., Veazey, P., Miles, G.A., Acosta, N., de Arias, A.R. and Miles, M.A. (2003). Mechanism of genetic exchange in American trypanosomes. Nature *421*, 936–9.

Gerton, J.L., DeRisi, J., Shroff, R., Lichten, M., Brown, P. O. and Petes, T. D. (2000). Inaugural article: global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA. *97* (21), 11383–11390.

Gibson, W. (1995). The significance of genetic exchange in trypanosmes. Parasitol. Today *11*, 465–468.

Gibson, W. and Garside, L. (1990). Kinetoplast DNA minicircles are inherited from both parents in genetic hybrids of *Trypanosoma brucei*. Mol. Biochem. Parasitol. *42*, 45–54.

Gibson, W. and Stevens, J. (1999). Genetic Exchange in the Trypanosomatidae. Adv. Parasitol. *43*, 1–45.

Gibson, W., Winters, K., Mizen, G., Kearns, J. and Bailey, M. (1997). Intraclonal mating in *Trypanosoma brucei* is associated with outcrossing. Microbiology *143*, 909–920.

Gibson, W.C. (1989). Analysis of a genetic cross between *Trypanosoma brucei rhodesiense* and *T. b. brucei*. Parasitology *99*, 391–402.

Gibson, W.C. and M. Bailey (1994). Genetic exchange in *Trypanosoma brucei*: evidence for meiosis from analysis of a cross between drug-resistant transformants. Mol. Biochem. Parasitol. *64*, 241–252.

Gibson, W.C., Garside, L. and Bailey, M. (1992). Trisomy and chromosome size changes in hybrid trypanosomes from a genetic cross between *Trypanosoma brucei rhodesiense* and *T. b. brucei*. Mol. Biochem. Parasitol. *51*, 189–199.

Gibson, W.C. and Garside, L. H. (1991). Genetic exchange in *Trypanosoma brucei brucei*: variable chromosomal location of housekeeping genes in different trypanosome stocks. Mol. Biochem. Parasitol. *42*, 45–54.

Gibson, W.C., Kanmogne, G. and Bailey, M. (1995). A successful backcross in *Trypanosoma brucei*. Mol. Biochem. Parasitol. *69*, 101–110.

Gibson, W.C., Marshall, T.F.d.C. and Godfrey, D.G. (1980). Numerical analysis of enzyme polymorphism: a new approach to the epidemiology and taxonomy of trypanosomes of the subgenus Trypanozoon. Adv. Parasitol. *18*, 175–246.

Gibson, W.C. and Mizen, V.H. (1997). Heritability of the trait for human infectivity in genetic crosses of *Trypanosoma brucei* spp. Trans. R. Soc. Trop. Med. Hyg. *91*, 236–7.

Gibson, W.C. and Whittington, H. (1993). Genetic exchange in *Trypanosoma brucei*: Selection of hybrid trypanosomes by introduction of genes conferring drug resistance. Mol. Biochem. Parasitol. *60*, 19–26.

Gottesdiener, K., Garcia-Anoveros, J., Lee, M.G. and Van der Pleog, L.H.T. (1990). Chromosomal organization of the protozoan *T. brucei*. Mol. Cell Biol. *10*, 6079–6083.

Gull, K., Alsford, S. and Ersfeld, K. (1998). Segregation of minichromosomes in trypanosomes: implications for mitotic mechanisms. Trends Micobiol *6*, 319–23.

Hall, N., Berriman, M., Lennard, N.J., Harris, B.R., Hertz-Fowler, C., Bart-Delabesse, E.N., Gerrard, C.S., Atkin, R.J., Barron, A.J., Bowman, S., *et al.*, (2003). The DNA sequence of chromosome I of an African trypanosome: gene content, chromosome organization, recombination and polymorphism. Nucleic Acids Res. *31* (16), 4864–4873.

Hassold, T. J. (1986). Chromosome abnormalities in human reproductive wastage. *Trends Genet. 2*, 105–110.

Hide, G., Welburn, S. C., Tait, A. and Maudlin, I. (1994). Epidemiological relationships of *Trypanosoma brucei* stocks from South East Uganda: evidence for different population structures in human infective and non-human infective isolates. Parasitology *109*, 95–111.

Hope, M., MacLeod, A., Leech, V., Melville, S., Sasse, J., Tait, A. and Turner, C.M. (1999). Analysis of ploidy (in megabase chromosomes) in *Trypanosoma brucei* after genetic exchange. Mol. Biochem. Parasitol. *104* (1), 1–9.

Jenni, L., Marti, S., Schweizer, J., Betschart, B., F., L. P. R. W., Wells, J.M., Tait, A., Paindavoine, P., Pays, E. and Steinert, M. (1986). Hybrid formation between African trypanosomes during cyclical transmission. Nature *322*, 173–175.

Kooy, R.F., Hirumi, H., Moloo, S.K., Nantulya, V.M., Dukes, P., Van der Linden, P.M., Duijudan, W.A.L., Janse, C.J. and Overdulve, J.P. (1989). Evidence for diploidy in metacyclic forms of African trypanosomes. Proc. Nat. Acad. Sci. USA. *86*, 5469–5472.

Le Page, R.W.F., Wells, J.M., Prospero, T.D. and Sternberg, J. (1988). Genetic analysis of hybrid *T. brucei*. Current communications in molecular biology: molecular genetics of parasitic protozoa. M. J. Turner and D. Arnott.

Cold Spring Harbor, Cold Spring Harbor Laboratory Press: 65–71.

MacLeod, A., Tait, A. and Turner, C.M. (2001). The population genetics of *Trypanosoma brucei* and the origin of human infectivity. Phil. Trans. R. Soc. Lond. B Biol. Sci. *356* (1411), 1035–1044.

MacLeod, A., Tweedie, A., McLellan, S., Hope, M., Taylor, S., Cooper, A., Sweeney, L., Turner, C. M. and Tait, A. (2005). Allelic segregation and independent assortment in *T. brucei* crosses: proof that the genetic system is Mendelian and involves meiosis. Mol. Biochem. Parasitol. *143* (1), 12–9.

MacLeod, A., Tweedie, A., McLellan, S., Taylor, S., Hall, N., Berriman, M., El-Sayed, N. M., Hope, M., Turner, C. M. and Tait, A. (2005). The genetic map and comparative analysis with the physical map of *Trypanosoma brucei*. Nucleic Acids Res. *33* (21), 6688–6693.

MacLeod, A., Tweedie, A., Welburn, S. C., Maudlin, I., Turner, C. M. and Tait, A. (2000). Minisatellite marker analysis of *Trypanosoma brucei*: reconciliation of clonal, panmictic, and epidemic population genetic structures. Proc. Natl. Acad. Sci. USA. *97* (24), 13442–13447.

Maynard-Smith, J., Smith, N.H., O'Rourke, M. and Spratt, B.G. (1993). How clonal are bacteria? Proc. Natl. Acad. Sci. USA. *90*, 4384–4388.

Melville, S.E., Gerrard, C. S. and Blackwell, J.M. (1999). Multiple causes of size variation in the diploid megabase chromosomes of African trypanosomes. Chromo Res. *7*, 191–203.

Melville, S.E., Leech, V., Gerrard, C.S., Tait, A. and Blackwell, J.M. (1998). The molecular karyotype of megabase chromosomes of *Trypanosoma brucei* and the assignment of chromosome markers. Mol. Biochem. Parasit *94*, 155–173.

Mu, J., Awadalla, P., Duan, J., McGee, K. M., Joy, D.A., McVean, G.A. and Su, X.Z. (2005). Recombination hotspots and population structure in *Plasmodium falciparum*. PLoS Biol. *3* (10), e335.

Obado, S.O., Taylor, M.C., Wilkinson, S.R., Bromley, E.V. and Kelly, J.M. (2005). Functional mapping of a trypanosome centromere by chromosome fragmentation identifies a 16-kb GC-rich transcriptional strand-switch domain as a major feature. Genome Res. *15* (1), 36–43.

Paindavoine, P., Zampetti-Bosseler, F., Pays, E., Schweizer, J., Guyaux, M., Jenni, L. and Steinert, M. (1986). Trypanosome hybrids generated in tsetse flies by nuclear fission. EMBO J. *5*, 3631–3636.

Panton, L.J., Tesh, R.B., Nadeau, K.C. and Beverley, S.M. (1991). A test for genetic exchange in mixed infections of *Leishmania major* in the sand fly *Phlebotomus papatasi*. J. Protozool *38* (3), 224–8.

Petes, T.D. (2001). Meiotic recombination hot spots and cold spots. Nature Rev. Genet. *2* (5), 360–369.

Roeder, G.S. (1997). Meiotic chromosomes: it takes two to tango. Genes Dev. *11* (20), 2600–2621.

Ross-Macdonald, P. and Roeder, G.S. (1994). Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell *79* (6), 1069–1080.

Schweizer, J. and Jenni, L. (1991). Hybrid formation in the life cycle of *Trypanosoma brucei*: detection of hybrid trypanosomes in a mid-gut derived isolate. Acta Tropica *48*, 319–321.

Schweizer, J., Pospichal, H., Hide, G., Buchannan, N., Tait, A. and Jenni, L. (1994). Analysis of a new genetic cross between two East African *Trypanosoma brucei* clones. Parasitol. *109*, 83–93.

Schweizer, Tait, A. and Jenni, L. (1988). The timing and frequency of hybrid formation in African trypanosomes during cyclical transmission. Parasitol. Res. *75*, 98–101.

Sternberg, J. and Tait, A. (1990). Genetic exchange in *Trypanosoma brucei*. Trends Genet. *6*, 317–322.

Sternberg, J., Tait, A., Haley, S., Schweizer, J. and Jenni, L. (1987). The inheritance of chromosomal and kinetoplast variants in *T. brucei* spp. Molecular strategies of parasitic invasion, UCLA symposia on molecular and cellular biology. Agabian, N. Goodman, H. and Nogeira, N. New York, Alan R. Liss, pp. 253–258.

Sternberg, J., Tait, A., Haley, S., Wells, J.M., Le Page, R. F. W., Schweizer, J. and Jenni, L. (1988). Gene exchange in African trypanosomes: characterization of a new hybrid genotype. Mol. Biochem. Parasitol. *27*, 191–200.

Sternberg, J., Turner, C.M.R., Wells, J.M., Ranford-Cartwright, L.C., Le Page, R.F.W. and Tait, A. (1989). Gene exchange in African trypanosomes: frequency and allelic segregation. Mol. Biochem. Parasitol. *34*, 269–280.

Su, X.-Z., Ferdig, M.T., Huang, Y., Huynh, C.Q., Lui, A., You, J., Wootton, J. C. and Wellems, T.E. (1999). A genetic map and recombination parameters of the human malaria parasite *Plasmodium falciparum*. Science *286*, 1351–3.

Tait, A. (1980). Evidence for diploidy and mating in trypanosomes. Nature *287*, 536–538.

Tait, A. (1983). Sexual processes in the Kinetoplastida. Parasitology *86*, 29–57.

Tait, A. (2000). *Trypanosomiasis*. London, Arnold.

Tait, A., Buchanan, N., Hide, G. and Turner, C. M. R. (1996). Self-fertilization in *Trypanosoma brucei*. Mol. Biochem. Parasitol. *76*, 31–42.

Tait, A., Masiga, D., Ouma, J., MacLeod, A., Sasse, J., Melville, S., Lindegard, G., McIntosh, A. and Turner, M. (2002). Genetic analysis of phenotype in *Trypanosoma brucei*: a classical approach to potentially complex traits. Phil. Tran.s R. Soc. Lond. B Biol. Sci. *357* (1417), 89–99.

Tait, A., Sternberg, J. and Turner, C. M. R. (1988). Genetic exchange in *Trypanosoma brucei*: allelic segregation and reassortment. Mol. Genet. Parasitic Protozoa. Turner, M.J. and Arnot, D. Cold Spring Harbor Laboratory.

Tait, A., Turner, C.M.R., Le Page, R.W.F. and Wells, J.M. (1989). Genetic evidence that metacyclic forms of *Trypanosoma brucei* are diploid. Mol. Biochem. Parasitol. *37*, 247–255.

Tibayrenc, M. and Ayala, F. J. (2002). The clonal theory of parasitic protozoa: 12 years on. Trends Parasitol. *18* (9), 405–410.

Tibayrenc, Kjellberg, F. and Ayala, F.J. (1990). A clonal theory of parasitic protozoa: the population structures of *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas* and *Trypanosoma* and their medical and taxonomic consequences. Proc. Natl. Acad. Sci. USA *87*, 2414–2418.

Turner, C.M.R., Hide, G., Buchanan, N. and Tait, A. (1995). *Trypanosoma brucei*: Inheritance of kinetoplast DNA maxicircles in a genetic cross and their segregation during vegetative growth. Exp. Parasitol. *80*, 234–241.

Turner, C.M.R., Sternberg, J., Buchanan, N., Smith, E., Hide, G. and Tait, A. (1990). Evidence that the mechanism of gene exchange in *Trypanosoma brucei* involves meiosis and syngamy. Parasitol. *101*, 377–386.

Tzung, K. W., Williams, R. M., Scherer, S., Federspiel, N., Jones, T., Hansen, N., Bivolarevic, V., Huizar, L., Komp, C., Surzycki, R., Tamse, R., Davis, R.W. and Agabian, N. (2001). Genomic evidence for a complete sexual cycle in Candida albicans. Proc. Natl. Acad. Sci. USA *98* (6), 3249–3253.

Van Den Abbeele, J., Claes, Y., Van Bockstaele, D., Le Ray, D. and Coosemans, M. (1999). *Trypanosoma brucei* spp. development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis. Parasitol. *118*, 469–478.

Van der Ploeg, L. H. T., Cornelissen, A. W. C. A., Barry, J.D. and Borst, P. (1984). Chromosomes of the kinetoplastida. EMBO J. *3*, 3109–3115.

Victoir, K. and Dujardin, J.C. (2002). How to succeed in parasitic life without sex? Asking Leishmania. Trends Parasitol. *18* (2), 81–85.

Walliker, D., Quakyi, I.A., Wellems, T.E., McCutchan, T.F., Szarfman, A., London, W.T., Corcoran, L.M., Burkot, T.R. and Carter, R. (1987). Genetic analysis of the human malaria parasite *Plasmodium falciparum*. Science *236* (4809), 1661–1666.

Wells, J.M., Prospero, T.D., Jenni, L. and Le Page, R. W. F. (1987). DNA contents and molecular karyotypes of hybrid *Trypanosoma brucei*. Mol. Biochem. Parasitol. *24*, 103–116.

Wickstead, B., Ersfeld, K. and Gull, K. (2003). The mitotic stability of the minichromosomes of *Trypanosoma brucei*. Mol. Biochem. Parasitol. *132* (2), 97–100.

Wickstead, B., Ersfeld, K. and Gull, K. (2004). The small chromosomes of *Trypanosoma brucei* involved in antigenic variation are constructed around repetitive palindromes. Genome Res. *14* (6), 1014–1024.

Zampetti-Bosseler, F., Schweizer, J., Pays, E., Jenni, L. and Steinert, M. (1986). Evidence for haploidy in metacyclic forms of *Trypanosoma brucei*. J. Cell Sci. *95*, 57–59.

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