Genome evolution in trypanosomatid parasites

ANDREW P. JACKSON*

*Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool,
Liverpool Science Park Ic2, 146 Brownlow Hill, Liverpool L3 5RF, UK*

SUMMARY

A decade of genome sequencing has transformed our understanding of how trypanosomatid parasites have evolved and provided fresh impetus to explaining the origins of parasitism in the Kinetoplastida. In this review, I will consider the many ways in which genome sequences have influenced our view of genomic reduction in trypanosomatids; how species-specific genes, and the genomic domains they occupy, have illuminated the innovations in trypanosomatid genomes; and how comparative genomics has exposed the molecular mechanisms responsible for innovation and adaptation to a parasitic lifestyle.

Key words: *Trypanosoma*, *Leishmania*, genome, evolution, phylogeny, parasitism.

INTRODUCTION

Trypanosomatids are unicellular flagellates and obligate parasites that infect various animals and plants. They include *Trypanosoma* and *Leishmania*, species of which cause potent vector-borne diseases in humans, livestock and wildlife; diseases that are responsible for substantial mortality and morbidity across the world. *Trypanosoma cruzi* causes Chagas disease in South and Central America; *Trypanosoma brucei* causes Human African Trypanosomiasis in sub-Saharan Africa (and, along with related species, a similar disease in livestock); while *Leishmania* spp. cause various forms of leishmaniasis in humans. Other species of *Trypanosoma* and *Leishmania* infect a wide range of vertebrate hosts, and all are transmitted by invertebrate vectors; predominantly these are biting insects, although some aquatic species are transmitted by leeches (Lom, 1979). *Phytomonas* spp. are plant parasites transmitted by phloem-sucking insects and are occasionally an agricultural problem in South and Central America (Camargo, 1999). Besides these dixenic (i.e. two-host) parasites that cycle between insect/leech and vertebrate/plant hosts, the trypanosomatids include various other genera, such as *Crithidia, Leptomonas, Herpetomonas, Angomonas* and *Strigomonas* that are cosmopolitan, monoxenic (i.e. one host) parasites of insects (Maslov *et al*. 2013). The diverse associations of trypanosomatids indicate that the origin of parasitism is singular and ancient (Simpson *et al*. 2006).

* Corresponding author: Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, Liverpool Science Park Ic2, 146 Brownlow Hill, Liverpool L3 5RF, UK. E-mail: a.p.jackson@liv.ac.uk

The order Trypanosomatidae is one part of the phylum Kinetoplastida; most other Kinetoplastids live freely or as commensals in marine, terrestrial and aquatic environments. The current consensus on Kinetoplastid phylogeny is summarized in Fig. 1; trypanosomatids are monophyletic and the sister clade to eubodonids (Callahan *et al*. 2002; Simpson *et al*. 2004; Moreira *et al*. 2004; von der Heyden *et al*. 2004; Deschamps *et al*. 2011). The closest known relative among eubodonids is *Bodo saltans*, a free-living bacteriovore of terrestrial and fresh-water microbiota. Hence, the phylogeny indicates that parasitism in trypanosomatids had a single origin; although the position of the fish parasites *Cryptobia* spp. and *Ichthyobodo* spp. show that parasitism has appeared on other occasions within the Kinetoplastida (Simpson *et al*. 2006; von der Heyden *et al*. 2004). This is the context in which I review the contribution of trypanosomatid genome sequences to our understanding of how parasitism evolved and subsequently diversified.

Since the publication of the 'TriTryp' genome sequences for *T. cruzi, T. brucei* and *Leishmania major* in 2005 (Berriman *et al*. 2005; El-Sayed *et al*. 2005*a*; Ivens *et al*. 2005), there has been much comparative analysis of these seminal resources. They have been complemented by transcriptomic (Holzer *et al*. 2006; Leifso *et al*. 2007; Saxena *et al*. 2007; Rochette *et al*. 2008, 2009; Alcolea *et al*. 2009, 2010; Depledge *et al*. 2009; Jensen *et al*. 2009; Kabani *et al*. 2009; Minning *et al*. 2009; Veitch *et al*. 2010; Adaui *et al*. 2011) and proteomic analyses (Atwood *et al*. 2005; Rosenzweig *et al*. 2008*a,b*; Alcolea *et al*. 2011; Eyford *et al*. 2011; Urbaniak *et al*. 2012; Butter *et al*. 2013) of gene expression at various life-cycle stages. Genome sequences for additional species of *Trypanosoma* (Jackson *et al*. 2009, 2012), *Leishmania*
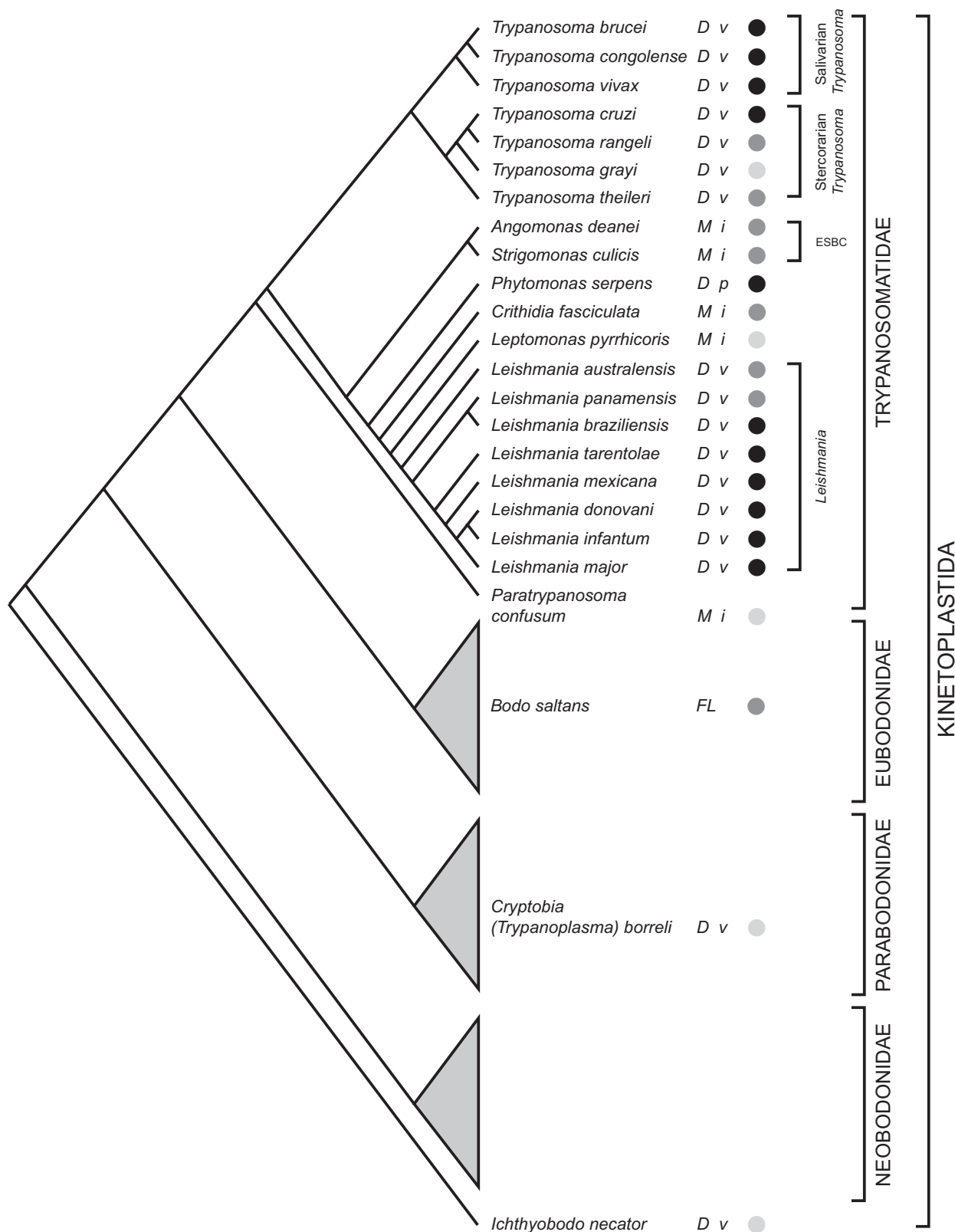
Fig. 1. Kinetoplastid phylogeny. A cladogram depicting the current consensus on Kinetoplastid phylogenetic relationships (adapted from von der Heyden *et al*. 2004; Simpson *et al*. 2004, 2006; Deschamps *et al*. 2011; Flegontov *et al*. 2013). Each bodonid order is shown as a grey triangle, representing an indeterminate, but large, number of species. The status of genome sequencing projects for each named species is indicated by filled circles (black: finished; dark grey: unfinished draft; light grey: sequencing in progress). The life cycle of each species is indicated (*D*: dixenic; *M*: monoxenic; *FL*: free-living), as well as the host type(s) (*v*: vertebrate; *i*: insect; *p*: plant). ESBC: 'Endosymbiont-bearing clade'.

(Peacock *et al.* 2007; Downing *et al.* 2011; Rogers *et al.* 2011; Raymond *et al.* 2012; Real *et al.* 2013) and *Phytomonas* (Porcel *et al.* 2014) have been produced, with several more in progress (see Fig. 1).

Comparison of the Tritryp genomes showed that both gene order and gene repertoire are broadly conserved within chromosomal cores (El-Sayed *et al.* 2005*b*). It is generally thought that the considerable co-linearity displayed by trypanosomatid genomes, despite their apparently ancient divergences, reflects strong and fundamental selective constraints on genome structure (Ghedin *et al.* 2004). Analysis of gene order conservation across Eukaryotic genomes indicates that highly conserved gene pairs are retained for both functional and transcriptional regulation (Dávila-López *et al.* 2010). While there is little to suggest that the conserved proximity of genes in trypanosomatids reflects their shared or related functions, it has been suggested that their polycistronic organization necessitates the co-directionality of replication and transcription (Ghedin *et al.* 2004), and that this structural peculiarity of trypanosomatids (the cause of which remains unsolved), is responsible for the strong purifying selection that maintains gene order.

Beyond the chromosomal cores, within sub-telomeric regions for instance, there are numerous species-specific features (El-Sayed *et al.* 2005*b*). From the outset it was appreciated that these genes are very often associated with disease mechanisms (El-Sayed *et al.* 2005*b*) and are the basis for the distinctive cell surface architectures displayed by each parasite (Acosta-Serrano *et al.* 2007; Handman *et al.* 2008). Thus, after 10 years of comparative and experimental analysis of these genomes the principal genomic features that distinguish the stem trypanosomatid lineages, and which are most likely to have been instrumental in the evolution of parasitism, are apparent.

GENOMIC REDUCTION

Parasites were once thought to be 'degenerate'; while this view is no longer prevalent, it remains intuitive that some characters vital to free-living organisms, but no longer necessary for parasites within a host environment, are lost when the selection pressure to retain them is removed. Hence, we expect phenotypic reduction, which is often observed of parasites, to be reflected in genomic reduction. For example, the genomes of both schistosomes and cestodes, which are phenotypically reduced relative to free-living platyhelminthes, lack elements of canonical metazoan metabolism and developmental regulation (Berriman *et al.* 2009; Tsai *et al.* 2013). Genome reduction reaches its apogee in the microsporidian parasites, which in some cases have reduced their genomes to the physiological minimum required for life, and this corresponds with their extreme host dependence (Nakjang *et al.* 2013). At such extremes, we also observe physical compaction of the genome, in addition to the loss of genes (Keeling and Slamovits, 2005).

Trypanosomatids do not appear to be reduced physically; the size of their genomes (25–35 mb in the haploid state) and the gene density (2·8–4·6 Kb/gene) is comparable with free-living unicellular eukaryotes, for instance *Saccharomyces cerevisiae* (12·5 mb/2·09 Kb/gene) and *Dictyostelium discoideum* (33·8 mb/2·72 Kb/gene). However, trypanosomatid genomes might still be functionally reduced, having lost genes essential to free-living Kinetoplastids.

Before the advent of genome sequences, it was known that trypanosomatids lacked certain common metabolic capabilities. For example, they are auxotrophic for pteridine and folate, which are essential co-factors in macromolecule biosynthesis, because they lack the ability to synthesize tetrahydrobiopterin (Beck and Ullman, 1990, Bello *et al.* 1994, Nare *et al.* 1997; Ouellette *et al.* 2002). Similarly, they must scavenge haem from their hosts (or obtain it from bacterial endosymbionts; Alves *et al.* 2011), because they lack a native haem biosynthesis pathway (Chang *et al.* 1975; Korený *et al.* 2010). Trypanosomatids are also auxotrophic for purines (Marr *et al.* 1978; Gutteridge and Gaborak, 1979), vital in the biosynthesis of nucleic acids and energy metabolism. Other aspects of model eukaryotic physiology are also absent, for example, a system of redox homoeostasis based on catalase and glutathione reductase. Instead, trypanosomatids rely on a unique thiol-based redox metabolism based on trypanothione for the deactivation of oxidizing agents (Oza *et al.* 2005; Krauth-Siegel and Comini, 2008; Comini and Flohé, 2013). The initial Tritryp comparison showed that trypanosomatids do not possess receptor-linked tyrosine kinases (Parsons *et al.* 2005), canonical mitochondrial import systems (Pusnik *et al.* 2009), known telomere end binding proteins such as POT1 (Lira *et al.* 2007), certain genes that regulate autophagy (Herman *et al.* 2006) and others controlling apoptosis (i.e. TNF-related family receptors, Bcl-2 family members and caspases; Smirlis *et al.* 2010).

The question relating to these and any other missing features is whether they represent evolutionary losses, or instead, reflect the branching position of the Kinetoplastida in the eukaryotic phylogeny. It may be that certain widely conserved genes are absent from trypanosomatids because Kinetoplastids separated from other eukaryotic lineages early in evolutionary history and before those genes evolved. Furthermore, it could be that we have systematically underestimated genomic and physiological diversity in eukaryotes, and the apparent deficiencies of trypanosomatids reflect a biased perception based on a narrow sampling of animal and plant genomes. In short, the absence of 'typical' features from
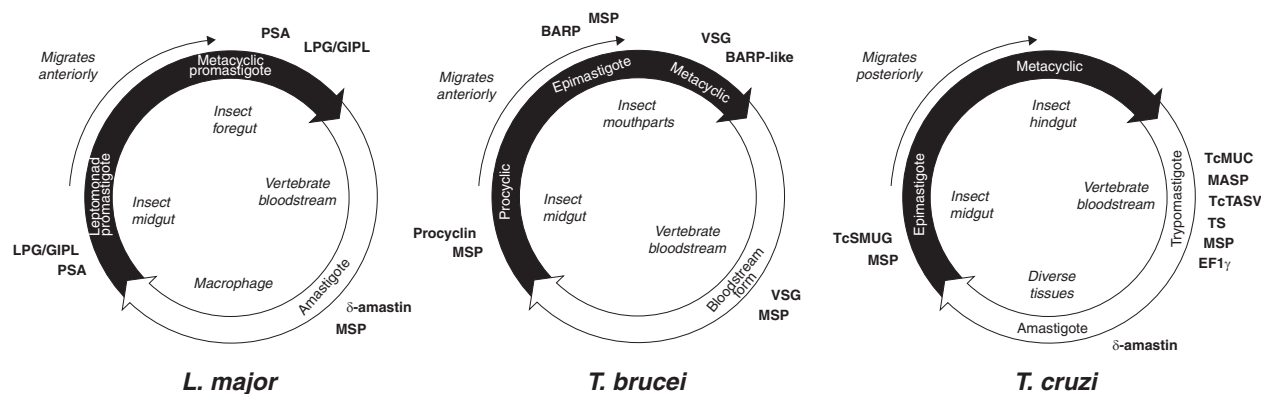
Fig. 2. Trypanosomatid life cycles. Each circle represents the movement of parasites between insect (black) and vertebrate (white) hosts, showing the transition of parasite life stages, the position of each stage within the hosts and the timing of expression of cell surface-expressed protein families mentioned in the text. Note that for *T. brucei* and *L. major*, the parasites move anteriorly from the insect gut as they develop, while *T. cruzi* migrates posteriorly as it prepares for transmission into the vertebrate.

trypanosomatids need not represent evolutionary loss. In fact, detailed comparisons in the years following publication of the Tritryp genome sequences showed that, while trypanosomatids often lack some conserved features and have numerous clade-specific derivations, they are nevertheless comparable to free-living protists in the number and diversity of protein kinases (Parsons *et al*. 2005; Bahia *et al*. 2009), phosphatases (Brenchley *et al*. 2007), GTPases and other genes involved in intracellular trafficking (Field, 2005; Field *et al*. 2007) and DNA helicases (Gargantini *et al*. 2012).

In summary, these genomes are not reduced in size or substantially reduced in function. While trypanosomatids employ unique solutions in redox homoeostasis, mitochondrial protein import and telomere regulation, they nonetheless have a broadly typical eukaryotic physiology. Where there are disparities, it is not clear whether these genes were lost or never existed and this will only become clear after we have sampled the genomes of free-living Kinetoplastids for comparison. Instead, there is abundant evidence that trypanosomatid genomes have expanded during their evolution both physically, through the evolution of sub-telomeres and accessory chromosomes, and functionally, with the acquisition of new genes through duplication and horizontal gene transfer.

GENOMIC INNOVATION: SPECIES-SPECIFIC GENE FAMILIES

Trypanosomatid cell surfaces include various polymorphic proteins combined with diverse glycolipid conjugates (Ferguson, 1997). These structures are enigmatic and their origins are mysterious because they are not seen in other organisms; indeed, the highly abundant cell-surface glycoproteins of *T. brucei, T. cruzi* and *L. major* are mutually exclusive, making it very hard to infer what the ancestral cell surface looked like (El-Sayed *et al*.

2005*b*). The Tritryp genomes revealed the genes that encode these surface features and their non-random distribution in the genome, which has been reviewed in detail elsewhere (Acosta-Serrano *et al*. 2007; Handman *et al*. 2008; De Pablos and Osuna, 2012). These cell surface proteins attract considerable interest because they are implicated in disease, virulence and mechanisms of pathogenesis (De Pablos and Osuna, 2012). Species-specific genes provide the clearest insight into genomic innovations associated with parasitism and the multi-copy gene families that encode these cell surface proteins dominate such species-specific genes in comparative analyses (El-Sayed *et al*. 2005*b*).

The life cycles of the Tritryp species and the points at which species-specific cell surface proteins are expressed are shown in Fig. 2. Species-specific genes in *T. cruzi* are dominated by gene families that encode the mucin-based surface coat during its trypomastigote stage (Cerqueira *et al*. 2008; Nakayasu *et al*. 2009; De Pablos and Osuna, 2012); primarily mucins (TcMUC; Acosta-Serrano *et al*. 2001; Buscaglia *et al*. 2006) and trans-sialidases (TS; Kim *et al*. 2005; Montagna *et al*. 2006; Freitas *et al*. 2011; Oppezzo *et al*. 2011; Ammar *et al*. 2013; Oliveira *et al*. 2014), but also a 'dispersed gene family' (DGF-1; El-Sayed *et al*. 2005*b*; Kawashita *et al*. 2009; Lander *et al*. 2010), the mucin-associated surface protein family (MASP; El-Sayed *et al*. 2005*b*; Bartholomeu *et al*. 2009; dos Santos *et al*. 2012), and the *T. cruzi* Trypomastigote Alanine, Serine and Valine-rich proteins (TcTASV; García *et al*. 2010; Bernabó *et al*. 2013). Gene families specifically expressed in the other life stages include amastin in the intracellular amastigote stage, and *T. cruzi* Small MUcin-like Genes (TcSMUG; Urban *et al*. 2011) in the replicative epimastigote. In addition to these developmentally regulated, surface-expressed gene families, expansions of Retrotransposon Hotspot (RHS) genes and Elongation Factor 1 gamma

(EF1γ) genes are prominent innovations of the *T. cruzi* genome.

In *T. brucei*, species-specific genes are dominated by those encoding the Variant Surface Glycoproteins (VSG) that form the surface glycocalyx of all salivarian trypanosomes during their bloodstream stage in the mammal host (Hutchinson *et al*. 2007; Marcello and Barry, 2007; Jackson *et al*. 2012; Weirather *et al*. 2012; Hall *et al*. 2013). Other species-specific genes like the Invariant Surface Glycoprotein (ISG) genes (Jackson *et al*. 1993; Ziegelbauer and Overath, 1993) and Expression-Site Associated Genes (ESAGs; Pays *et al*. 2001; see below) are also preferentially expressed in the bloodstream stage. In the insect host, species-specific genes are dominated by procyclin, encoding the major surface glycoprotein of the procyclic stage while in the insect midgut (Roditi *et al*. 1998; Berriman *et al*. 2005), and the Brucei Alanine-Rich Protein (BARP) that, along with related forms, is specifically expressed by the epimastigote and metacyclic stages while in the insect mouthparts (Urwyler *et al*. 2007; Jackson *et al*. 2013).

The cell surface of *Leishmania* is dominated by non-protein lipophosphoglycan (LPG) and glycoinositolphospholipid (GIPL) molecules (de Assis *et al*. 2012). The LPG/GIPL coat is complemented by species-specific, multi-copy proteins such as δ-amastin, which is specifically expressed during the intracellular amastigote stage (Rochette *et al*. 2005). While its function is unknown, the evolution of δ-amastin is thought to be an adaptation for infection of, or survival within, macrophages since it is absent from monoxenic species (*Crithidia* and *Leptomonas* spp.) lacking a vertebrate stage (Jackson, 2010) and less abundant in *Leishmania* species that do not routinely infect macrophages (Raymond *et al*. 2012). Furthermore, a parallel expansion of δ-amastin has occurred in *T. cruzi*, which also has an amastigote stage, and this is associated with virulence (Kangussu-Marcolino *et al*. 2013). Another *Leishmania*-specific family, tuzin (Ivens *et al*. 2005), is linked to δ-amastin loci physically and phylogenetically (Jackson, 2010); hence, tuzin might be involved in the same adaptation. In the insect life stage, the promastigote surface antigen (PSA or gp46) is preferentially expressed in metacyclic promastigotes (Handman *et al*. 1995) and is encoded by a diverse gene family in human-infecting species (Devault and Bañuls, 2008). Also specifically expressed in metacyclics are the HASP (Hydrophilic Acylated Surface Protein) and SHERP (Small Hydrophilic ER-associated Protein) gene families (Depledge *et al*. 2010; Sádlová *et al*. 2010).

While the precise functions of these enigmatic gene families are unknown, several contribute to parasite fitness. This may be because they initiate infection, for instance, the TcMUC and TS proteins interact to transfer host sialic acid residues to parasite mucins, which is essential for attachment and invasion by *T. cruzi* trypomastigotes (Acosta-Serrano *et al*. 2001; Oliveira *et al*. 2014). Other cell surface protein families are essential for parasite development and transmission through the insect host; for example, HASP and SHERP are required for *L. major* to form infective metacyclics while in the insect foregut (Sádlová *et al*. 2010). However, given their prominent roles at the cell surface, most of these species-specific proteins are likely to have immunological roles. These may be in suppressing innate responses, for example by degrading antimicrobial peptides or other effectors of complement-mediated lysis, as has been shown for PSA (Lincoln *et al*. 2004), or in manipulating cell-mediated immune responses. For instance, TcMUC represses T-cell expansion and cytokine production (Nunes *et al*. 2013). Salivarian trypanosomes employ VSG in antigenic variation, and have evolved sophisticated mechanisms for regulating VSG expression (see below). The abundance and variety of TcMUC, TS and MASP genes has led some to suggest that a subtler form of antigenic variation operates in *T. cruzi* as well (Buscaglia *et al*. 2004, 2006; dos Santos *et al*. 2012).

GENOMIC INNOVATION: CONTINGENCY ZONES

Trypanosomatids have substantially modified the genome to accommodate these abundant families of cell-surface effectors, by creating genomic sub-domains segregated from the core genome by distance, but also by sequence composition and epigenetic modification (Figueiredo *et al*. 2009; Rudenko, 2010). We can call these sub-domains 'contingency zones' because they provide the environment for flexible expression of what are known as contingency genes (Deitsch *et al*. 1997). In this trypanosomatids are not alone; diverse parasites possess polymorphic effector protein families that display specialized expression profiles across a wide range of physiological conditions (Deitsch *et al*. 1997; Kissinger and DeBarry, 2011). It has often been observed that contingency genes aggregate towards the telomeres, a position that promotes both the specific regulation of their expression and their diversification through recombination and gene duplication (Barry *et al*. 2003; Kissinger and DeBarry, 2011). Thus, both *T. brucei* and *T. cruzi* have expanded sub-telomeric regions to contain and regulate their diverse contingency genes (Berriman *et al*. 2005; El-sayed *et al*. 2005*a*,*b*; Moraes Barros *et al*. 2012). It is likely that the strand-switch regions that occur between polycistrons on trypanosomatid chromosomes also serve as incubators of novelty, since they often harbour species-specific genes (Peacock *et al*. 2007; Jackson *et al*. 2009).

Perhaps the best example of structural innovation in trypanosomatid genomes is the VSG expression site (ES) in *T. brucei*. African trypanosomes evade the

humoral immune response by periodically switching the VSG monolayer that masks their cell surfaces. This demands that only a single VSG is expressed at a time, while all others are silenced (i.e. monoallelic expression). The function of the ES is to ensure monoallelic expression by providing a dedicated locus for VSG transcription. Thus, the active VSG is transcribed solely from one of several, alternative ESs and antigenic switching occurs when a different VSG from among the many hundreds of silent, sub-telomeric loci, replaces the ES copy through ectopic gene conversion, or by activating an alternative ES (Horn and McCulloch, 2010; Rudenko, 2011). Analysis of ES sequences from several *T. brucei* strains has identified a canonical ES structure (Graham *et al*. 1999; Berriman *et al*. 2002; Hertz-Fowler *et al*. 2008), which includes not only the VSG and repeat sequences required to promote recombination with sub-telomeric VSG loci, but also the ESAGs (reviewed in Pays *et al*. 2001; McCulloch and Horn, 2009). The functions of most ESAGs are unclear; however, all are transcribed preferentially in the bloodstream stage (Jensen *et al*. 2009; Siegel *et al*. 2010; Veitch *et al*. 2010) and it is known that they are *T. brucei*-specific innovations, often derived from conserved gene families with pre-existing cell surface roles (Barker *et al*. 2008; Barnwell *et al*. 2010; Salmon *et al*. 2012; Jackson *et al*. 2013). Hence, it may be that they support antigenic variation or that the specific regulatory environment of the ES has been exploited secondarily to up-regulate proteins with established and diverse roles during the bloodstream stage.

GENOMIC INNOVATION: THE MAJOR SURFACE PROTEASES

Alongside the many species-specific cell surface proteins, there is one family conserved in all trypansomatid genomes that must have experienced substantial evolution since the origin of parasitism. The Major Surface Protease (MSP) gene family encode a range of metalloproteases that are implicated in various aspects of pathogenesis and virulence in *Leishmania* (Yao, 2010). MSP subverts the normal host defensive mechanisms by degrading components of immune cell signalling pathways (Gomez *et al*. 2009; Hallé *et al*. 2009; Contreras *et al*. 2010), and suppresses other aspects of innate immunity (Kulkarni *et al*. 2006; Lieke *et al*. 2008). In *Trypanosoma*, MSP is equally abundant in gene copy number and protein abundance but its function is less well understood; it is known to remove the VSG coat from the *T. brucei* surface during differentiation into the procyclic form (PCF) (Grandgenett *et al*. 2007) and is thought to have a role in cell invasion by *T. cruzi* (Cuevas *et al*. 2003; Kulkarni *et al*. 2009). As it is present in all trypanosomatids, we can infer the diversification of MSP from its

phylogeny, and this too indicates that MSP has been instrumental in parasite adaptation.

The MSP phylogeny is described in [Fig. 3]. It shows how, beginning from a much smaller gene repertoire, MSP has differentiated into distinct clades in both *Leishmania* and *Trypanosoma* (Victoir *et al*. 2005; Ma *et al*. 2011); each clade is associated with a conserved locus, and we know that some of these distinct lineages are developmentally regulated (Yao, 2010). For instance, MSP-A and MSP-C are up-regulated in bloodstream form (BSF) *T. brucei*, while MSP-B is predominantly seen in the procyclic form (LaCount *et al*. 2003; Urbaniak *et al*. 2012). Hence, the trypanosomatids have elaborated their MSP repertoire by creating new loci at least in part to regulate function during the life cycle. Moreover, these different forms have been duplicated to create multiple isoforms, often in species-specific ways; for instance, MSP-C is polymorphic in *Trypanosoma vivax* while single copy in other salivarian species, and the single-copy MSP gene found on chromosome 28 in *Leishmania* has been greatly expanded in *Phytomonas*. However, the phylogeny also demonstrates that MSP in *Leishmania* and *Trypanosoma* cluster by genus, and therefore, there is no orthologous MSP shared by all. Thus, MSP repertoires in *Leishmania* and *Trypanosoma* have evolved independently, and their similarities in genomic structure, developmental regulation and pathogenesis represent parallel evolution, reflecting a common need for diverse surface proteases throughout trypanosomatid diversification.

DEVELOPMENTAL REGULATION OF GENE EXPRESSION

Trypanosomatids display morphological plasticity that is often associated with developmental transition through a complex life cycle. This is important for the origins of parasitism but not an issue that comparative genomics can illuminate dramatically, without including a comparator lacking developmental complexity. The recent discovery of *Paratrypanosoma confusum* parasitizing the gut of a *Culex pipiens* mosquito strengthens the argument that the ancestral trypanosomatid was a monoxenic insect parasite, since *P. confusum* is a robust outgroup to all other trypanosomatids (Flegontov *et al*. 2013). As long as *P. confusum* has no second host, this shows that a dixenic life cycle has evolved on three separate occasions in *Trypanosoma, Leishmania* and *Phytomonas*. Trypanosomatids are capable of assuming multiple developmental forms and transition between forms coincides with passing between distinct environments, whether they are in different hosts or a single host, for example from the hindgut to the foregut of an insect. Experimental approaches are beginning to reveal the non-coding sequences (Bringaud *et al*. 2007; Holzer *et al*. 2008; Smith *et al*.
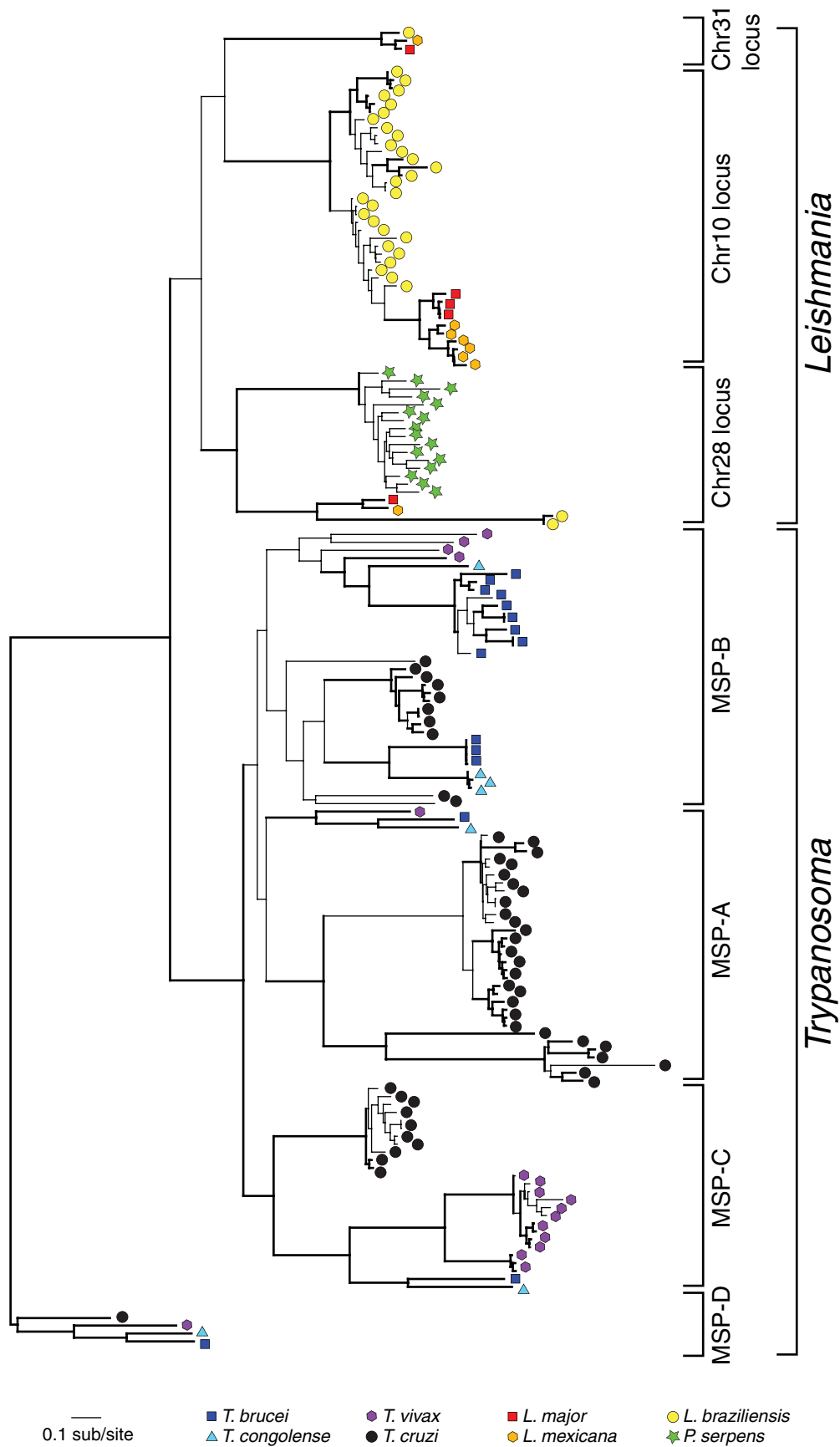
Fig. 3. Major Surface Protease (MSP) gene family phylogeny. This maximum likelihood phylogeny of MSP amino acid sequences sampled from completed genome sequences was estimated using PHYML and a LG+Γ model of amino acid substitution (Guindon *et al.* 2010). Node robustness was assessed with non-parametric bootstraps; branches with bootstrap support >75 are shown with bold lines. Sequences are labelled with coloured symbols, according to the key. The tree is mid-point rooted, which corresponds with the *Trypanosoma*-specific MSP-D locus (Marcoux *et al.* 2010).

2009; Li *et al.* 2012; Pastro *et al.* 2013) and RNA-binding proteins (reviewed in Kolev *et al.* 2014) that interact to regulate gene expression, as well as genes specifically required for differentiation from one life stage to another (Goldenberg and Avila, 2011; Kolev *et al.* 2012; Rico *et al.* 2013). Comparison of life-stage-specific transcriptomes (Holzer *et al.* 2006; Leifso *et al.* 2007; Saxena *et al.* 2007; Rochette *et al.* 2008, 2009; Alcolea *et al.* 2009, 2010; Depledge *et al.* 2009; Jensen *et al.* 2009; Kabani *et al.* 2009; Minning *et al.* 2009; Veitch *et al.* 2010; Adaui *et al.* 2011;) and proteomes (Atwood *et al.* 2005; Rosenzweig *et al.* 2008*a*,*b*; Alcolea *et al.* 2011; Urbaniak *et al.* 2012; Gunasekera *et al.* 2012; Butter *et al.* 2013) in various species have estimated the proportion of genes showing preferential expression in the insect or vertebrate stages to be between 2 and 44%; the breadth of these values reflects the diverse conditions and approaches employed. However, it is clear that a significant minority of genes are developmentally regulated. We can predict that this regulation is achieved with layers of interaction between genomic loci, mRNA, non-coding RNA and DNA and RNA-binding proteins. Hence, to understand the origins of complex life cycles we will need to compare the interaction networks of free-living, monoxenic and dixenic Kinetoplastids, and in this *P. confusum* and the free-living *Bodo saltans* will be instrumental.

MECHANISMS OF GENOMIC EVOLUTION: GENE DUPLICATION

Besides the genomic innovations themselves, comparative analysis also reveals the molecular mechanisms that create them. These evolutionary events range in size from single amino acid substitutions to chromosomal duplications, and include both coding and non-coding regions, but it is gene duplication above all that creates the raw material for evolutionary novelty (Ohno, 1970). After duplication, paralogs may acquire new functions (neofunctionalization), segregate existing functions (subfunctionalization) or lose function under mutation pressure (pseudogenization) (Lynch and Conery, 2000). Since developmental regulation of gene expression is widespread, it is unsurprising that many gene duplicates are distinguished in the timing or location of their expression. For example, TcMCA5 is an epimastigote-specific metacaspase implicated in programmed cell death of *T. cruzi* that has evolved from a constitutively expressed metacaspase gene family (Kosec *et al.* 2006). In *Leishmania*, Zinoviev *et al.* (2012) identified two functionally redundant RNA helicases that have evolved purely to perform the same role in insect and vertebrate stages respectively. By contrast, TcPRACA and TcPRACB are two paralogous proline racemases involved in immune-suppression by *T. cruzi* (Reina-San-Martín *et al.*

2000); here, function is segregated by location, TcPRACB being expressed intracellularly and TcPRACA secreted (Chamond *et al.* 2005).

Of course, the derivation of many gene duplicates may be multifactorial; in the example of proline racemases, secretion of TcPRACA may coincide with a new role in the differentiation of infective meta-cyclics (Chamond *et al.* 2005). Thus, it is difficult to unambiguously distinguish neofunctionalization from the segregation of the same function by time, space or substrate. However, the transferrin receptor (TFR) in *T. brucei*, which is required for salvaging haem from the host and is homologous to the VSG (Salmon *et al.* 1997), is one example. Recently, it was confirmed that the TFR had evolved from an a-type VSG in the ancestor of *T. brucei* and *Trypanosoma congolense*, and that, despite their homology, TFR and VSG genes do not recombine, supporting a functionally distinct role from the variant antigen repertoire (Jackson *et al.* 2012, 2013). As suggested above, the conspicuous abundance and diversity of certain *T. cruzi* gene families, such as TS, EF1γ and MSP, could indicate that these genes have secondarily evolved a novel role in immune evasion as a consequence of being at the cell surface for their pre-existing functions, i.e. to transfer sialic acid to TcMUC in the case of TS (Oliveira *et al.* 2014). Furthermore, many TS, EF1γ and MSP genes in *T. cruzi* are not predicted to encode proteins capable of their putative functions (El-Sayed *et al.* 2005). At first sight, this would appear to indicate frequent pseudogenization, yet a population of pseudogenes acquiring substitutions under neutral conditions would be expected to display a spectrum of mutational decay that is not seen (El-Sayed *et al.* 2005). This suggests that these genes may remain under purifying selection for another role, which could represent neofunctionalization.

The evolution of gene duplicates is particularly obvious in the abundant tandem gene arrays of trypanosomatid genomes. Tandem duplication is very common in trypanosomatids, perhaps as a means of increasing transcript abundance for highly expressed genes in the presence of polycistronic transcription. Comparative analysis of homologous arrays shows that tandem duplicates can evolve new functions, despite the propensity for concerted evolution of tandemly arrayed genes (Jackson, 2007*a*), and that this follows a consistent pattern of structural segregation. Figure 4 shows two examples of functional divergence within tandem gene arrays. The expression profiles of adenylate cyclase gene paralogues from the *rac* array of *Leishmania* spp. correspond with their position in the array. The 3′-most gene (*rac*-A) and the gene positioned upstream of *rac*-A in the array (*rac*-B1) are expressed specifically in the promastigote (Sanchez *et al.* 1995; Akopyants *et al.* 2004), while transcripts for the remaining copies are more abundant in the
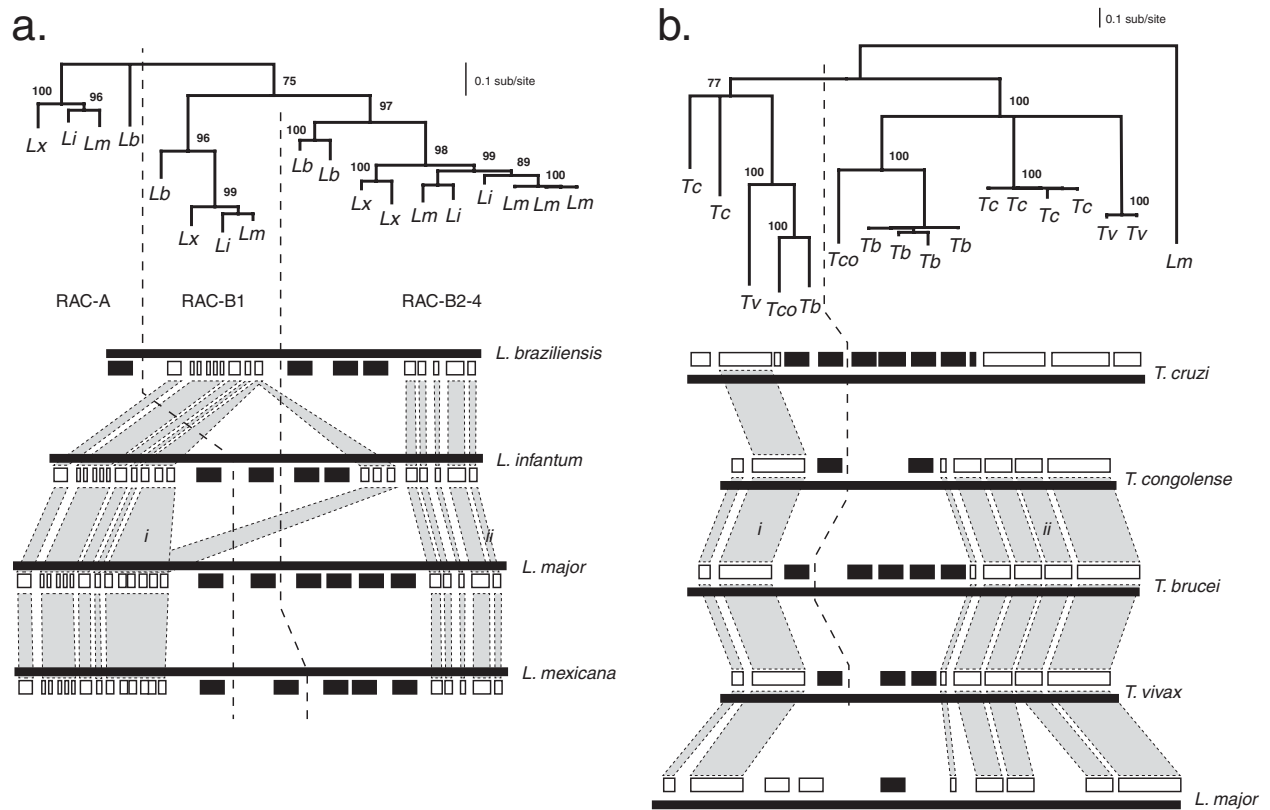
Fig. 4. Structural differentiation of paralogues in tandem gene arrays. a. Receptor-type adenylate cyclase (*rac*) genes in four *Leishmania* species. The *rac* array (i.e. LmjF.17·0200) is located at the extreme left-end of chromosome 17 in *L. major*; its conserved position is defined downstream by an EF1α gene array (*i*) and upstream by a metalloprotease (*ii*). The structure of the array in four species is depicted, *rac* genes are shown in black, and other loci are shown in white. Vertical grey shading represents homology between flanking loci, to demonstrate positional conservation. The dashed black line separates array positions that correspond with distinct clades in the phylogeny. A maximum likelihood phylogeny estimated from amino acid sequences using a LG + Γ model is shown, with non-parametric bootstraps applied to nodes where support is >75. Terminal nodes are labelled with species name initials. The tree is midpoint rooted. **b**. Cation transporter genes in four *Trypanosoma* species. The transporter array (i.e. Tb927.11.9000) is located on chromosome 11 in *T. brucei*; its conserved position is defined downstream by a palmitoyl acyltransferase 4 gene (*i*) and upstream by an EF1γ2 gene (*ii*). The phylogeny and genome comparison are as depicted in **a**. except that the tree is rooted with a single-copy orthology from *L. major*.

amastigote (Akopyants *et al.* 2004). Interestingly, *rac*-A and *rac*-B1 may have differentiated in a complementary fashion, since *rac*-B1 negatively regulates the activity of *rac*-A in the promastigote (Sanchez *et al.* 1995). In *Trypanosoma*, the 5′-most copy of a cation transporter gene array is preferentially expressed in the PCF (Jensen *et al.* 2009; Urbaniak *et al.* 2012) (indeed, it is essential to its growth; Alsford *et al.* 2011), while transcripts for all downstream copies are up-regulated in the bloodstream stage (Jensen *et al.* 2009; Veitch *et al.* 2010).

The phylogenies of these gene duplicates show that those gene copies that are functionally differentiated retain orthology across species (i.e. they cluster together despite being in different genomes), while undifferentiated copies cluster by species. This shows that gene duplicates that have diverged in their structures and expression for a novel function are preserved by selection over the course of trypanosomatid evolution, despite the pressure exerted by allelic gene conversion in these situations. In fact,

when tandem gene duplicates differentiate, this often occurs at either end of the array (Jackson, 2007*a*), even occurring in otherwise invariant arrays that are exposed to frequent gene conversion; for example, differentiation of the terminal 3′UTR in the β-tubulin array in *Leishmania* spp. has created a promastigote-specific β-tubulin isoform (Jackson *et al.* 2006).

Duplication events do not only affect individual genes. A 0·5 mb segmental duplication in *T. brucei* was identified that has created duplicons shared by chromosomes 4 and 8 (Jackson, 2007*b*). Originally, this region contained approximately 158 genes but subsequent deletions from either duplicon have returned many loci to their original copy number. However, 74 loci have been retained as paralogues in both duplicons. Comparison of their coding and flanking sequences indicated that substantial divergence had occurred and this was assumed to reflect functional divergence (Jackson, 2007*b*). They include CAP5.5, a cysteine peptidase essential for cell

Locus	Identifer:		Description	Sequence identity[#]:		Fold-change PCF/BSF:	
	Chr4	Chr8		CDS	3'UTR	Chr4	Chr8
6	Tb927.4.5340	Tb927.8.6980	hypothetical protein	0.824	0.523	5.62	0.66*
12	Tb927.4.5220	Tb927.8.7190	chromosome segregation protein-like	0.402	0.09	0.85	3.81**
13	Tb927.4.5190	Tb927.8.7290	hypothetical protein	0.751	0.418	17.54	0.05**
18	Tb927.4.5120	Tb927.8.7260	kinetoplast-associated protein	0.368	0.365	1.90	1.21*
23	Tb927.4.5010	Tb927.8.7410	calreticulin	0.987	0.714	0.29	0.41*
24	Tb927.4.5000	Tb927.8.7420	C2 calcium/lipid-binding region protein	0.995	0.966	0.55	0.60*
32	Tb927.4.4910	Tb927.8.7530	3,2-trans-enoyl-CoA isomerase	0.717	0.185	1.17	11.96*
36	Tb927.4.4870	Tb927.8.7600	amino acid transporter	0.702	0.182	0.15	10.76*
39	Tb927.4.4740	Tb927.8.7730	dihydroceramide synthase	0.730	0.207	0.41	24.53**
49	Tb927.4.4470	Tb927.8.7860	adenylate cyclase	0.592	0.131	0.12	0.06**
52	Tb927.4.4370	Tb927.8.8000	hypothetical protein	0.701	0.278	2.88	0.08**
53	Tb927.4.4360	Tb927.8.8020	monoglyceride lipase	0.783	0.199	1.01	0.81*
54	Tb927.4.4350	Tb927.8.8030	cell-surface protein	0.541	0.15	5.61	0.10*
56	Tb927.4.4310	Tb927.8.8050	spectrin repeat protein	0.274	0.204	1.06	0.74*
62	Tb927.4.4160	Tb927.8.8170	CheY-like domain protein	0.773	0.901	2.34	2.53*
65	Tb927.4.4130	Tb927.8.8280	prefoldin domain protein	0.948	0.658	0.96	1.03**
68	Tb927.4.4040	Tb927.8.8280	hypothetical protein	0.436	0.209	1.06	0.06
71	Tb927.4.3950	Tb927.8.8330	cytoskeleton-associated protein CAP5.5	0.748	0.258	18.8	0.05*

Fig. 5. Peptide abundance in procyclic form (PCF) and bloodstream form (BSF) *T. brucei* for selected paralogues resulting from a segmental duplication. 74 loci are present in two forms in *T. brucei* due to a segmental duplication. The loci listed here are those detected in proteomic analyses. Locus number and sequence identity values refer to the segmental duplication described in Jackson (2007*b*). Fold change in peptide abundance between PCF and BSF cells taken from * Urbaniak *et al*. (2012) or ** Butter *et al*. (2013). Preferential expression in PCF and BSF is indicated by blue and red shading respectively. Constitutive expression is indicated by orange shading.

morphogenesis, which has been shown to have two paralogues expressed specifically in the insect and vertebrate stages respectively (Hertz-Fowler *et al*. 2001; Olego-Fernandez *et al*. 2009). Figure 5 shows how recent proteomic evidence now confirms that several of the paralogues retained after segmental duplication have evolved stage-specific expression profiles, indicating subfunctionalization by life stage. Gene expression in trypanosomatids is largely regulated by sequences within the 3′ untranslated region (UTR) of transcripts (Vanhamme and Pays, 1995; Haile and Papadopoulou, 2007). Accordingly, it is the paralogous pairs with no sequence identity in their 3′ UTRs that have the greatest differences (loci #13, 36, 39, 49 and 71 in Fig. 5), while those paralogues with similar 3′ UTR sequences display similar abundance in both cases (loci #23, 24, 62 and 65 in Fig. 5).

MECHANISMS OF GENOMIC EVOLUTION: HORIZONTAL GENE TRANSFER

Horizontal gene transfer (HGT) is another mechanism by which many eukaryotic genomes have acquired new functionality. Berriman *et al*. (2005) identified 49 putative HGT from bacteria and other eukaryotes in trypanosomatid genomes. Confirming HGT rests on sound phylogenetic reconstruction; the most convincing cases are those where the donated gene is closely related to donor genes in unrelated genomes, and nested among these in a phylogeny. Some putative HGT in trypanosomatids achieve this, notably the haem-biosynthesis pathway, absent from *Trypanosoma* but partially restored in *Leishmania* and related genera through HGT of three genes (*hemF, hemG* and *hemH* encoding coproporphyrinogen oxidase, protoporphyrinogen oxidase and ferrochelatase, respectively) from gamma-proteobacteria. In phylogenies, *HemF-H* are nested among bacterial homologues and apart from related eukaryotic genes (Ivens *et al*. 2005, Korený *et al*. 2010). In salivarian trypanosomes, a phospholipase A1 (*PLA1*) gene is thought to have been acquired from proteobacteria (Richmond and Smith, 2007). In support of this, the PLA1 gene is absent from all other Kinetoplastids (indeed most other eukaryotes) and it nests among proteobacterial sequences in sequence comparisons. Moreover, the *PLA*1 locus (Tb927.1.4830) occurs precisely at the boundary between chromosomal core and sub-telomere in African trypanosome genomes, suggesting perhaps that it was recently transposed.

Other good examples of HGT include a cytosolic dihydroorotate dehydrogenase in the pyrimidine bio-synthetic pathway, which is unique to Kinetoplastids, and replaces the mitochondrial dihydroorotate dehydrogenase that is typical of euglenids and other eukaryotes. In phylogenies, the cytosolic genes are nested among bacterial taxa, while the mitochondrial genes form a eukaryotic clade (Annoura *et al*. 2005). Likewise, ornithine decarboxylase genes from salivarian trypanosomes do not cluster with homologues from other trypanosomatids, but instead they are nested among metazoan genes and are the sister taxon to ornithine decarboxylase from vertebrates (Steglich and Schaeffer, 2006). In fact, ornithine decarboxylase is known to be absent from *T. cruzi* (Carrillo *et al*. 1999), indicating that this HGT from vertebrates has restored function in African trypanosomes that was lost after the origin of *Trypanosoma*. However, since the African trypanosome genes are not nested *within* the vertebrate clade, we can rule out any recent transfer from contemporary hosts and suggest instead a more distant transfer from an ancient chordate.

In other cases of putative HGT the donated gene is not nested among would-be donors, just closest to them in phylogenies. Here, it is possible that the punctate distribution is due to lineage sorting, i.e. patchy inheritance of an ancestral lineage by daughter lineages. When, as is common, eukaryotic diversity is inadequately sampled, it is difficult to distinguish HGT and lineage sorting. For example, trypanosomatid genomes possess four superoxide dismutase genes required for antioxidant defence (*soda, sodb1, sodb2* and *sodc*), which localize to distinct cellular compartments (Dufernez *et al*. 2006). The four *sod* genes do not cluster together; *soda/sodc* cluster most closely to *Trichomonas vaginalis*, while *sodb1/sodb2* cluster with diverse eukaryotes (Dufernez *et al*. 2006). This suggests sorting of ancestral *sod* lineages but not necessarily HGT. Similarly, two metallo-carboxypeptidases (TcMCP-1 and TcMCP-2) in *T. cruzi* are found only in Kinetoplastids and prokaryotes, but homologues from the two taxa are sister clades, rather than nested (Niemirowicz *et al*. 2007). While the original study recognized the possibility of both HGT and lineage sorting, they rejected the latter due to the number of deletions this would require. These losses may not be necessary, however, if eukaryotic diversity were exhaustively sampled. Finally, an uncharacterized protein, META1, is up-regulated in *Leishmania* metacyclics and is homologous to a bacterial heat-inducible protein, itself similar to a component of the type III secretion system in *Shigella* (Puri *et al*. 2011). META1 is hypothesized to have evolved via HGT and may be involved in secretory processes in *Leishmania* since mutagenesis of select hydrophobic residues in META1 affects the secretion of the secreted acid phosphatase (Puri *et al*. 2011). However, META1 is not nested among bacterial sequences and, at this stage, the HGT hypothesis rests on it remaining absent from all other eukaryotes.

Although poor sampling continues to limit our ability to distinguish HGT and lineage sorting (Opperdoes and Michels, 2007), HGT has clearly contributed to trypanosomatid genomes; for example, substantial integration of genes from a bacterial endosymbiont has recently been demonstrated in *Angomonas deanei* (Alves *et al*. 2011). The role of HGT in the origins of parasitism will be clarified through comparison of trypanosomatids with free-living Kinetoplastids and other neglected unicellular eukaryotes, to reject the lineage sorting hypothesis and to confirm that the HGT is uniquely associated with parasites, such as *hemF-H* or *PLA1*, and not Kinetoplastids generally.

CONCLUSION

The genetic content of trypanosomatid genomes indicates that they have been elaborated relative to their common ancestor in terms of both physical structure and physiological capacity. Species-specific gene families, instrumental in cell surface architecture, are central to this history of innovation, and implicitly linked to the origins of complex life cycles and disease. By definition, these unique innovations are mutually exclusive, yet there are themes that cut across species. These gene families are functionally differentiated to perform multiple roles in different host environments through the parasite life cycle. They are positioned in sub-telomeres, tandem gene arrays or other contingency zones that perhaps promote regulatory flexibility and sequence diversity. Their sequences are diverse and often contain low complexity repeats that may promote greater diversity through recombination. In their phylogenies, these gene families display rapid turnover – the gain and loss of lineages – that hint at the importance of host-parasite interactions in genomic evolution. These themes, which would, in fact, apply to parasites of all kinds, suggest how each trypanosomatid lineage has used similar molecular mechanisms to meet the demands of transmission and survival. There are issues in comparative analysis we have not addressed, like protein-protein interactions, the regulatory roles of non-coding regions and regulatory proteins, genomic plasticity or indeed the $\sim$50% of trypanosomatid genes that have no known function. There are also some genes, such as the TcMUC family in *T. cruzi*, procyclin in *T. brucei* and *T. congolense*, and the HASP and SHERP families in *L. major*, that defy any explanation using a comparative approach, and which may have evolved *de novo* from non-coding regions. Yet, we have learned enough from the structure and content of trypanosomatid genomes to conclude that becoming parasitic was more an innovative and elaborative process, than one of loss and reduction. With the

addition of free-living Kinetoplastids to our comparative analyses, the mechanisms by which these enigmatic genomic adaptations for parasitism came about will be revealed.

REFERENCES

Acosta-Serrano, A., Almeida, I. C., Freitas-Junior, L. H., Yoshida, N. and Schenkman, S. (2001). The mucin-like glycoprotein super-family of *Trypanosoma cruzi*: structure and biological roles. *Molecular and Biochemical Parasitology* **114**, 143–150.

Acosta-Serrano, A., Hutchinson, C., Nakayasu, E. S., Almeida, I. and Carrington, M. (2007). Comparison and evolution of the surface architecture of trypanosomatid parasites. In *African Trypanosomes: After the Genome* (ed. Barry, J. D., McCulloch, R., Mottram, J. C. and Acosta-Serrano, A.), pp. 319–338. Horizon Bioscience, Wymondham, UK.

Adaui, V., Castillo, D., Zimic, M., Gutierrez, A., Decuypere, S., Vanaerschot, M., De Doncker, S., Schnorbusch, K., Maes, I., Van der Auwera, G., Maes, L., Llanos-Cuentas, A., Arevalo, J. and Dujardin, J. C. (2011). Comparative gene expression analysis throughout the life-cycle of *Leishmania braziliensis*: diversity of expression profiles among clinical isolates. *PLOS Neglected Tropical Diseases* **5**, e1021.

Akopyants, N. S., Matlib, R. S., Bukanova, E. N., Smeds, M. R., Brownstein, B. H., Stormo, G. D. and Beverley, S. M. (2004). Expression profiling using random genomic DNA microarrays identifies differentially expressed genes associated with three major developmental stages of the protozoan parasite *Leishmania major*. *Molecular and Biochemical Parasitology* **136**, 71–86.

Alcolea, P. J., Alonso, A., Sánchez-Gorostiaga, A., Moreno-Paz, M., Gómez, M. J., Ramos, I., Parro, V. and Larraga, V. (2009). Genome-wide analysis reveals increased levels of transcripts related with infectivity in peanut lectin non-agglutinated promastigotes of *Leishmania infantum*. *Genomics* **93**, 551–564.

Alcolea, P. J., Alonso, A., Gómez, M. J., Moreno, I., Domínguez, M., Parro, V. and Larraga, V. (2010). Transcriptomics throughout the life-cycle of *Leishmania infantum*: high down-regulation rate in the amastigote stage. *International Journal for Parasitology* **40**, 1497–1516.

Alcolea, P. J., Alonso, A. and Larraga, V. (2011). Proteome profiling of *Leishmania infantum* promastigotes. *Journal of Eukaryotic Microbiology* **58**, 352–358.

Alsford, S., Turner, D. J., Obado, S. O., Sanchez-Flores, A., Glover, L., Berriman, M., Hertz-Fowler, C. and Horn, D. (2011). High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome. *Genome Research* **21**, 915–924.

Alves, J. M., Voegtly, L., Matveyev, A. V., Lara, A. M., da Silva, F. M., Serrano, M. G., Buck, G. A., Teixeira, M. M. and Camargo, E. P. (2011). Identification and phylogenetic analysis of heme synthesis genes in trypanosomatids and their bacterial endosymbionts. *PLOS ONE* **6**, e23518.

Ammar, Z., Plazolles, N., Baltz, T. and Coustou, V. (2013). Identification of trans-sialidases as a common mediator of endothelial cell activation by African trypanosomes. *PLOS Pathogens* **9**, e1003710.

Annoura, T., Nara, T., Makiuchi, T., Hashimoto, T. and Aoki, T. (2005). The origin of dihydroorotate dehydrogenase genes of kinetoplastids, with special reference to their biological significance and adaptation to anaerobic, parasitic conditions. *Journal of Molecular Evolution* **60**, 113–127.

Atwood, J. A., 3rd, Weatherly, D. B., Minning, T. A., Bundy, B., Cavola, C., Opperdoes, F. R., Orlando, R. and Tarleton, R. L. (2005). The *Trypanosoma cruzi* proteome. *Science* **309**, 473–476.

Bahia, D., Oliveira, L. M., Lima, F. M., Oliveira, P., Silveira, J. F., Mortara, R. A. and Ruiz, J. C. (2009). The TryPIKinome of five human pathogenic trypanosomatids: *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, *Leishmania braziliensis* and *Leishmania infantum* – new tools for designing specific inhibitors. *Biochemical and Biophysical Research Communications* **390**, 963–970.

Barker, A. R., Wickstead, B., Gluenz, E. and Gull, K. (2008). Bioinformatic insights to the ESAG5 and GRESAG5 gene families in kinetoplastid parasites. *Molecular and Biochemical Parasitology* **162**, 112–122.

Barnwell, E. M., van Deursen, F. J., Jeacock, L., Smith, K. A., Maizels, R. M., Acosta-Serrano, A. and Matthews, K. (2010). Developmental regulation and extracellular release of a VSG expression-site-associated gene product from *Trypanosoma brucei* bloodstream forms. *Journal of Cell Science* **123**, 3401–3411.

Barry, J. D., Ginger, M. L., Burton, P. and McCulloch, R. (2003). Why are parasite contingency genes often associated with telomeres? *International Journal of Parasitology* **33**, 29–45.

Bartholomeu, D. C., Cerqueira, G. C., Leão, A. C., daRocha, W. D., Pais, F. S., Macedo, C., Djikeng, A., Teixeira, S. M. and El-Sayed, N. M. (2009). Genomic organization and expression profile of the mucin-associated surface protein (masp) family of the human pathogen *Trypanosoma cruzi*. *Nucleic Acids Research* **37**, 3407–3417.

Beck, J. T. and Ullman, B. (1990). Nutritional requirements of wild-type and folate transport-deficient *Leishmania donovani* for pterins and folates. *Molecular and Biochemical Parasitology* **43**, 221–230.

Bello, A. R., Nare, B., Freedman, D., Hardy, L. and Beverley, S. M. (1994). PTR1: a reductase mediating salvage of oxidized pteridines and methotrexate resistance in the protozoan parasite *Leishmania major*. *Proceedings of the National Academy of Sciences USA* **91**, 11442–11446.

Bernabó, G., Levy, G., Ziliani, M., Caeiro, L. D., Sánchez, D. O. and Tekiel, V. (2013). TcTASV-C, a protein family in *Trypanosoma cruzi* that is predominantly trypomastigote-stage specific and secreted to the medium. *PLOS ONE* **8**, e71192.

Berriman, M., Hall, N., Sheader, K., Bringaud, F., Tiwari, B., Isobe, T., Bowman, S., Corton, C., Clark, L., Cross, G. A., Hoek, M., Zanders, T., Berberof, M., Borst, P. and Rudenko, G. (2002). The architecture of variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **122**, 131–140.

Berriman, M., Ghedin, E., Hertz-Fowler, C., Blandin, G., Renauld, H., Bartholomeu, D. C., Lennard, N. J., Caler, E., Hamlin, N. E., Haas, B., Böhme, U., Hannick, L., Aslett, M. A., Shallom, J., Marcello, L., Hou, L., Wickstead, B., Alsmark, U. C., Arrowsmith, C., Atkin, R. J., Barron, A. J., Bringaud, F., Brooks, K., Carrington, M., Cherevach, I., Chillingworth, T. J., Churcher, C., Clark, L. N., Corton, C. H., Cronin, A., Davies, R. M., Doggett, J., Djikeng, A., Feldblyum, T., Field, M. C., Fraser, A., Goodhead, I., Hance, Z., Harper, D., Harris, B. R., Hauser, H., Hostetler, J., Ivens, A., Jagels, K., Johnson, D., Johnson, J., Jones, K., Kerhornou, A. X., Koo, H., Larke, N., Landfear, S., Larkin, C., Leech, V., Line, A., Lord, A., Macleod, A., Mooney, P. J., Moule, S., Martin, D. M., Morgan, G. W., Mungall, K., Norbertczak, H., Ormond, D., Pai, G., Peacock, C. S., Peterson, J., Quail, M. A., Rabbinowitsch, E., Rajandream, M. A., Reitter, C., Salzberg, S. L., Sanders, M., Schobel, S., Sharp, S., Simmonds, M., Simpson, A. J., Tallon, L., Turner, C. M., Tait, A., Tivey, A. R., Van Aken, S., Walker, D., Wanless, D., Wang, S., White, B., White, O., Whitehead, S., Woodward, J., Wortman, J., Adams, M. D., Embley, T. M., Gull, K., Ullu, E., Barry, J. D., Fairlamb, A. H., Opperdoes, F., Barrell, B. G., Donelson, J. E., Hall, N., Fraser, C. M., Melville, S. E. and El-Sayed, N. M. (2005). The genome of the African trypanosome *Trypanosoma brucei*. *Science* **309**, 416–422.

Berriman, M., Haas, B. J., LoVerde, P. T., Wilson, R. A., Dillon, G. P., Cerqueira, G. C., Mashiyama, S. T., Al-Lazikani, B., Andrade, L. F., Ashton, P. D., Aslett, M. A., Bartholomeu, D. C., Blandin, G., Caffrey, C. R., Coghlan, A., Coulson, R., Day, T. A., Delcher, A., DeMarco, R., Djikeng, A., Eyre, T., Gamble, J. A., Ghedin, E., Gu, Y., Hertz-Fowler, C., Hirai, H., Hirai, Y., Houston, R., Ivens, A., Johnston, D. A., Lacerda, D., Macedo, C. D., McVeigh, P., Ning, Z., Oliveira, G., Overington, J. P., Parkhill, J., Pertea, M., Pierce, R. J., Protasio, A. V., Quail, M. A., Rajandream, M. A., Rogers, J., Sajid, M., Salzberg, S. L., Stanke, M., Tivey, A. R., White, O., Williams, D. L., Wortman, J., Wu, W., Zamanian, M., Zerlotini, A., Fraser-Liggett, C. M., Barrell, B. G. and El-Sayed, N. M. (2009). The genome of the blood fluke *Schistosoma mansoni*. *Nature* **460**, 352–358.

Brenchley, R., Tariq, H., McElhinney, H., Szöor, B., Huxley-Jones, J., Stevens, R., Matthews, K. and Tabernero, L. (2007). The TriTryp phosphatome: analysis of the protein phosphatase catalytic domains. *BMC Genomics* **8**, 434.

Bringaud, F., Müller, M., Cerqueira, G. C., Smith, M., Rochette, A., El-Sayed, N. M., Papadopoulou, B. and Ghedin, E. (2007). Members of a large retroposon family are determinants of post-transcriptional gene expression in *Leishmania*. *PLOS Pathogens* **3**, 1291–1307.

Buscaglia, C. A., Campo, V. A., Di Noia, J. M., Torrecilhas, A. C., De Marchi, C. R., Ferguson, M. A., Frasch, A. C. and Almeida, I. C. (2004). The surface coat of the mammal-dwelling infective trypomastigote

stage of *Trypanosoma cruzi* is formed by highly diverse immunogenic mucins. *Journal of Biological Chemistry* **279**, 15860–15869.

Buscaglia, C. A., Campo, V. A., Frasch, A. C. and Di Noia, J. M. (2006). *Trypanosoma cruzi* surface mucins: host-dependent coat diversity. *Nature Reviews Microbiology* **4**, 229–236.

Butter, F., Bucerius, F., Michel, M., Cicova, Z., Mann, M. and Janzen, C. J. (2013). Comparative proteomics of two life-cycle stages of stable isotope-labeled *Trypanosoma brucei* reveals novel components of the parasite's host adaptation machinery. *Molecular and Cellular Proteomics* **12**, 172–179.

Callahan, H. A., Litaker, R. W. and Noga, E. J. (2002). Molecular taxonomy of the suborder Bodonina (Order Kinetoplastida), including the important fish parasite, *Ichthyobodo necator*. *Journal of Eukaryotic Microbiology* **49**, 119–128.

Camargo, E. P. (1999). *Phytomonas* and other trypanosomatid parasites of plants and fruit. *Advances in Parasitology* **42**, 29–112.

Carrillo, C., Cejas, S., González, N. S. and Algranati, I. D. (1999). *Trypanosoma cruzi* epimastigotes lack ornithine decarboxylase but can express a foreign gene encoding this enzyme. *FEBS Letters* **454**, 192–196.

Cerqueira, G. C., Bartholomeu, D. C., DaRocha, W. D., Hou, L., Freitas-Silva, D. M., Machado, C. R., El-Sayed, N. M. and Teixeira, S. M. (2008). Sequence diversity and evolution of multigene families in *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology* **157**, 65–72.

Chamond, N., Goytia, M., Coatnoan, N., Barale, J. C., Cosson, A., Degrave, W. M. and Minoprio, P. (2005). *Trypanosoma cruzi* proline racemases are involved in parasite differentiation and infectivity. *Molecular Microbiology* **58**, 46–60.

Chang, K. P., Chang, C. S. and Sassa, S. (1975). Heme biosynthesis in bacterium-protozoon symbioses: enzymic defects in host hemoflagellates and complemental role of their intracellular symbiotes. *Proceedings of the National Academy of Sciences USA* **72**, 2979–2983.

Comini, M. A. and Flohé, L. (2013). The trypanothione-based redox metabolism of trypanosomatids. In *Trypanosomatid Diseases: Molecular Routes to Drug Discovery* (ed. Jäger, T., Oliver, K. and Flohé, L.), pp. 167–200. Wiley-VCH, Weinheim, Germany.

Contreras, I., Gómez, M. A., Nguyen, O., Shio, M. T., McMaster, R. W. and Olivier, M. (2010). *Leishmania*-induced inactivation of the macrophage transcription factor AP-1 is mediated by the parasite metalloprotease GP63. *PLOS Pathogens* **6**, e1001148.

Cuevas, I. C., Cazzulo, J. J. and Sánchez, D. O. (2003). *Gp63* homologues in *Trypanosoma cruzi*: surface antigens with metalloprotease activity and a possible role in host cell infection. *Infection and Immunity* **71**, 5739–5749.

Dávila-López, M., Martínez-Guerra, J. J. and Samuelsson, T. (2010). Analysis of gene order conservation in eukaryotes identifies transcriptionally and functionally linked genes. *PLOS ONE* **5**, e10654.

Deitsch, K. W., Moxon, E. R. and Wellems, T. E. (1997). Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections. *Microbiology and Molecular Biology Review* **61**, 281–293.

de Assis, R. R., Ibraim, I. C., Nogueira, P. M., Soares, R. P. and Turco, S. J. (2012). Glycoconjugates in New World species of Leishmania: polymorphisms in lipophosphoglycan and glycoinositolphospholipids and interaction with hosts. *Biochimica et Biophysica Acta* **1820**, 1354–1365.

De Pablos, L. M. and Osuna, A. (2012). Multigene families in *Trypanosoma cruzi* and their role in infectivity. *Infection and Immunity* **80**, 2258–2264.

Depledge, D. P., Evans, K. J., Ivens, A. C., Aziz, N., Maroof, A., Kaye, P. M. and Smith, D. F. (2009). Comparative expression profiling of *Leishmania*: modulation in gene expression between species and in different host genetic backgrounds. *PLOS Neglected Tropical Diseases* **3**, e476.

Depledge, D. P., MacLean, L. M., Hodgkinson, M. R., Smith, B. A., Jackson, A. P., Ma, S., Uliana, S. R. and Smith, D. F. (2010). *Leishmania*-specific surface antigens show sub-genus sequence variation and immune recognition. *PLOS Neglected Tropical Diseases* **4**, e829.

Deschamps, P., Lara, E., Marande, W., López-García, P., Ekelund, F. and Moreira, D. (2011). Phylogenomic analysis of kinetoplastids supports that trypanosomatids arose from within bodonids. *Molecular Biology and Evolution* **28**, 53–58.

Devault, A. and Bañuls, A. L. (2008). The promastigote surface antigen gene family of the *Leishmania* parasite: differential evolution by positive selection and recombination. *BMC Evolutionary Biology* **8**, 292.

dos Santos, S. L., Freitas, L. M., Lobo, F. P., Rodrigues-Luiz, G. F., Mendes, T. A., Oliveira, A. C., Andrade, L. O., Chiari, E., Gazzinelli, R. T., Teixeira, S. M., Fujiwara, R. T. and Bartholomeu, D. C. (2012). The MASP family of *Trypanosoma cruzi*: changes in gene expression and antigenic profile during the acute phase of experimental infection. *PLOS Neglected Tropical Diseases* **6**, e1779.

Downing, T., Imamura, H., Decuypere, S., Clark, T. G., Coombs, G. H., Cotton, J. A., Hilley, J. D., de Doncker, S., Maes, I., Mottram, J. C., Quail, M. A., Rijal, S., Sanders, M., Schönian, G., Stark, O., Sundar, S., Vanaerschot, M., Hertz-Fowler, C., Dujardin, J. C. and Berriman, M. (2011). Whole genome sequencing of multiple *Leishmania donovani* clinical isolates provides insights into population structure and mechanisms of drug resistance. *Genome Research* **21**, 2143–2156.

Dufernez, F., Yernaux, C., Gerbod, D., Noël, C., Chauvenet, M., Wintjens, R., Edgcomb, V. P., Capron, M., Opperdoes, F. R. and Viscogliosi, E. (2006). The presence of four iron-containing superoxide dismutase isozymes in trypanosomatidae: characterization, subcellular localization, and phylogenetic origin in *Trypanosoma brucei*. *Free Radical Biology and Medicine* **40**, 210–225.

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., Westenberger, S. J., Caler, E., Cerqueira, G. C., Branche, C., Haas, B., Anupama, A., Arner, E., Aslund, L., Attipoe, P., Bontempi, E., Bringaud, F., Burton, P., Cadag, E., Campbell, D. A., Carrington, M., Crabtree, J., Darban, H., da Silveira, J. F., de Jong, P., Edwards, K., Englund, P. T., Fazelina, G., Feldblyum, T., Ferella, M., Frasch, A. C., Gull, K., Horn, D., Hou, L., Huang, Y., Kindlund, E., Klingbeil, M., Kluge, S., Koo, H., Lacerda, D., Levin, M. J., Lorenzi, H., Louie, T., Machado, C. R., McCulloch, R., McKenna, A., Mizuno, Y., Mottram, J. C., Nelson, S., Ochaya, S., Osoegawa, K., Pai, G., Parsons, M., Pentony, M., Pettersson, U., Pop, M., Ramirez, J. L., Rinta, J., Robertson, L., Salzberg, S. L., Sanchez, D. O., Seyler, A., Sharma, R., Shetty, J., Simpson, A. J., Sisk, E., Tammi, M. T., Tarleton, R., Teixeira, S., Van Aken, S., Vogt, C., Ward, P. N., Wickstead, B., Wortman, J., White, O., Fraser, C. M., Stuart, K. D. and Andersson, B. (2005*a*). The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. *Science* **309**, 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., Peacock, C., Bartholomeu, D. C., Haas, B. J., Tran, A. N., Wortman, J. R., Alsmark, U. C., Angiuoli, S., Anupama, A., Badger, J., Bringaud, F., Cadag, E., Carlton, J. M., Cerqueira, G. C., Creasy, T., Delcher, A. L., Djikeng, A., Embley, T. M., Hauser, C., Ivens, A. C., Kummerfeld, S. K., Pereira-Leal, J. B., Nilsson, D., Peterson, J., Salzberg, S. L., Shallom, J., Silva, J. C., Sundaram, J., Westenberger, S., White, O., Melville, S. E., Donelson, J. E., Andersson, B., Stuart, K. D. and Hall, N. (2005*b*). Comparative genomics of trypanosomatid parasitic protozoa. *Science* **309**, 404–409.

Eyford, B. A., Sakurai, T., Smith, D., Loveless, B., Hertz-Fowler, C., Donelson, J. E., Inoue, N. and Pearson, T. W. (2011). Differential protein expression throughout the life-cycle of *Trypanosoma congolense*, a major parasite of cattle in Africa. *Molecular and Biochemical Parasitology* **177**, 116–125.

Ferguson, M. A. (1997). The surface glycoconjugates of trypanosomatid parasites. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **352**, 1295–1302.

Field, M. C. (2005). Signalling the genome: the Ras-like small GTPase family of trypanosomatids. *Trends in Parasitology* **21**, 447–450.

Field, M. C., Natesan, S. K., Gabernet-Castello, C. and Koumandou, V. L. (2007). Intracellular trafficking in the trypanosomatids. *Traffic* **8**, 629–639.

Figueiredo, L. M., Cross, G. A. M. and Janzen, C. J. (2009). Epigenetic regulation in African trypanosomes: a new kid on the block. *Nature Reviews Microbiology* **7**, 504–513.

Flegontov, P., Votýpka, J., Skalický, T., Logacheva, M. D., Penin, A. A., Tanifuji, G., Onodera, N. T., Kondrashov, A. S., Volf, P., Archibald, J. M. and Lukeš, J. (2013). *Paratrypanosoma* is a novel early-branching trypanosomatid. *Current Biology* **23**, 1787–1793.

Freitas, L. M., dos Santos, S. L., Rodrigues-Luiz, G. F., Mendes, T. A., Rodrigues, T. S., Gazzinelli, R. T., Teixeira, S. M., Fujiwara, R. T. and Bartholomeu, D. C. (2011). Genomic analyses, gene expression and antigenic profile of the trans-sialidase superfamily of *Trypanosoma cruzi* reveal an undetected level of complexity. *PLOS ONE* **6**, e25914.

García, E. A., Ziliani, M., Agüero, F., Bernabó, G., Sánchez, D. O. and Tekiel, V. (2010). TcTASV: a novel protein family in *Trypanosoma cruzi* identified from a subtractive trypomastigote cDNA library. *PLOS Neglected Tropical Diseases* **4**, e841.

Gargantini, P. R., Lujan, H. D. and Pereira, C. A. (2012). *In silico* analysis of trypanosomatids' helicases. *FEMS Microbiology Letters* **335**, 123–129.

Ghedin, E., Bringaud, F., Peterson, J., Myler, P., Berriman, M., Ivens, A., Andersson, B., Bontempi, E., Eisen, J., Angiuoli, S., Wanless, D., Von Arxa, A., Murphy, L., Lennard, N., Salzberg, S., Adams, M. D., White, O., Hall, N., Stuart, K., Fraser, C. M. and El-Sayed, N. M. A. (2004). Gene synteny and evolution of genome architecture in trypanosomatids. *Molecular and Biochemical Parasitology* **134**, 183–191.

Goldenberg, S. and Avila, A. R. (2011). Aspects of *Trypanosoma cruzi* stage differentiation. *Advances in Parasitology* **75**, 285–305.

Gomez, M. A., Contreras, I., Hallé, M., Tremblay, M. L., McMaster, R. W. and Olivier, M. (2009). Leishmania GP63 alters host signaling through cleavage-activated protein tyrosine phosphatases. *Science Signaling* **2**, ra58.

Graham, S. V., Terry, S. and Barry, J. D. (1999). A structural and transcription pattern for variant surface glycoprotein gene expression sites used in metacyclic stage *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **103**, 141–154.

Grandgenett, P. M., Otsu, K., Wilson, H. R., Wilson, M. E. and Donelson, J. E. (2007). A function for a specific zinc metalloprotease of African trypanosomes. *PLOS Pathogens* **3**, 1432–1445.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**, 307–321.

Gunasekera, K., Wüthrich, D., Braga-Lagache, S., Heller, M. and Ochsenreiter, T. (2012) Proteome remodelling during development from blood to insect-form *Trypanosoma brucei* quantified by SILAC and mass spectrometry. *BMC Genomics* **13**, 556.

Gutteridge, W. E. and Gaborak, M. (1979). A re-examination of purine and pyrimidine synthesis in the three main forms of *Trypanosoma cruzi*. *International Journal of Biochemistry* **10**, 415–422.

Haile, S. and Papadopoulou, B. (2007). Developmental regulation of gene expression in trypanosomatid parasitic protozoa. *Current Opinion in Microbiology* **10**, 569–577.

Hall, J. P., Wang, H. and Barry, J. D. (2013). Mosaic VSGs and the scale of *Trypanosoma brucei* antigenic variation. *PLOS Pathogens* **9**, e1003502.

Hallé, M., Gomez, M. A., Stuible, M., Shimizu, H., McMaster, W. R., Olivier, M. and Tremblay, M. L. (2009). The *Leishmania* surface protease GP63 cleaves multiple intracellular proteins and actively participates in p38 mitogen-activated protein kinase inactivation. *Journal of Biological Chemistry* **284**, 6893–6908.

Handman, E., Osborn, A. H., Symons, F., van Driel, R. and Cappai, R. (1995). The *Leishmania* promastigote surface antigen 2 complex is differentially expressed during the parasite life-cycle. *Molecular and Biochemical Parasitology* **74**, 189–200.

Handman, E., Papenfuss, A. T., Speed, T. P. and Goding, J. W. (2008) *Leishmania* surface proteins. In *Leishmania: After the Genome* (ed. Myler, P. J. and Fasel, N.), pp. 177–204. Caister Academic Press, Norfolk, UK.

Herman, M., Gillies, S., Michels, P. A. and Rigden, D. J. (2006). Autophagy and related processes in trypanosomatids: insights from genomic and bioinformatic analyses. *Autophagy* **2**, 107–118.

Hertz-Fowler, C., Ersfeld, K. and Gull, K. (2001). CAP5.5, a life-cycle-regulated, cytoskeleton-associated protein is a member of a novel family of calpain-related proteins in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **116**, 25–34.

Hertz-Fowler, C., Figueiredo, L. M., Quail, M. A., Becker, M., Jackson, A., Bason, N., Brooks, K., Churcher, C., Fahkro, S., Goodhead, I., Heath, P., Kartvelishvili, M., Mungall, K., Harris, D., Hauser, H., Sanders, M., Saunders, D., Seeger, K., Sharp, S., Taylor, J. E., Walker, D., White, B., Young, R., Cross, G. A., Rudenko, G., Barry, J. D., Louis, E. J. and Berriman, M. (2008). Telomeric expression sites are highly conserved in *Trypanosoma brucei*. *PLOS ONE* **3**, e3527.

Holzer, T. R., McMaster, W. R. and Forney, J. D. (2006). Expression profiling by whole-genome interspecies microarray hybridization reveals differential gene expression in procyclic promastigotes, lesion-derived amastigotes and axenic amastigotes in *Leishmania mexicana*. *Molecular and Biochemical Parasitology* **146**, 198–218.

Holzer, T. R., Mishra, K. K., LeBowitz, J. H. and Forney, J. D. (2008). Coordinate regulation of a family of promastigote-enriched mRNAs by the 3′UTR PRE element in *Leishmania mexicana*. *Molecular Biochemical Parasitology* **157**, 54–64.

Horn, D. and McCulloch, R. (2010). Molecular mechanisms underlying the control of antigenic variation in African trypanosomes. *Current Opinion in Microbiology* **13**, 700–705.

Hutchinson, O. C., Picozzi, K., Jones, N. G., Mott, H., Sharma, R., Welburn, S. C. and Carrington, M. (2007). Variant surface glycoprotein

gene repertoires in *Trypanosoma brucei* have diverged to become strain-specific. *BMC Genomics* **8**, 234.

Ivens, A. C., Peacock, C. S., Worthey, E. A., Murphy, L., Aggarwal, G., Berriman, M., Sisk, E., Rajandream, M. A., Adlem, E., Aert, R., Anupama, A., Apostolou, Z., Attipoe, P., Bason, N., Bauser, C., Beck, A., Beverley, S. M., Bianchettin, G., Borzym, K., Bothe, G., Bruschi, C. V., Collins, M., Cadag, E., Ciarloni, L., Clayton, C., Coulson, R. M., Cronin, A., Cruz, A. K., Davies, R. M., De Gaudenzi, J., Dobson, D. E., Duesterhoeft, A., Fazelina, G., Fosker, N., Frasch, A. C., Fraser, A., Fuchs, M., Gabel, C., Goble, A., Goffeau, A., Harris, D., Hertz-Fowler, C., Hilbert, H., Horn, D., Huang, Y., Klages, S., Knights, A., Kube, M., Larke, N., Litvin, L., Lord, A., Louie, T., Marra, M., Masuy, D., Matthews, K., Michaeli, S., Mottram, J. C., Müller-Auer, S., Munden, H., Nelson, S., Norbertczak, H., Oliver, K., O'neil, S., Pentony, M., Pohl, T. M., Price, C., Purnelle, B., Quail, M. A., Rabbinowitsch, E., Reinhardt, R., Rieger, M., Rinta, J., Robben, J., Robertson, L., Ruiz, J. C., Rutter, S., Saunders, D., Schäfer, M., Schein, J., Schwartz, D. C., Seeger, K., Seyler, A., Sharp, S., Shin, H., Sivam, D., Squares, R., Squares, S., Tosato, V., Vogt, C., Volckaert, G., Wambutt, R., Warren, T., Wedler, H., Woodward, J., Zhou, S., Zimmermann, W., Smith, D. F., Blackwell, J. M., Stuart, K. D., Barrell, B. and Myler, P. J. (2005). The genome of the kinetoplastid parasite, *Leishmania major*. *Science* **309**, 436–442.

Jackson, A. P. (2007*a*). Tandem gene arrays in *Trypanosoma brucei*: comparative phylogenomic analysis of duplicate sequence variation. *BMC Evolutionary Biology* **7**, 54.

Jackson, A. P. (2007*b*). Evolutionary consequences of a large duplication event in *Trypanosoma brucei*: chromosomes 4 and 8 are partial duplicons. *BMC Genomics* **8**, 432.

Jackson, A. P. (2010). The evolution of amastin surface glycoproteins in trypanosomatid parasites. *Molecular Biology and Evolution* **27**, 33–45.

Jackson, A. P., Vaughan, S. and Gull, K. (2006). Comparative genomics and concerted evolution of beta-tubulin paralogs in *Leishmania* spp. *BMC Genomics* **7**, 137.

Jackson, A. P., Sanders, M., Berry, A., McQuillan, J., Aslett, M. A., Quail, M. A., Chukualim, B., Capewell, P., MacLeod, A., Melville, S. E., Gibson, W., Barry, J. D., Berriman, M. and Hertz-Fowler, C. (2009). The genome sequence of *Trypanosoma brucei gambiense*, causative agent of chronic human African trypanosomiasis. *PLOS Neglected Tropical Diseases* **4**, e658.

Jackson, A. P., Berry, A., Aslett, M., Allison, H. C., Burton, P., Vavrova-Anderson, J., Brown, R., Browne, H., Corton, N., Hauser, H., Gamble, J., Gilderthorp, R., Marcello, L., McQuillan, J., Otto, T. D., Quail, M. A., Sanders, M. J., van Tonder, A., Ginger, M. L., Field, M. C., Barry, J. D., Hertz-Fowler, C. and Berriman, M. (2012). Antigenic diversity is generated by distinct evolutionary mechanisms in African trypanosome species. *Proceedings of the National Academy of Sciences USA* **109**, 3416–3421.

Jackson, A. P., Allison, H. C., Barry, J. D., Field, M. C., Hertz-Fowler, C. and Berriman, M. (2013). A cell-surface phylome for African trypanosomes. *PLOS Neglected Tropical Diseases* **7**, e2121.

Jackson, D. G., Windle, H. J. and Voorheis, H. P. (1993). The identification, purification, and characterization of two invariant surface glycoproteins located beneath the surface coat barrier of bloodstream forms of *Trypanosoma brucei*. *Journal of Biological Chemistry* **268**, 8085–8095.

Jensen, B. C., Sivam, D., Kifer, C. T., Myler, P. J. and Parsons, M. (2009). Widespread variation in transcript abundance within and across developmental stages of *Trypanosoma brucei*. *BMC Genomics* **10**, 482.

Kabani, S., Fenn, K., Ross, A., Ivens, A., Smith, T. K., Ghazal, P. and Matthews, K. (2009). Genome-wide expression profiling of *in vivo*-derived bloodstream parasite stages and dynamic analysis of mRNA alterations during synchronous differentiation in *Trypanosoma brucei*. *BMC Genomics* **10**, 427.

Kangussu-Marcolino, M. M., de Paiva, R. M., Araújo, P. R., de Mendonça-Neto, R. P., Lemos, L., Bartholomeu, D. C., Mortara, R. A., daRocha, W. D. and Teixeira, S. M. (2013). Distinct genomic organization, mRNA expression and cellular localization of members of two amastin sub-families present in *Trypanosoma cruzi*. *BMC Microbiology* **13**, 10.

Kawashita, S. Y., da Silva, C. V., Mortara, R. A., Burleigh, B. A. and Briones, M. R. (2009). Homology, paralogy and function of DGF-1, a highly dispersed *Trypanosoma cruzi* specific gene family and its implications for information entropy of its encoded proteins. *Molecular and Biochemical Parasitology* **165**, 19–31.

Keeling, P. J. and Slamovits, C. H. (2005). Causes and effects of nuclear genome reduction. *Current Opinion in Genetics and Development* **15**, 601–608.

Kim, D., Chiurillo, M. A., El-Sayed, N., Jones, K., Santos, M. R., Porcile, P. E., Andersson, B., Myler, P., da Silveira, J. F. and Ramírez, J. L. (2005). Telomere and subtelomere of *Trypanosoma cruzi* chromosomes are enriched in (pseudo)genes of retrotransposon hot spot and trans-sialidase-like gene families: the origins of *T. cruzi* telomeres. *Gene* **346**, 153–161.

Kissinger, J. C. and DeBarry, J. (2011). Genome cartography: charting the apicomplexan genome. *Trends in Parasitology* **27**, 345–354.

Kolev, N. G., Ramey-Butler, K., Cross, G. A. M., Ullu, E. and Tschudi, C. (2012). Developmental progression to infectivity in *Trypanosoma brucei* triggered by an RNA-binding protein. *Science* **338**, 1352–1353.

Kolev, N. G., Ullu, E. and Tschudi, C. (2014). The emerging role of RNA-binding proteins in the life-cycle of *Trypanosoma brucei*. *Cellular Microbiology* **16**, 482–489.

Korený, L., Lukes, J. and Oborník, M. (2010). Evolution of the haem synthetic pathway in kinetoplastid flagellates: an essential pathway that is not essential after all? *International Journal for Parasitology* **40**, 149–156.

Kosec, G., Alvarez, V. E., Agüero, F., Sánchez, D., Dolinar, M., Turk, B., Turk, V. and Cazzulo, J. J. (2006). Metacaspases of *Trypanosoma cruzi*: possible candidates for programmed cell death mediators. *Molecular and Biochemical Parasitology* **145**, 18–28.

Krauth-Siegel, R. L. and Comini, M. A. (2008). Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochimica et Biophysica Acta* **1780**, 1236–1248.

Kulkarni, M. M., McMaster, W. R., Kamysz, E., Kamysz, W., Engman, D. M. and McGwire, B. S. (2006). The major surface-metalloprotease of the parasitic protozoan, *Leishmania*, protects against antimicrobial peptide-induced apoptotic killing. *Molecular Microbiology* **62**, 1484–1497.

Kulkarni, M. M., Olson, C. L., Engman, D. M. and McGwire, B. S. (2009). *Trypanosoma cruzi* GP63 proteins undergo stage-specific differential posttranslational modification and are important for host cell infection. *Infection and Immunity* **77**, 2193–2200.

LaCount, D. J., Gruszynski, A. E., Grandgenett, P. M., Bangs, J. D. and Donelson, J. E. (2003). Expression and function of the *Trypanosoma brucei* major surface protease (GP63) genes. *Journal of Biological Chemistry* **278**, 24658–24664.

Lander, N., Bernal, C., Diez, N., Añez, N., Docampo, R. and Ramírez, J. L. (2010). Localization and developmental regulation of a dispersed gene family 1 protein in *Trypanosoma cruzi*. *Infection and Immunity* **78**, 231–240.

Leifso, K., Cohen-Freue, G., Dogra, N., Murray, A. and McMaster, W. R. (2007). Genomic and proteomic expression analysis of *Leishmania* promastigote and amastigote life-stages: the *Leishmania* genome is constitutively expressed. *Molecular and Biochemical Parasitology* **152**, 35–46.

Li, Z. H., De Gaudenzi, J. G., Alvarez, V. E., Mendiondo, N., Wang, H., Kissinger, J. C., Frasch, A. C. and Docampo, R. (2012). A 43-nucleotide U-rich element in 3′-untranslated region of large number of *Trypanosoma cruzi* transcripts is important for mRNA abundance in intracellular amastigotes. *Journal of Biological Chemistry* **287**, 19058–19069.

Lieke, T., Nylén, S., Eidsmo, L., McMaster, W. R., Mohammadi, A. M., Khamesipour, A., Berg, L. and Akuffo, H. (2008). *Leishmania* surface protein gp63 binds directly to human natural killer cells and inhibits proliferation. *Clinical and Experimental Immunology* **153**, 221–230.

Lincoln, L. M., Ozaki, M., Donelson, J. E. and Beetham, J. K. (2004). Genetic complementation of *Leishmania* deficient in PSA (GP46) restores their resistance to lysis by complement. *Molecular and Biochemical Parasitology* **137**, 185–189.

Lira, C. B., Giardini, M. A., Neto, J. L., Conte, F. F. and Cano, M. I. (2007). Telomere biology of trypanosomatids: beginning to answer some questions. *Trends in Parasitology* **23**, 357–362.

Lom, J. (1979). Biology of fish trypanosomes and trypanoplasms. In *Biology of Kinetoplastida Volume II* (ed. Lumsden, W. H. R. and Evans, D. A.), pp. 269–377. Academic Press, New York, NY, USA.

Lynch, M. and Conery, J. S. (2000). The evolutionary fate and consequences of duplicate genes. *Science* **290**, 1151–1155.

Ma, L., Chen, K., Meng, Q., Liu, Q., Tang, P., Hu, S. and Yu, J. (2011). An evolutionary analysis of trypanosomatid GP63 proteases. *Parasitology Research* **109**, 1075–1084.

Marcello, L. and Barry, J. D. (2007). Analysis of the VSG gene silent archive in *Trypanosoma brucei* reveals that mosaic gene expression is prominent in antigenic variation and is favored by archive substructure. *Genome Research* **17**, 1344–1352.

Marcoux, V., Wei, G., Tabel, H. and Bull, H. J. (2010). Characterization of major surface protease homologues of *Trypanosoma congolense*. *Journal of Biomedicine and Biotechnology* **2010**, 418157.

Marr, J. J., Berens, R. L. and Nelson, D. J. (1978). Purine metabolism in *Leishmania donovani* and *Leishmania braziliensis*. *Biochimica et Biophysica Acta* **544**, 360–371.

Maslov, D. A., Votýpka, J., Yurchenko, V. and Lukeš, J. (2013). Diversity and phylogeny of insect trypanosomatids: all that is hidden shall be revealed. *Trends in Parasitology* **29**, 43–52.

McCulloch, R. and Horn, D. (2009). What has DNA sequencing revealed about the VSG expression sites of African trypanosomes? *Trends in Parasitology* **25**, 359–363.

Minning, T. A., Weatherly, D. B., Atwood, J., 3rd, Orlando, R. and Tarleton, R. L. (2009). The steady-state transcriptome of the four major life-cycle stages of *Trypanosoma cruzi*. *BMC Genomics* **10**, 370.

Montagna, G. N., Donelson, J. E. and Frasch, A. C. (2006). Procyclic *Trypanosoma brucei* expresses separate sialidase and trans-sialidase enzymes on its surface membrane. *Journal of Biological Chemistry* **281**, 33949–33958.

Moraes Barros, R. R., Marini, M. M., Antônio, C. R., Cortez, D. R., Miyake, A. M., Lima, F. M., Ruiz, J. C., Bartholomeu, D. C., Chiurillo, M. A., Ramirez, J. L. and da Silveira, J. F. (2012). Anatomy and evolution of telomeric and subtelomeric regions in the human protozoan parasite *Trypanosoma cruzi*. *BMC Genomics* **13**, 229.

Moreira, D., López-García, P. and Vickerman, K. (2004). An updated view of kinetoplastid phylogeny using environmental sequences and a closer outgroup: proposal for a new classification of the class Kinetoplastea. *International Journal of Systematics and Evolutionary Microbiology* **54**, 1861–1875.

Nakayasu, E. S., Yashunsky, D. V., Nohara, L. L., Torrecilhas, A. C., Nikolaev, A. V. and Almeida, I. C. (2009). GPIomics: global analysis of glycosylphosphatidylinositol-anchored molecules of *Trypanosoma cruzi*. *Molecular Systems Biology* **5**, 261.

Nakjang, S., Williams, T. A., Heinz, E., Watson, A. K., Foster, P. G., Sendra, K. M., Heaps, S. E., Hirt, R. P. and Embley, T. M. (2013). Reduction and expansion in microsporidian genome evolution: new insights from comparative genomics. *Genome Biology and Evolution* **5**, 2285–2303.

Nare, B., Hardy, L. W. and Beverley, S. M. (1997). The roles of pteridine reductase 1 and dihydrofolate reductase-thymidylate synthase in pteridine metabolism in the protozoan parasite *Leishmania major*. *Journal of Biological Chemistry* **272**, 13883–13891.

Niemirowicz, G., Parussini, F., Agüero, F. and Cazzulo, J. J. (2007). Two metallocarboxypeptidases from the protozoan *Trypanosoma cruzi* belong to the M32 family, found so far only in prokaryotes. *Biochemistry Journal* **401**, 399–410.

Nunes, M. P., Fortes, B., Silva-Filho, J. L., Terra-Granado, E., Santos, L., Conde, L., de Araújo Oliveira, I., Freire-de-Lima, L., Martins, M. V., Pinheiro, A. A., Takiya, C. M., Freire-de-Lima, C. G., Todeschini, A. R., Dosreis, G. A. and Morrot, A. (2013). Inhibitory effects of *Trypanosoma cruzi* sialoglycoproteins on CD4(+) T cells are associated with increased susceptibility to infection. *PLOS ONE* **8**, e77568.

Ohno, S. (1970). *Evolution by Gene Duplication*. Springer-Verlag, New York, NY, USA.

Olego-Fernandez, S., Vaughan, S., Shaw, M. K., Gull, K. and Ginger, M. L. (2009). Cell morphogenesis of *Trypanosoma brucei* requires the paralogous, differentially expressed calpain-related proteins CAP5.5 and CAP5.5V. *Protist* **160**, 576–590.

Oliveira, I. A., Freire-de-Lima, L., Penha, L. L., Dias, W. B. and Todeschini, A. R. (2014). *Trypanosoma cruzi* trans-sialidase: structural features and biological implications. *Subcellular Biochemistry* **74**, 181–201.

Opperdoes, F. R. and Michels, P. A. (2007). Horizontal gene transfer in trypanosomatids. *Trends in Parasitology* **23**, 470–476.

Oppezzo, P., Obal, G., Baraibar, M. A., Pritsch, O., Alzari, P. M. and Buschiazzo, A. (2011). Crystal structure of an enzymatically inactive trans-sialidase-like lectin from *Trypanosoma cruzi*: the carbohydrate binding mechanism involves residual sialidase activity. *Biochimica et Biophysica Acta* **1814**, 1154–1161.

Ouellette, M., Drummelsmith, J., El-Fadili, A., Kündig, C., Richard, D. and Roy, G. (2002). Pterin transport and metabolism in *Leishmania* and related trypanosomatid parasites. *International Journal for Parasitology* **32**, 385–398.

Oza, S. L., Shaw, M. P., Wyllie, S. and Fairlamb, A. H. (2005). Trypanothione biosynthesis in *Leishmania major*. *Molecular and Biochemical Parasitology* **139**, 107–116.

Parsons, M., Worthey, E. A., Ward, P. N. and Mottram, J. C. (2005). Comparative analysis of the kinomes of three pathogenic

trypanosomatids: *Leishmania major, Trypanosoma brucei* and *Trypanosoma cruzi. BMC Genomics* **6**, 127.

Pastro, L., Smircich, P., Pérez-Díaz, L., Duhagon, M. A. and Garat, B. (2013). Implication of CA repeated tracts on post-transcriptional regulation in *Trypanosoma cruzi. Experimental Parasitology* **134**, 511–518.

Pays, E., Lips, S., Nolan, D., Vanhamme, L. and Pérez-Morga, D. (2001). The VSG expression sites of *Trypanosoma brucei*: multipurpose tools for the adaptation of the parasite to mammalian hosts. *Molecular and Biochemical Parasitology* **114**, 1–16.

Peacock, C. S., Seeger, K., Harris, D., Murphy, L., Ruiz, J. C., Quail, M. A., Peters, N., Adlem, E., Tivey, A., Aslett, M., Kerhornou, A., Ivens, A., Fraser, A., Rajandream, M. A., Carver, T., Norbertczak, H., Chillingworth, T., Hance, Z., Jagels, K., Moule, S., Ormond, D., Rutter, S., Squares, R., Whitehead, S., Rabbinowitsch, E., Arrowsmith, C., White, B., Thurston, S., Bringaud, F., Baldauf, S. L., Faulconbridge, A., Jeffares, D., Depledge, D. P., Oyola, S. O., Hilley, J. D., Brito, L. O., Tosi, L. R., Barrell, B., Cruz, A. K., Mottram, J. C., Smith, D. F. and Berriman, M. (2007). Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. *Nature Genetics* **39**, 839–847.

Porcel, B. M., Denoeud, F., Opperdoes, F., Noel, B., Madoui, M. A., Hammarton, T. C., Field, M. C., Da Silva, C., Couloux, A., Poulain, J., Katinka, M., Jabbari, K., Aury, J. M., Campbell, D. A., Cintron, R., Dickens, N. J., Docampo, R., Sturm, N. R., Koumandou, V. L., Fabre, S., Flegontov, P., Lukeš, J., Michaeli, S., Mottram, J. C., Szöőr, B., Zilberstein, D., Bringaud, F., Wincker, P. and Dollet, M. (2014). The streamlined genome of *Phytomonas* spp. relative to human pathogenic kinetoplastids reveals a parasite tailored for plants. *PLOS Genetics* **10**, e1004007.

Puri, V., Goyal, A., Sankaranarayanan, R., Enright, A. J. and Vaidya, T. (2011). Evolutionary and functional insights into *Leishmania* META1: evidence for lateral gene transfer and a role for META1 in secretion. *BMC Evolutionary Biology* **11**, 334.

Pusnik, M., Charrière, F., Mäser, P., Waller, R. F., Dagley, M. J., Lithgow, T. and Schneider, A. (2009). The single mitochondrial porin of *Trypanosoma brucei* is the main metabolite transporter in the outer mitochondrial membrane. *Molecular Biology and Evolution* **26**, 671–680.

Raymond, F., Boisvert, S., Roy, G., Ritt, J. F., Légaré, D., Isnard, A., Stanke, M., Olivier, M., Tremblay, M. J., Papadopoulou, B., Ouellette, M. and Corbeil, J. (2012). Genome sequencing of the lizard parasite *Leishmania tarentolae* reveals loss of genes associated to the intracellular stage of human pathogenic species. *Nucleic Acids Research* **40**, 1131–1147.

Real, F., Vidal, R. O., Carazzolle, M. F., Mondego, J. M., Costa, G. G., Herai, R. H., Würtele, M., de Carvalho, L. M., E Ferreira, R. C., Mortara, R. A., Barbiéri, C. L., Mieczkowski, P., da Silveira, J. F., Briones, M. R., Pereira, G. A. and Bahia, D. (2013). The genome sequence of *Leishmania (Leishmania) amazonensis*: functional annotation and extended analysis of gene models. *DNA Research* **20**, 567–581.

Reina-San-Martín, B., Degrave, W., Rougeot, C., Cosson, A., Chamond, N., Cordeiro-Da-Silva, A., Arala-Chaves, M., Coutinho, A. and Minoprio, P. (2000). A B-cell mitogen from a pathogenic trypanosome is a eukaryotic proline racemase. *Nature Medicine* **6**, 890–897.

Richmond, G. S. and Smith, T. K. (2007). A novel phospholipase from *Trypanosoma brucei. Molecular Microbiology* **63**, 1078–1095.

Rico, E., Rojas, F., Mony, B. M., Szoor, B., MacGregor, P. and Matthews, K. R. (2013). Bloodstream form pre-adaptation to the tsetse fly in *Trypanosoma brucei. Frontiers in Cellular and Infection Microbiology* **3**, 78.

Rochette, A., McNicoll, F., Girard, J., Breton, M., Leblanc, E., Bergeron, M. G. and Papadopoulou, B. (2005). Characterization and developmental gene regulation of a large gene family encoding amastin surface proteins in *Leishmania* spp. *Molecular and Biochemical Parasitology* **140**, 205–220.

Rochette, A., Raymond, F., Ubeda, J. M., Smith, M., Messier, N., Boisvert, S., Rigault, P., Corbeil, J., Ouellette, M. and Papadopoulou, B. (2008). Genome-wide gene expression profiling analysis of *Leishmania major* and *Leishmania infantum* developmental stages reveals substantial differences between the two species. *BMC Genomics* 9, 255.

Rochette, A., Raymond, F., Corbeil, J., Ouellette, M. and Papadopoulou, B. (2009). Whole-genome comparative RNA expression profiling of axenic and intracellular amastigote forms of *Leishmania infantum. Molecular and Biochemical Parasitology* **165**, 32–47.

Roditi, I., Furger, A., Ruepp, S., Schürch, N. and Bütikofer, P. (1998). Unravelling the procyclin coat of *Trypanosoma brucei. Molecular and Biochemical Parasitology* **91**, 117–130.

Rogers, M. B., Hilley, J. D., Dickens, N. J., Wilkes, J., Bates, P. A., Depledge, D. P., Harris, D., Her, Y., Herzyk, P., Imamura, H.,

Otto, T. D., Sanders, M., Seeger, K., Dujardin, J. C., Berriman, M., Smith, D. F., Hertz-Fowler, C. and Mottram, J. C. (2011). Chromosome and gene copy number variation allow major structural change between species and strains of *Leishmania. Genome Research* **21**, 2129–2142.

Rosenzweig, D., Smith, D., Opperdoes, F., Stern, S., Olafson, R. W. and Zilberstein, D. (2008*a*). Retooling *Leishmania* metabolism: from sand fly gut to human macrophage. *FASEB Journal* **22**, 590–602.

Rosenzweig, D., Smith, D., Myler, P. J., Olafson, R. W. and Zilberstein, D. (2008*b*). Post-translational modification of cellular proteins during *Leishmania donovani* differentiation. *Proteomics* **8**, 1843–1850.

Rudenko, G. (2010). Epigenetics and transcriptional control in African trypanosomes. *Essays in Biochemistry* **48**, 201–219.

Rudenko, G. (2011). African trypanosomes: the genome and adaptations for immune evasion. *Essays in Biochemistry* **51**, 47–62.

Sádlová, J., Price, H. P., Smith, B. A., Votýpka, J., Volf, P. and Smith, D. F. (2010). The stage-regulated HASPB and SHERP proteins are essential for differentiation of the protozoan parasite *Leishmania major* in its sand fly vector, *Phlebotomus papatasi. Cellular Microbiology* **12**, 1765–1779.

Salmon, D., Hanocq-Quertier, J., Paturiaux-Hanocq, F., Pays, A., Tebabi, P., Nolan, D. P., Michel, A. and Pays, E. (1997). Characterization of the ligand-binding site of the transferrin receptor in *Trypanosoma brucei* demonstrates a structural relationship with the N-terminal domain of the variant surface glycoprotein. *EMBO Journal* **16**, 7272–7278.

Salmon, D., Bachmaier, S., Krumbholz, C., Kador, M., Gossmann, J. A., Uzureau, P., Pays, E. and Boshart, M. (2012). Cytokinesis of *Trypanosoma brucei* bloodstream forms depends on expression of adenylyl cyclases of the ESAG4 or ESAG4-like subfamily. *Molecular Microbiology* **84**, 225–242.

Sanchez, M. A., Zeoli, D., Klamo, E. M., Kavanaugh, M. P. and Landfear, S. M. (1995). A family of putative receptor-adenylate cyclases from *Leishmania donovani. Journal of Biological Chemistry* **270**, 17551–17558.

Saxena, A., Lahav, T., Holland, N., Aggarwal, G., Anupama, A., Huang, Y., Volpin, H., Myler, P. J. and Zilberstein, D. (2007). Analysis of the *Leishmania donovani* transcriptome reveals an ordered progression of transient and permanent changes in gene expression during differentiation. *Molecular and Biochemical Parasitology* **152**, 53–65.

Siegel, T. N., Hekstra, D. R., Wang, X., Dewell, S. and Cross, G. A. (2010). Genome-wide analysis of mRNA abundance in two life-cycle stages of *Trypanosoma brucei* and identification of splicing and polyadenylation sites. *Nucleic Acids Research* **38**, 4946–4957.

Simpson, A. G., Gill, E. E., Callahan, H. A., Litaker, R. W. and Roger, A. J. (2004). Early evolution within kinetoplastids (euglenozoa), and the late emergence of trypanosomatids. *Protist* **15**, 407–422.

Simpson, A. G., Stevens, J. R. and Lukes, J. (2006). The evolution and diversity of kinetoplastid flagellates. *Trends in Parasitology* **22**, 168–174.

Smirlis, D., Duszenko, M., Ruiz, A. J., Scoulica, E., Bastien, P., Fasel, N. and Soteriadou, K. (2010). Targeting essential pathways in trypanosomatids gives insights into protozoan mechanisms of cell death. *Parasites and Vectors* **3**, 107.

Smith, M., Bringaud, F. and Papadopoulou, B. (2009). Organization and evolution of two SIDER retroposon subfamilies and their impact on the *Leishmania* genome. *BMC Genomics* **10**, 240.

Steglich, C. and Schaeffer, S. W. (2006). The ornithine decarboxylase gene of *Trypanosoma brucei*: evidence for horizontal gene transfer from a vertebrate source. *Infection Genetics and Evolution* **6**, 205–219.

Tsai, I. J., Zarowiecki, M., Holroyd, N., Garciarrubio, A., Sanchez-Flores, A., Brooks, K. L., Tracey, A., Bobes, R. J., Fragoso, G., Sciutto, E., Aslett, M., Beasley, H., Bennett, H. M., Cai, J., Camicia, F., Clark, R., Cucher, M., De Silva, N., Day, T. A., Deplazes, P., Estrada, K., Fernández, C., Holland, P. W., Hou, J., Hu, S., Huckvale, T., Hung, S. S., Kamenetzky, L., Keane, J. A., Kiss, F., Koziol, U., Lambert, O., Liu, K., Luo, X., Luo, Y., Macchiaroli, N., Nichol, S., Paps, J., Parkinson, J., Pouchkina-Stantcheva, N., Riddiford, N., Rosenzvit, M., Salinas, G., Wasmuth, J. D., Zamanian, M., Zheng, Y., Taenia solium Genome Consortium, Cai, X., Soberón, X., Olson, P. D., Laclette, J. P., Brehm, K. and Berriman, M. (2013). The genomes of four tapeworm species reveal adaptations to parasitism. *Nature* **496**, 57–63.

Urban, I., Santurio, L. B., Chidichimo, A., Yu, H., Chen, X., Mucci, J., Agüero, F. and Buscaglia, C. A. (2011). Molecular diversity of the

Trypanosoma cruzi TcSMUG family of mucin genes and proteins. *Biochemistry Journal* **438**, 303–313.

Urbaniak, M. D., Guther, M. L. and Ferguson, M. A. (2012). Comparative SILAC proteomic analysis of *Trypanosoma brucei* bloodstream and procyclic lifecycle stages. *PLOS ONE* **7**, e36619.

Urwyler, S., Studer, E., Renggli, C. K. and Roditi, I. (2007). A family of stage-specific alanine-rich proteins on the surface of epimastigote forms of *Trypanosoma brucei*. *Molecular Microbiology* **63**, 218–228.

Vanhamme, L. and Pays, E. (1995). Control of gene expression in trypanosomes. *Microbiological Review* **59**, 223–240.

Veitch, N. J., Johnson, P. C., Trivedi, U., Terry, S., Wildridge, D. and MacLeod, A. (2010). Digital gene expression analysis of two life-cycle stages of the human-infective parasite, *Trypanosoma brucei gambiense* reveals differentially expressed clusters of co-regulated genes. *BMC Genomics* **11**, 124.

Victoir, K., Arevalo, J., De Doncker, S., Barker, D. C., Laurent, T., Godfroid, E., Bollen, A., Le Ray, D. and Dujardin, J. C. (2005). Complexity of the major surface protease (msp) gene organization in *Leishmania* (*Viannia*) *braziliensis*: evolutionary and functional implications. *Parasitology* **131**, 207–214.

Von der Heyden, S., Chao, E. E., Vickerman, K. and Cavalier-Smith, T. (2004). Ribosomal RNA phylogeny of bodonid and diplonemid flagellates and the evolution of euglenozoa. *Journal of Eukaryotic Microbiology* **51**, 402–416.

Weirather, J. L., Wilson, M. E. and Donelson, J. E. (2012). Mapping of VSG similarities in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **181**, 141–152.

Yao, C. (2010). Major surface protease of trypanosomatids: one size fits all? *Infection and Immunity* **78**, 22–31.

Ziegelbauer, K. and Overath, P. (1993). Organization of two invariant surface glycoproteins in the surface coat of *Trypanosoma brucei*. *Infection and Immunity* **61**, 4540–4545.

Zinoviev, A., Akum, Y., Yahav, T. and Shapira, M. (2012). Gene duplication in trypanosomatids – two DED1 paralogs are functionally redundant and differentially expressed during the life-cycle. *Molecular and Biochemical Parasitology* **185**, 127–136.