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REVIEW ARTICLE

Excavata-Kinetoplastea Trypanosomatidae Parasites and the Interaction with their Hosts

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Abstract

Kinetoplastea Trypanosomatidae are unicellular flagellated parasites of different kind of hosts. They cause diseases in plants, animals and humans. These parasites are transmitted by insect vectors in a wide range of geographic areas. In this communication we review the interactions between trypanosomatid parasites, their hosts and thoughts about their impact on environmental health, especially in those regions where hosts are natural reserves of many species. The interactions of these parasites and their hosts in some cases have great economic and environmental impact. We also address the genetic mechanisms that determine variability in some specific taxa. Two cell fusion and recombination of nuclear genomes and mitochondrial introgression seems to be the mechanisms that produce the greatest variability. However, these organisms most frequently reproduce clonally. Finally the mechanisms of the trypanosomatids response to environmental changes in their host are also analyzed.

Introduction

Excavata is a supergroup of Protist (Eukaryotic domain, Protist kingdom, Excavata sub-kingdom) typified with the unique discoidal cristae mitochondrial which exhibit a paddle-like morphology present in groups such as euglenids and kinetoplastids [1]. Besides among them it is possible to mention the trypanosomatid parasite such as *Leishmania tarentolae*, *L. major*, *Bodo saltans*, *Trypanosoma cruzi*, *Euglena gracilis* and other [2]. The Trypanosomatidae are a diverse family of protozoan parasites that are predominately monoxenous (development restricted to one host species). Nonetheless, some trypanosomatids occupy a dixenous niche [3]. Plant-infecting trypanosomes are grouped under the single genus *Phytomonas* example of a dixenous trypanosomatid. However, the trypanosomatids were extensively studied thanks to two genera of human pathogens *Trypanosoma* and *Leishmania.* They are obligatorily dixenous, possess zoonotic or anthroponotic life-cycles, and are transmitted by hematophagous insects. Until 2001, monoxenous trypanosomatids had been identified from roughly 350 insect species only, while more than 900 vertebrate hosts had been identified for the dixenous genera [4]. The monoxenous trypanosomatids infect a broad range of insects, including those of the orders Diptera, Hemiptera, Hymenoptera and Siphonaptera [5]. Invertebrate hosts of monoxenous trypanosomatids may become infected *via* multiple routes including ingestion of cyst-like amastigotes from the faeces of other infected hosts [6], food sharing, predating other infected insect species, or cannibalism [7]. *Trypanosoma* species employ one of two methods of development within their invertebrate host, termed Salivaria and Stercoraria. Salivaria is characterised by development within the frontal portion of the invertebrates' digestive system and transmitted through the bite of an insect. Stercoraria is characterised by development of parasites within the posterior region of the invertebrates' hindgut and transmitted through the excretion of faeces [8,9]. Wildlife has been a major source of infectious diseases transmissible to humans [10,11]. The zoonoses or infectious diseases are transmissible between animals and humans [12], with a wildlife reservoir representing an important public health problem in all continents. It is thought that trypanosomatids had a single origin as exclusively insect-borne parasites and later become digenetic parasites when vertebrates emerged since the Mesozoic era 230 mya [13]. The top-

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ics chosen for this review show some aspects in common among them that have been decisive for classifying this group of parasites (Kinetoplastid parasites). It stands out in this group of parasites its ubiquity in the different continents and the enormous diversity of hosts. The high economic cost for the developing countries of the research and control of the diseases they cause in their hosts (Environmental and economical impact of trypanosomatids; *Phytomonas*). We also wanted to emphasize that the introduction of these parasites has had devastating consequences on the local host populations of some communities (Trypanosomatids in emerging environmental health situations). The presence of these parasites generates in their hosts a great diversity of clinical manifestations (*Leishmania*). Despite being evolutionarily primitive of asexual reproduction they have developed molecular mechanisms of genetic recombination with which they respond efficiently to environmental changes (Genetic variability by recombination within trypanosomatids; Mechanism of trypanosomatids response to environmental changes).

Kinetoplastid Parasites

Kinetoplast DNA (kDNA) is the most structurally complex mitochondrial DNA of the order Kinetoplastida; kDNA is a giant network of thousands of catenated circular DNAs. The kDNA circles are of two types, maxicircles and minicircles. Maxicircles usually range from 20 to 40 kb, homogeneous in sequence, depending on the species, and are present in 20-30 identical copies/cell. Minicircles, present in several thousand copies per network, are usually nearly identical in size (0.5 to 2.5 kb, depending on the species), and are frequently heterogeneous in sequence. Maxicircles encode typical mitochondrial gene products (e.g. rRNAs and subunits of respiratory chain complexes), but remarkably, some of the protein-coding genes are encoded. To generate functional mRNAs, transcripts undergo posttranscriptional modification via an intricate RNA editing process that involves insertion and deletion of uridine residues at specific sites in the transcripts [14,15]. The genetic information for editing is provided by guide RNAs (gRNAs) with about 50-70 nucleotides, that are mostly encoded by minicircles, with few in the maxicircles. Different trypanosomatids have different numbers of gRNA genes/minicircles (1-4), therefore since a species require many different gRNAs for editing; it also has a diverse set of minicircle sequence classes, each with a certain copy number in an individual. kDNA is fragile, sometimes altered or even partially or completely lost in nature, becoming diskinetoplastic (Dk) or akinetoplastic (Ak). This has led to the notion that kDNA is dispensable for certain stages of the life cycle or species of trypanosomatids, especially in the salivarian trypanosomes such as *Trypanosoma evansi.* The loss of kDNA sequences in Dk or Ak strains results in the loss of function of mitochondrial genes encoded in maxicircles. Notably, all Dk trypanosome strains are able to survive with glucose as the only source of energy in the bloodstream forms of the vertebrate host, but not as in the procyclical forms present in the vector insect where mitochondrial activity generates ATP [16].

Environmental and Economical Impact of Trypanosomatids

Numerous economically important agricultural crops such as almonds, apples, melons and another plant species that enrich the diversity of both agricultural and non-agricultural landscapes are pollinated by the western honey bee *Apis mellifera.* Increased annual losses of commercially managed honey bee colonies have been associated with higher pathogen among them it is possible to mention the trypanosomatid parasite *Crithidia mellificae.* It is now appreciated that *C. mellificae* likely infects *A. mellifera* throughout the globe [17]. *C. mellificae* was discovered in Australia and has subsequently been detected in *A. mellifera* samples from the USA [18,19] Belgium [20], China [21], and Japan [22]. *Leishmania* species are other trypanosomatids with clinical manifestations for diverse mammals and also present in reptiles (*L. tarentolae*). Animal- and human-infecting trypanosomes are grouped in several clades. They cause diseases in humans and animals, causing severe economical loss among which the following are particularly important: a) *Trypanosoma brucei* causes African sleeping sickness; the East African form of disease (Rhodesian sleeping sickness) is acute, while the West African form (Gambian sleeping sickness) is more chronic. It is restricted to tropical Africa and largely rural areas. Wild and domestic animals act as reservoir hosts of disease, which is transmitted and developed in the salivary glands of the tsetse fly. Case finding and treatment of African trypanosomiasis cost $12-24 as measure by disability-adjusted life-years (DALY) averted in developing countries [23]; b) *Trypanosoma evansi* is an Ak trypanosome that causes a disease named Surra; it has the widest geographical distribution worldwide and exhibits highly variable clinical effects, depending on the host and the geographical area. Surra disease affects a large number of wild and domesticated animal species in Africa, Asia, and Central and South America. The principal host species varies geographically, but camels, horses, buffalos and cattle are particularly affected, although other animals including wildlife are also susceptible. Several species of haematophagous flies, including Tabanids and Stomoxes, are implicated in transferring infection from host to host, acting as mechanical vectors. These characteristics make Surra not only a multispecies but also a polymorphic disease [24]; c) *Trypanosoma vivax*: This is a heteroxenous parasite infecting camelids and domestic animals, present in areas of tse-tse flies and develops in the proboscis of the vector. However, outside of tse-tse areas such as in South America the parasite is carried by other hematophagous insects (stable flies) where transmission is non-cyclical [25]; the

parasite is transmitted mechanically with bloodstream forms. d) *Trypanosoma congolense*: It is genetically related to *T. brucei* but evolutionary distinct, as the life cycle does not involve infection of the salivary gland of the tse-tse fly. e) *Trypanosoma cruzi*: This is a stercorarian trypanosome transmitted by insect feces. *Trypanosoma cruzi* causes Chagas' disease, with an initial acute phase of several weeks that subsides into chronic phase which may continue for decades and can generate in human mega-organ syndromes as megacolon, or heart damage and sudden death. The disease remains the most important parasitic disease in the Western Hemisphere, with an estimated disease burden, as measured by DALY, which is 7.5 times as great as that of malaria [26]. A substantial proportion of the burden emerges from the loss in productivity due to cardiovascular alterations [27]. Opossums and other wild animals of South America are particularly important in this zoonotic infection. The vectors are triatomine bugs of several genera. f) *Leishmania* is very diverse all over the world. Their primary hosts are vertebrates; *Leishmania* commonly infects hyraxes, canids, rodents, and humans. Case finding and treatment of leishmaniasis cost $11-22 per DALY averted in developing countries [23]. The relationships between trypanosomes showed them to be monophyletic, with the genus split into eight well supported clades [28]. These include: 1) The aquatic clade, comprising trypanosomes of mainly aquatic and amphibious vertebrates; 2) The *T. cruzi* clade, which includes several infective mammalian trypanosomes (*T. cruzi, T. rangeli*, bat trypanosomes from both the Old and New Worlds and a trypanosome from an Australian kangaroo); 3) The *T. brucei* clade, many of which are pathogens of domestic livestock; 4) The *T. lewisi* clade, which contains trypanosomes from a wide range of rodents, a lagomorph and an insectivore; 5) The *T. theileri* clade, which contains trypanosomes from marsupial and placental mammals; 6) The *T. avium* clade ; 7) The '*T. corvi*' clade; and 8) The 'lizard clade', which contains trypanosomes of squamate reptiles. According to recent molecular phylogenetic studies, bird trypanosomes form three distinct clades named after the principal species: *T. avium*, *T. corvi* and *T. bennetti*. The vectors of avian trypanosomes are blackflies, hippoboscid flies and mosquitoes. Phylogenetic trees clustered the snake trypanosomes together in a clade closest to lizard trypanosomes, forming a strongly supported monophyletic assemblage (i.e., lizard- snake clade) [29,30]. Trypanosomes have adapted to all classes of vertebrates and to a variety of invertebrate blood-sucking leeches and bedbugs, some of which act as vectors [31-33]. Closer examination of the trypanosome hosts in each clade suggests absence of strict co-speciation with the vertebrate host. One example is the parasite *T. cruzi*, which is transmitted by blood-sucking insect vectors (*Reduviidae*, subfamily *Triatominae*) to a variety of vertebrates including small mammals and humans. *Cavernicola pilosa* transmits *T. cruzi marenkellei*, *T. dionisii* and *T. vespertilionis* exclusively to bats, the two first present in the New and the last in the Old World.

Trypanosomatids in Emerging Environmental Health Situations

Increasing interaction between wildlife and humans or domestic animals may lead to disease emergence and require innovative methods and strategies for disease surveillance and management in wildlife. This apparently increased involvement of wildlife in livestock and human disease is likely due to several changing factors, most of them anthropogenic. When animals were translocated ago into a new environment only 100-200 years, not just a single species was introduced but rather an entire micro-ecosystem consisting of the target species and all its accompanying parasites, such as *T. evansi* and *T. vivax*. In both cases the transformation into the Dk and eventually Ak cells enabled these trypanosomatids to spread out of Africa and become the most dangerous and widespread trypanosomes. Any significant change occurring in the environment and management of the population, or even other populations in the same ecosystem, may imbalance the equilibrium of the host, infectious agent and environment, allowing a newly introduced agent or subclinical infection to manifest as an emerging disease [34]. Parasitic diseases by nature are chronic and difficult to detect in host populations, often able to use several host species, and easily transported in infected hosts that show no overt symptoms of infection. One case is *T. evansi* in pigs, which are common in Papua New Guinea. Rats and bats were the only placental mammals on the island until pigs were brought in by humans [35]. Another example is in Australia. Its long isolation resulted in the evolution of a unique, extremely rich and varied native fauna that is potentially susceptible to introduced parasites [36,37]. Concern has been expressed that should *T. evansi* be introduced into Australia it could devastate native mammalian fauna [38]. Many small and medium sized mammal species, for example, those were once widespread across the continent are now restricted to isolated areas in the southwest or on offshore islands. In the last 10 years there has been a further decline of many of these native mammals, with evidence that parasitic diseases may be implicated [39]. Longitudinal molecular ecological studies have demonstrated an overall pattern of widespread distribution, with *Trypanosoma* genotypes/species occurring in many different host species, often at high prevalence, on the mainland of Australia as well as on off-shore islands [40]. Another emerging situation occurs with Chagas disease in non-endemic countries free of vectors and with low prevalence, which now report a growing number of cases [41,42]. This is the consequence of increased human immigration worldwide in the last decade *via* congenital transmission. There is a great

challenge for clinicians in non-endemic countries to be able to recognize the disease symptoms and perform diagnosis and treatment. The recognition of species of trypanosomes has represented great difficulty, but now the development of numerous molecular techniques has facilitated this goal. *T. vivax* in the New World is an example of a pathogen that has spread beyond its original distribution range though human intervention-both in spanning the thousands of miles between Africa and South America and in propagating itself on arrival. The parasite has now spread to ten of the 13 countries of the South American continent, often resulting in severe wasting disease and death. The ancestral monoxenous lifestyle of trypanosomatids evolved at least three times independently into a dixenous strategy, in *Trypanosoma*, *Leishmania* and *Phytomonas* [43,44].

Leishmania

Over 20 *Leishmania* species have different animal reservoirs and insect vectors (over 90 sandfly species). The parasites have two main forms in their life cycle: Promastigotes in the insect gut, and amastigotes that infect the lymphoid macrophage system of the mammalian host. These species generate three main forms of leishmaniasis: visceral (VL), cutaneous (CL), and mucocutaneous.

The distribution of leishmaniasis given by WHO [45] is the following

African Region: Visceral, cutaneous and mucocutaneous leishmaniasis are endemic in Algeria and countries in East Africa which are highly endemic. In East Africa, outbreaks of visceral leishmaniasis occur frequently. Region of the Americas: The epidemiology of cutaneous leishmaniasis in the Americas is very complex, with variations in transmission cycles, reservoir hosts, sandfly vectors, clinical manifestations and response to therapy, and multiple circulating *Leishmania* species in the same geographical area. Brazil represents over 90% of the VL cases in that region. Eastern Mediterranean Region: This region accounts for 70% of the cutaneous leishmaniasis cases worldwide. Visceral leishmaniasis is highly endemic in Iraq, Somalia and Sudan. European Region: Cutaneous and visceral leishmaniasis are endemic in this region. There are also imported cases mainly from Africa and the Americas. South-East Asia Region: Visceral leishmaniasis is the main form of the disease in this region, also endemic for cutaneous leishmaniasis. The region is the only one with a regional initiative to eliminate visceral leishmaniasis as a public health problem.

Leishmania tropica and *L. major*, and *L. mexicana, L. brasiliensis* complex presumably diverged before the breakup of Gondwanaland some 80 mya ago [46,47]. The relevance of identification of *Leishmania* species will depend on the biology of the parasite and the epidemiology of the disease. Clinical applications are relevant in leishmaniasis since established links exist between some *Leishmania* and disease severity and treatment outcome [48]. *L. donovani* causes visceral disease; *L. infantum* is the causative agent of infantile visceral leishmaniasis [49]; in Latin America has been called *Leishmania chagasi* [50,51]. It is also an unusual cause of cutaneous leishmaniasis [4], which is normally caused by specific lineages (or zymodemes). Wild canids and domestic dogs are the natural reservoir of this organism *L. tropica* causes cutaneous leishmaniasis (oriental sore); *L. mexicana* and *L. braziliensis* cause muco-cutaneous leishmaniasis. Dogs and rodents are particularly important in zoonotic infections which are transmitted by sandflies of the genus *Phlebotomus* and *Lutzomyia.* In visceral leishmaniasis, *L. infantum and L. donovani* have different epidemiological profiles, being transmitted zoonotically and anthropologically, respectively. Therefore treatments of the disease depend on rapid, accurate identification of the *Leishmania* species in biological samples. *Leishmania* can be divided into four morphologically similar main groups, including the visceral and cutaneous types that infect man, geographical provenance, definitive hosts and the sand fly vector. Human leishmaniasis causes a wide range of clinical symptoms. Lesions of cutaneous leishmaniasis caused by *L. tropica, L. major* and *L. aethiopica* often heal spontaneously, whereas muco-cutaneous leishmaniasis caused by *L. brasiliensis brasiliensis* and *L. mexicana* tend to metastasize, causing terrible disfigurement and even death. Visceral leishmaniasis or Kala Azar due to *L. donovani* has a high mortality rate, as does *L. infantum* and *L. chagasi*.

Phytomonas

Phytomonas are a ubiquitous and diverse group of plant parasites that exhibit both pathogenic and endophytic lifestyles. Species of the genus *Phytomonas* are found in a wide range of geographical areas, including North and Central Africa, China, India, several European countries and the American continent [52-54]. *Phytomonas* spp. is adapted to sap-sucking insects as primary hosts and plants as secondary hosts [55]. *Phytomonas* spp. was first described from the latex of Mediterranean spurge (*Euphorbia pilulifera*) [56]. Currently, the genus *Phytomonas* includes more than 200 species that colonize over 20 plant families [57]. The transmission to plants occurs through the saliva of phytophagous hemipterans. When these insects become infected while feeding on an infected plant, the parasites colonize the midgut; once a midgut infection is established they cross the midgut epithelium, thereby gaining access to the hemocoel of the lymph, when systemic infection occurs. In the final step of insect colonization, parasites then bind to and invade the insect salivary glands [58]. They are distributed primarily in tropical and subtropical zones, multiplying in latex tubes, fruits and seeds or colonizing the phloem sap inside the sieve tubes. *Phytomonas* infection can occur without apparent pathogenicity, but conversely it can cause lethal

disease in plants of substantial economic value, including the coffee tree, coconut and oil palms. This results in important economic losses in Latin America and the Caribbean [59]. The species definitively known to cause plant disease are: *P. staheli* causes palm wilt and *P. leptovasorum* causes coffee phloem necrosis; both are problematic in areas of South America. *P. staheli* and *P. leptovasorum* exclusively inhabit the phloem during the plant stage of their life cycle. Individual *Phytomonas* species may be spread between plant hosts by a broad range of different insects. However, the natural vector was shown to be the nocturnal coreid spurge bug *Dicranocephalus agilis* [52]. The earliest descriptions of *Phytomonas* noted that these parasites exhibit extreme morphological polymorphism. The majority of *Phytomonas* species isolated exhibit promastigote morphology [60]. Analysis of the principal salivary glands of the predatory bug *Troilus luridus* revealed four major morphotypes corresponding to the developmental stages of *P. nordicus*: free promastigotes, promastigotes within vacuoles, haptomonads attached to the microvilli of the salivary gland epithelium, and endomastigotes [61]. During the infection of *Oncopeltus fasciatus* with *P. serpens*, the parasites multiply in the hemolymph and modify their morphology inducing long slender parasites, a common morphotype observed during the life cycle of *Phytomonas* species [62]. A unifying feature of phylogenetic analyses of *Phytomonas* is that there appears to be large diversity within the group. For example, the kDNA sequence data shows that the divergence between the phloem-limited *Phytomonas* HART1 and the fruit parasite *Phytomona serpens* is greater than that spanning all sampled *Leishmania* species and similar to the divergence between *T. cruzi* and *T. brucei*. The *Phytomona* nuclear genomes analyzed to date are much smaller than those of their *Leishmania* and *Trypanosoma* relatives; for example, the genome of *Phytomonas* EM1 is 17.8 mega bases (Mb) in comparison to 32.9 Mb of *L. major*, 62.3 Mb of *T. brucei* and 32.5 Mb of *T. cruzi* [63-65]. Analysis of the genes encoding metabolic proteins in the *Phytomonas* genome sequences revealed a cohort of enzymes consistent with life in a plant environment. Both HART1 and EM1 genomes encode glucoamylase, alpha-glucosidase, and alpha-trehalose phosphorylase genes, allowing them to utilize plant carbohydrates. Interestingly, only the phloem-restricted pathogen *Phytomonas* HART1 encodes invertase genes for degradation of the disaccharide sucrose. Another remarkable feature of *Phytomonas* parasites is the loss of genes encoding the cytochrome c oxidase subunits I-III (COI, COII, COIII), and cytochrome b (cytB) of the bc1 complex, as in the Dk *T. evansi* [66,67].

Genetic Variability by Recombination within Trypanosomatids

The genetic variability of the parasite is fundamental to understand trypanosomatids biology and diversity and the evolution of meiosis in these ancient eukaryotes. Drug resistance to the available trypanocides is an increasing problem for trypanosomes infecting cattle and its spread is a major concern for the sustainable control of diseases. Thus the existence of mating would also be important at a practical level in terms of the spread of such traits. Trypanosomatids are diploids and do not generate gametes as other more evolved eukaryotic organisms, therefore genetic variability can be explained by point mutations during the clonal reproduction event or by recombination and hybridization events. Probably these last events generate more variability than that obtained by point mutations.

The case of *Trypanosoma brucei* and *Trypanosoma congolense*

Sleeping sickness in Africa affects large mammal fauna. The outbreaks of sleeping sickness correlate with massively infected tsetse flies which transmit trypanosome parasites. Huge variability was found in the *T. brucei* isolates circulating in the flies. A genetic exchange could satisfactorily explain the great diversity of *T. brucei* isolates and the appearance of new human infective isolates associated with outbreaks [68,69]. However an alternative explanation is that selection was the major factor in generating parasite diversity, since large deviations from Hardy-Weinberg equilibrium were found; this is observed mostly in organisms with clonal reproduction. The first prerequisite for mating to occur in trypanosomes is a doubly infected fly. Such a fly would probably have fed on a mammalian host with a mixed infection, most probably in a wild animal, rather than humans, the favored tsetse food. It is currently accepted that reproduction involving meiosis explains *T. brucei* genetic variability. The question is how frequently this kind of event occurs in nature. Whether *T. congolense* also undergoes mating is unclear because evidence on this question is limited. However *T. congolense* is clearly evolutionary distinct from *T. brucei*, as the life cycle does not involve infection of the salivary glands of the tsetse fly but proliferate in the insect. The population structure of *T. congolense* clearly reveals genetic heterogeneity, high levels of heterozygosity and linkage disequilibrium, which leads to the conclusion that this species reproduces fundamentally in clonal form, but with occasional recombination events, currently a subject of interesting debate [70-72]. In other parasites such as the agent causing malaria, *Plasmodium falciparum*, sex is obligatory part of the life cycle; mating occurs during every transmission through a mosquito vector, and where reproductive clonality readily occurs. In trypanosomes, clonal refers to populations with a few predominant genotypes and in which genetic recombination occurs very rarely and is not obligatory during vector transmission. Returning to the *T. brucei/T. congolense* question, it seems that in *T. brucei* genetic recombination is more frequent than in *T. congolense*, since the former recombine in the salivary gland where

conditions for mating are more appropriate.

The case of *Trypanosoma cruzi*

The interaction between *T. cruzi* and its invertebrate host have been explored studying life history outcomes with different *T. cruzi* genotypes [73]. Structured genetic diversity in *T. cruzi* is designated as divergent taxonomic units or subgroups, with variation defined as discrete typing units (DTUs). Six DTUs have been described (TcI-TcVI) [74]. TcI is the most abundant and widely dispersed. TcI and TcII are the most ancient, heterogeneous and with smaller genome sizes [75,76]. TcIII and TcIV have genetic characteristics of hybrids between TcI and TcII that homogenized their genomes. TcV and TcVI have characteristics of a hybrid of TcII and TcIII [77]. These results demonstrate that hybrid generation is consistent with the hypothesis that genetic recombination has occurred more than once within *T.cruzi* genomes. There are estimations of the reconstruction of the evolutionary history of *T. cruzi* by nucleotide sequences from several unlinked loci. The estimations demonstrated that the current extant of *T. cruzi* derived within the last 3 million years and the major hybridization leading to the hybrid TcV-TcVI occurred less than 1 million years ago [78]. However another study estimated the origin of *T. cruzi* hybrids TcV-TcVI within the last 60,000 years [75]. After the early nuclear fusion mechanism [79], the two parental nuclei fuse, resulting in polyploid progeny that can undergo recombination between alleles which later through subsequent chromosomal loss and nuclear erosion eventually tend to return to the diploid stage. This so-called parasexual pathway resembles the mechanism of genetic exchange observed in certain fungi [80]. Experimenal *T. cruzi* hybrids appear to be aneuploid, containing 1.6-1.7 times more DNA than the parental cells.

What is known about mitochondrial genomes among the resulting *T. cruzi* hybrids? The uniparental inheritance of kinetoplast maxicircle DNA (mitochondrial DNA) emerged after experimental fusion of *T. cruzi* [79]. Describing the diversity in a large panel of TcI populations with nuclear loci and mitochondrial loci with a highly resolvable multilocus sequence of maxicircle revealed incongruent phylogenetic histories among populations from different geographic regions [81,82]. The results also provided evidence of heteroplasmy (heterogeneous mitochondrial genomes or maxicircles) in a *T. cruzi* individual parasite. Both kinds of result are indicative that genetic recombination is geographically widespread in nature. A similar result has been obtained in the copies of minicircles of *T. brucei* kDNA which are inherited from both parents in the genetic hybrids [83].

The problem among *Leishmania*

Cell fusion between promastigote forms of *Leishmania* has been recorded by videomicroscopy. Suspected or definite hybrids have been found in nature with heterozygous isoenzyme patterns and pulse field electrophoresis studies (molecular karyotypes) with *Leishmania* isolates. Amphimixis, the sexual reproduction between two unrelated parasite populations, and conjugation, a process not involving gametes but rather the temporary union or fusion of two single cells with exchange of genetic material, seems to explain *Leishmania* variability. However, amphimixis between unrelated parasite populations may be greatly limited in nature since double infestations of mammal hosts or vectors (sand flies) by different parasite genotypes probably are rare in hypoendemic areas. Therefore this model of genetic variability for *Leishmania* seems infrequent or rare [84,85].

Mechanism of Trypanosomatids Response to Environmental Changes

Trypanosomatids do not regulate gene expression at transcription, therefore they can only down-regulate their expression or not. However, they encountered a solution by means of gene amplification. Under conditions of extreme stress *Leishmania* may even amplify single-copy genes and obtain a higher quantity of a specific protein. Regulation of this process could involve pre-mRNA processing, mRNA stability and translation, protein stability and/or post-translational control. In experimentally-induced drug resistant *Leishmania* strains, it is common to encounter circular episomes resulting from the amplification of short chromosomal regions containing key enzymes of nucleotide metabolism of DNA (dihydrofolate reductase and thymidilate synthase). Over-expression of specific genes also appears to be the main mechanism underlying clinical resistance by *Leishmania donovani* to first-line drugs like antimonials [86].

In ancient eukaryotes as yeast and fungus-related organisms a conserved pathway has been described named stress protein activated kinases (SPAK) which involved MAPK protein and AP-1-like transcription factor [60]. In the case of trypanosomatids, they must respond to extracellular and intracellular signals as they adapt rapidly to new environmental within their varied hosts. The molecualar mechanisms of parasite response to oxidative stress, heat shock and other environmental changes are still unknown. However the major mechanisms of gene regulation in trypanosomatids appear to be post transcriptional since transcriptional units are polycistronic and promoters do not have regulatory sequences as in higher eukaryotic organisms [87]. One example is *T. brucei* under rapid shifts to low temperature which allows differentiation of bloodstream to procyclic forms [88]. Studies on the same *T. brucei* from procyclic differentiation to mammalian infective forms after heat shock shows interesting changes in the compartmentalization of mRNA, from untranslated and associated with proteins from the cytosolic fraction to polysomal fraction after 1 hour of heat shock treatment

[89]. Several proteins are involved in this mechanism of mRNA storage and degradation of non-translated mRNAs [89]. These factors are RNA-binding proteins and the hallmark of mRNA stabilization, affecting the gene expression in trypanosomatids. One of these is the protein ZC3 H11 which binds to the 3'-UTR of chaperone mRNA and is required for both target mRNA retention and for cell survival after heat shock [90,91]. ZC3 H11 has been shown regulated by phosphorilation, a rapid mechanism of protein modification by protein kinases [92]. Small Heat Shock Protein (sHSPs) bearing alpha crystalline domains (ACD) participated in defense against heat and oxidative stress and play important roles in cell cycle, cytoskeleton dynamics and protein translation in eukaryotes. ACD-like proteins (ACDP) were investigated in 61 species of 12 kinetoplastidic genera. Phylogeny and genome organization revealed a kinetoplastid evolutionary repertoire and proteomic profiles suggested that most ACDPS may be species and stages regulated [93]. When *T. cruzi* changes from one to another host in the presence of an oxidative burst is another example of rapid adaptation to oxidative stress. It has been shown that phosphorilation of DNA polymerase β is activated few minutes later after an acute exposition to hydrogen peroxide [94].

Concluding Remarks

It has been suggested that parasite evolution is influenced by biology and opportunity [95]. It is now widely accepted that novel infectious disease can be a leading cause of serious population decline [96]. In the case of mammals, however, there are still no well-corroborated instances of such diseases having caused or significantly contributed to the complete collapse of species. However, during 2008 the first molecular evidence for a pathogen emerging in a native mammal species immediately prior to its final collapse was reported [97]. It is also important to note that thanks to advances in molecular biology and phylogenetics, the taxonomy of trypanosomatids has been corrected by assigning some of these parasites to different genera and establishing some new genera [98]. Despite these advances, the systematics of the trypanosomids still remain imperfect and propose new challenges to scientists interested in this topic.

There is no unconditional acceptance which factors definitely affect the ecology of the parasite, vector competence and parasite interactions within an intermediate host or permanent rather keep in mind that global issues including climate change, population migration, environmental changes and other factors serve to exacerbate parasite zoonoses and this problem continues to have a significant impact on public health throughout the world [99].

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