Trends in Parasitology

CellPress REVIEWS

Review

Beyond Blood: African Trypanosomes on the Move

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While the African trypanosomes are among the best-studied parasites, almost everything we know about them is based on the *brucei* group, which includes the human-infective sleeping sickness parasites and the causative agent of the cattle plague nagana. The past decades have seen an ever-more detailed molecular dissection of *Trypanosoma brucei*, which today is an accepted cell biological model system. Therefore, recent work on some fundamental aspects of trypanosome biology surprises, as we realise that our knowledge about parasite motility and tropism in the changing host microenvironments is far from definitive. In this review, we highlight a few examples of neglected parasitological questions, which may open (or reopen) a new chapter of trypanosome research.

Highlights

The bloodstream may not be the primary habitat for bloodstream trypanosomes.

The interstitial system may serve as a systemic and well-connected trypanosome niche.

The motion pattern of the diverse trypanosome life cycle stages represent adaptations to changing microenvironments.

Little is known about life cycle stages in the brain.

The tsetse fly is a tractable model system for studies on the mechanobiology of host–parasite interactions.

Parasites Are Motile and Manoeuvre to Spread in Their Hosts

Parasites are expert travellers. They explore their environment by swimming or crawling, to infest their habitats once the destination has been reached. At first sight this seems trivial. But do we really know how parasites navigate, and how the life conditions in the rather diverse microenvironments influence their motion? When compared to free-living species, parasites thrive in small worlds. Their host habitats are secluded, largely homeostatic, and thus fairly predictable. Furthermore, parasites deal with a comparatively limited set of cues. This does not mean that they live in safe havens though. On the contrary, the host body provides diverse and very hostile environments with extreme conditions. While much is known about immune and inflammatory responses, we rarely consider the mechanics and physicochemistry of the parasites' world. How do they explore their natural surroundings? Do they communicate in the host, and if so, how? How do they tackle physical barriers? For these tasks and many more, motility is decisive. African trypanosomes are wonderful examples for the multifunctional roles of flagellate motion. Throughout their life cycle, they show varying motility patterns, as adaptive responses to the changing microenvironments in the mammalian host and the tsetse vector [1,2]. Trypanosomes probe their surroundings with the free tip of their flagellum, which is thought to be armoured with a battery of sensors and receptors [3]. They swim responsively, which means that they can switch gears, accelerating, halting, and reversing direction instantaneously [4]. Trypanosomes can move collectively, at least in the fly [1]. The mammalian stages exploit directional swimming to escape the immune system [5]. And without the mechanical forces exerted by the incessant beat of the cell body-aligned flagellum, the cells could not divide properly [6].

Trypanosomes are considered prototypic blood parasites. While this is true, a significant portion of the complex life cycle takes place in the tsetse fly (Figure 1, Key Figure). In fact, we should ask how important blood actually is as a trypanosome habitat. In other words, are African trypanosomes really obligate blood parasites? In this review, we focus on motility in the context of trypanosome development in the changing host habitats. We summarise recent advances, but also highlight work, conducted many decades ago, that somehow escaped our radar.

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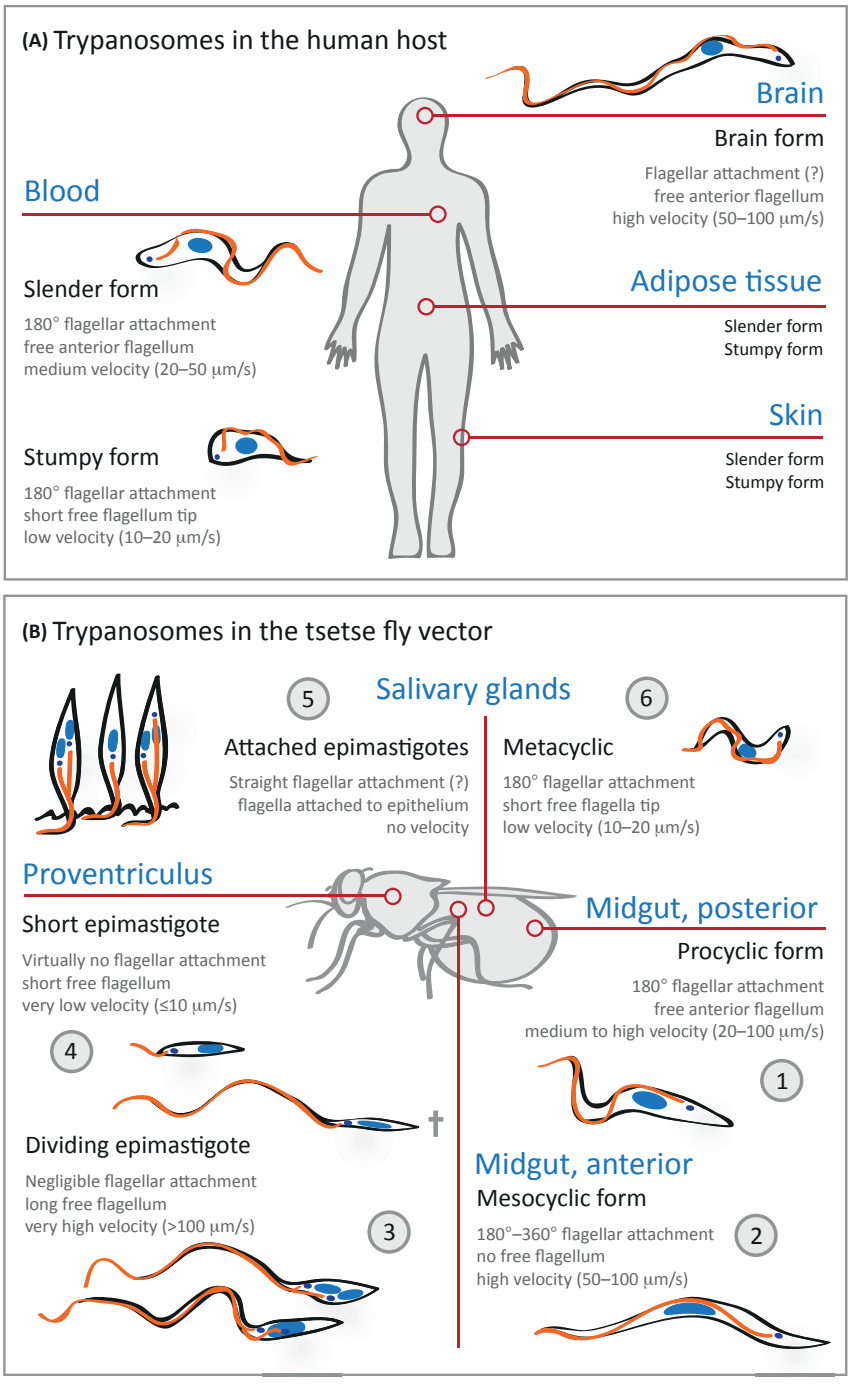
Key Figure

Developmental Stages of the *Trypanosoma brucei* Life Cycle



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Glossary

Ectoperitrophic space: the volume within insect midguts that is separated by the peritrophic matrix from the food bolus.
Interstitial space: contains the main part of extracellular body fluid and fills the spaces between cells and tissue-specific structures.
Peritrophic matrix: a dynamic extracellular sheet that lines the midgut of the tsetse fly and other insects.
Proventriculus: a muscular organ that is connected to the crop of some insects (such as the tsetse fly) and assists with the grinding of food.

The Trypanosome Swimming Apparatus

The structure of the *T. brucei* flagellum and its attachment to the cell body have been concisely reviewed [7,8].

The basic morphological architecture is modified in a series of developmental stage transitions, possibly in order to adapt to specific niches of the host bodies. The dynamic extension of the microtubule corset allows the repositioning of the flagellar pocket with the connected basal body along the anterior–posterior axis. This changes the emergence point of the flagellum and its course along the cell body from the posterior end. In addition, growth, and the extent of the free anterior part of the flagellum, is variable. Furthermore, at least two divisions occur asymmetrically and result in different morphotypes, ranging from trypomastigote cells, with flagella attached to the largest part, to epimastigote cells with free flagella appending [1,9–11].

Whereas the morphological parameters of axoneme bending, paraflagellar rod (PFR) structure, flagellar adhesion (Box 1), and cytological diversity in trypanosomatids have been described individually, the motile functionality of all aspects combined has not been sufficiently considered. In order to explain the developmental and evolutionary adaptations to host microhabitats, the cellular waveform needs to be considered.

The Cellular Waveform Describes the Dynamic Pleomorphism of Trypanosomes

The complex three-dimensional movement of the various trypanosome stages has been analysed in some detail. Cellular waveforms describe the combined, effective oscillatory and rotational actuation relevant to the characteristic locomotion of a specific morphotype [1,2]. The chirality of the attached flagellum determines the rotational motion of trypomastigotes, while the flagellar wave that is generated by axonemal oscillations deforms the attached, elastic cell body with characteristic frequencies. These forces result in the eponymous auger-like propulsion of cells along helical trajectories during persistent forward swimming. The reversal of flagellar waves results in cell twisting and tumbling motion as well as changes in swimming path direction.

The differences in cellular waveforms should account for specific reactions of cells to environmental influences, that is, mechanical resistance changes, due to viscosity or tissues (Box 2).

The detailed cellular mechanisms regulating cellular waveform are still far from clear. Specifically, the influence of the flagellar wave on the microtubule corset of the elastic cell body, via the intricate PFR and transmembrane filament connectors, needs to be elucidated as a prerequisite for tackling further questions. How does the PFR influence flagellar wave propagation and

Figure 1. The major morphotypes are depicted with an indication of their niches in the hosts, mammals (A) and the tsetse fly (B). Data relevant to the motility and propagation capabilities of the parasites are listed. The directional swimming speed was classified according to the maximum measured swimming velocity under natural conditions. Drawings are based on original micrographs. Nuclei and kinetoplasts are depicted in blue, and the traced flagella are shown in orange (original data from [1,2,62]). The infection process in mammals is initiated with the trypomastigote, slender bloodstream form parasites that propagate in the circulation (A). In the course of infection, they are able to infest various tissues and organs, including skin, adipose tissue, and the brain, thereby varying their motile capabilities. At high parasitaemia, the cell cycle is arrested and slowly swimming stumpy form cells develop. The numeration in (B) represents the progression of development in the fly. The mammalian stage parasites, ingested during the bloodmeal, transform into procyclic form cells (1). These infest the midgut, where the cell cycle arrested, mesocyclic cells (2) develop in high numbers and migrate to the proventriculus. Here, the long and fast epimastigote form (3) develops; it divides asymmetrically to produce short epimastigote parasites (4), which, in turn, become attached epimastigotes in the salivary glands (5). The metacyclic cells (6) that differentiate in the salivary glands are finally expelled by the biting tsetse and injected into the skin of mammals.

Box 1. The Trypanosome's Swimming Apparatus

The core feature of the trypanosome's flagellum is the axoneme, the conserved force-producing structure of all cilia and flagella across the eukaryotic kingdom [41]. The analysis of axonemal fine structure and the composition of hundreds of proteins is ongoing [42,43], and the trypanosome is firmly established as a model organism for these purposes [44,45]. A recent study even utilised RNAi and tagging of conserved proteins in trypanosomes to elucidate the conserved axonemal function of specific proteins involved in human spermatogenesis defects and infertility [46].

The common features of the trypanosomal flagellum extend to the anchoring and construction platform of axonemes, the basal body [47], as well as the assembly of flagella by intraflagellar transport (IFT) [48], the discovery of which has defined a whole new era of ciliary research [49]. This research prominently concerns sensory and signalling features, of which there is some evidence in the trypanosome flagellum, but still surprisingly little direct proof [40].

Flagellar shape and movement are strongly dependent on the specific attachment to the paraflagellar rod (PFR), which is characteristic for flagellates belonging to the euglenida and kinetoplastida groups [50,51]. This highly organised, lattice-like structure is longitudinally attached to the axonemes microtubules and dynein motors between doublets 4 and 7 [52]. These connections result in a restriction of flagellar oscillations, are necessary for directional swimming, and offer potential control points for trypanosome motility [52–54].

The tandem arrangement of axoneme and PFR composes the trypanosome flagellum that emanates from the basal body, which is embedded in the flagellar pocket [9,55]. As in the ciliary organelles of other eukaryotes, the flagellar compartment is elaborately separated from the main cytoplasm and is surrounded by the flagellar membrane with its specific sensory equipment [40]. In most trypanosome stages the flagellar pocket is tightly attached longitudinally to a large extent of the cell body through transmembrane connections to a filamentous system, the flagellar attachment zone (FAZ; [56]). This zone includes four microtubules that are part of a subpellicular microtubule corset that establishes and maintains trypanosomal morphology.

direction? Does the PFR relay information from the environment to the axoneme? What influence does the tightly controlled connection to the cells microtubule scaffold signify in any given physical environment? How is the elasticity of the cell body specified by the microtubule corset? Generally, what combination of flagellum and trypanosome cell body is ideally adapted to which microhabitat in its hosts?

Box 2. In the Trypanosome's World, Viscosity Rules and Inertia Is Irrelevant

The world of microscopic organisms is characterised by low Reynolds numbers, which means that the dimension and the velocity of all swimmers therein are small in comparison to the viscosity of the surrounding fluid. Therefore, parasite microswimmers experience negligible inertia in the body fluids of mammals or flies. This, in turn, means that any reaction of the swimming cell to its environment is immediate. Thus, when the propelling force, for example, the flagellar beat, stops, the cell comes to an instantaneous standstill. And the microswimmer accelerates to full speed with the first beat of the flagellum. Thus, many large-scale phenomena are likely to be explained in a relatively straightforward manner, provided that data of sufficient spatiotemporal resolution are available [1,2,4,57].

Such data include the effects of mechanical resistance to the probing flagellum while it oscillates. Trypanosome velocity can be measured, beat for beat, and can be seen to diminish immediately when the flagellar tip hits an object frontally, or be significantly increased when the flagellum laterally rebounds from rigid objects to swim faster and in a more directional manner. In the no-inertia environment, the cells exhibit versatile manoeuvring capabilities that allow them to adapt to all kinds of fluid and solid environments, that is, habitats as diverse as the bloodstream, vertebrate tissue interstitia, the fly gut, and various other insect ducts and tissues.

It remains to be analysed how the parasites respond on the cellular level to the mechanical forces impinging on them. Then we can also ask to what extent they are able to react directly, or if they are mainly preadapted in order to be optimally flexible in a variety of body niches. In other words, versatility, as realised with the trypanosome cellular waveform, could be a major evolutionary advantage for extracellular parasites in the divers confined realms of their hosts and even directly drive the evolution of developmental life cycles.

Lifelong Motion

As strictly extracellular parasites, African trypanosomes do not feature an immotile life cycle stage, which distinguishes them from, for example, *Plasmodium*, *Trypanosoma cruzi* or *Leishmania*. An active flagellum is always present, albeit varying in length from a few μm to several tens of μm. Hence, the most obvious function of transporting a cell through body fluids is accomplished with largely varying efficiency [1,2].

Swimming in Blood

Trypanosomes have a salient ability to navigate in and out of the circulatory system. There are, however, only few studies that experimentally consider the bloodstream as a physical environment. Parasite motion has been mainly studied with cultivated parasites, which ignores the fact that blood is an extraordinarily crowded environment. Almost half of the volume is packed with cells, and blood fluidity critically depends on the response of red blood cells to hydrodynamic stress, that is, on their deformability and orientation in fluid flow. For example, when shear rates are low, the shape of erythrocytes promotes the formation of cell stacks, so-called rouleaux, which increases blood viscosity [12]. At higher shear rates, the erythrocytes are stretched and singled, which decreases the viscosity of blood [13]. Thus, the rheological properties of erythrocytes directly influence the functionality of blood [14,15]. In other words, the viscoelasticity of blood cells is crucial for blood flow. This is an important concept, as we know that trypanosomes instantly and dynamically react to changing viscosities and mechanical obstacles, at least in the laboratory [2,4] (Box 2). The parasites are subject to the same shear stresses experienced by the erythrocytes. While there are many studies on blood cells, we have no idea about the elastic properties of the trypanosome itself. As a matter of fact, the parasites squeeze through very narrow microfluidic channels and are very flexible in three dimensions, even under strong confinement. Thus, the dense subcellular microtubule corset and the attached flagellum with its paraflagellar rod must make bloodstream-stage trypanosomes virtually as flexible and robust as blood cells.

When compared to blood velocity, however, blood trypanosomes move slowly, that is, they are not able to actively outswim the flow prevailing in the circulation [4]. Hence, the benefit from directionally swimming in the blood is not the ability to manoeuvre towards a specific destination. It is rather the relative motion that is important, as this produces hydrodynamic drag on the parasite's cell surface, which pushes host-derived antibodies towards the posterior cell pole, where they are internalised and destroyed by localised endocytosis [5].

Although different species of African trypanosomes are able to survive in the bloodstream, they exhibit very different motility behaviours [2]. They show diverging reactions to viscosity and crowding effects and thus appear to be differentially evolved for movement in the vertebrate microhabitats. This correlates well with the preferential annidation of various species in the blood and lymphatic fluids, or in **interstitial spaces** (see Glossary) [2]. Actually, though *T. brucei* is a capable blood swimmer, it turns out to be outperformed by *T. vivax*, which is known for its vigorous motility and preferred occurrence in the circulation. *T. brucei* on the other hand, is a versatile species, equally well adapted to swimming in the bloodstream as well as in confined tissue spaces.

Leaving the Circulation

Common knowledge has it that trypanosomes are blood-inhabiting protists. This view however, is a long-standing oversimplification. Almost 50 years ago, L.G. Goodwin addressed the Royal Society of Tropical Medicine and Hygiene in a thoughtful (and entertaining) speech, in which one chapter was devoted to 'Trypanosomes in the tissues' [16]. In the same year, W.E.

Ormerod drew the attention to ' . . . the fact that in man the growth of the parasite occurs mainly in tissues' [17].

K.C. Willett and R.M. Gordan had demonstrated in 1957 that the majority of tsetse fly-inoculated trypanosomes become entangled in tissue spaces [18]. More than 50 years before, in 1911, W. Yorke had suggested that 'perhaps the tissue juices form a more favourable nidus for the growth of the parasites' [19]. Goodwin commented in 1970: 'The trypanosomes take to this environment like ducks to water. They multiply, and spread through the tissue spaces and the lymphatics and are soon to be found in swollen lymph nodes, not only in the drainage area of the chancre, but throughout the body.'. The above studies and their unambiguous results have largely disappeared from our radar, which may have several reasons. First, the continued use of rodents as model hosts for trypanosomes has provided a biased picture of the infection, an insight that was also remarked on by Goodwin: '(the occurrence of parasites in tissues is) . . . often forgotten if one is used to dealing with laboratory strains adapted to cause an overwhelming parasitaemia that kills a mouse in a few days.'.

Today, the use of more natural animal models is chiefly excluded, also due to ever increasing regimentations. An alternative would be going back to the roots by studying infections in the field. But natural infections are not only difficult to access, they are, by modern scientific standards, often uncontrollable. Another important obstacle that has prevented the analysis of trypanosome tropism is that even with state-of-the-art technology, tracing trypanosomes in tissues is very demanding, let alone attempting time-resolved quantitative analysis at single-cell resolution (see burning questions). Thus, it is of importance that a series of recent publications have redirected the attention to the extravascular occurrence of African trypanosomes. Their presence in the interstitial fluid of adipose tissue and skin has been elegantly analysed, and it has been shown that skin trypanosomes can be transmitted directly to tsetse flies, even if no parasites are detectable in the blood [20–23]. Likewise, trypanosomes have been observed on the 'lane to the brain' [24,25]. However, it appears that we have no clear picture of what tissue invasion actually means. The life conditions in tissue spaces are rather unexplored. Extracellular flow velocities have rarely been measured *in vivo*, but existing numbers are around 30–50 μm/s [26,27]. In such a range of fluid flow rates, trypanosomes can effectively navigate by swimming. Thus, when considering parasite motion, the extracellular space provides a completely different, and maybe more physiological, environment. Furthermore, the question arises whether adipose tissues or skin are actually secluded parasite niches. Per definition, the interstitium is a contiguous fluid-filled space between the skin and other body organs, including muscles and the circulatory system [28]. A recent publication has by far extended this view, suggesting that fluid-filled macroscopic spaces are possibly larger and more widespread than previously known and that they undergo intermittent or rhythmic compression [29]. According to these findings, the interstitium appears not simply as a network of silent, local waters, but rather as an extensive river system that traverses our body. The ultimate challenge would now be to follow individual parasites on all potential paths through the host system, not only analyse them in specific, supposedly secluded tissues (Figure 2, Box 3).

A holistic view of the parasitic transport infrastructure has always been of special interest for the brain stages of trypanosomiasis. In recent models of brain invasion, it is discussed which pathway trypanosomes might use, via the blood–brain barrier (BBB) or the blood–cerebral spinal fluid (CSF) barrier. Further, the reinvasion of the bloodstream (relapse) from specific hiding places in brain regions has been described, which is likely to occur along new, alternative

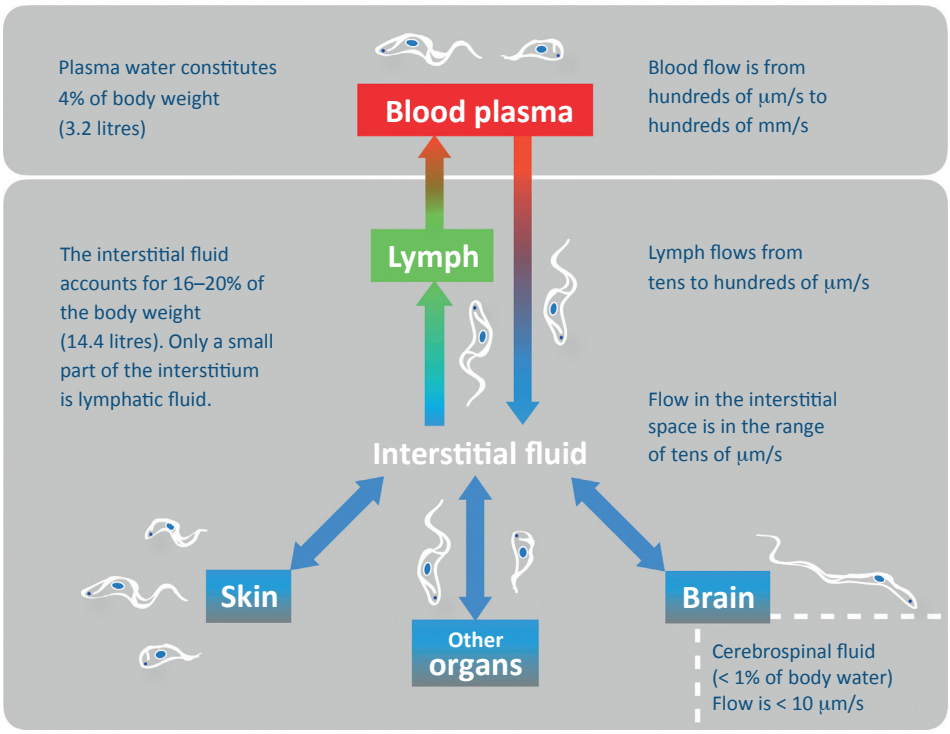

Figure 2. Volumes and Flow Velocities of Trypanosome-Containing Body Fluids. In adult humans, the intracellular space accounts for about 75% of body weight [63]. Of the remaining 25%, only 4% constitutes the blood volume. Thus, a very high proportion of extracellular body fluid is found in the interstitial space, of which the lymph occupies just a minor volume. The interstitium is continuous with the extracellular space of the grey and white matter of the brain [64], and partly drains into the cerebrospinal fluid. The flow regimes in the different body fluids vary over many orders of magnitude [28], challenging the swimming capabilities of trypanosomes, which have been found in all fluids.

Box 3. The Larger Volume: Interstitial Space and Lymph

The interstitium is the systemic fluid space between organs. It connects the skin with other body organs, and is actively drained into the lymphatic system. The interstitial fluid, together with the connected lymph, account for more than 8 litres of body fluid, more than twice the volume of the blood system. Fluid flow into the interstitium occurs through leaky blood capillaries, which are spread throughout the body. The interstitial fluid is actually at subatmospheric pressure [58], which means that hydraulic pressure drives fluid flow into the tissue spaces, and thus, opens an easy route for bloodstream trypanosome entry. The quantitative composition of interstitial fluid changes between tissues, which is another interesting aspect, as it renders the presence of (chemotactic) gradients likely. How wide the paths of the interstitium are remains an open question. The fact that long, slender bloodstream trypanosomes can turn in confinements of around 1 μm, however, means that the parasites can reach virtually any location within the interstitial space. Within the interstitial space a net fluid flow towards the lymphatics exists, which provides the system with a general topological directionality. When trypanosomes follow the interstitial flow, they will reach the lymph, and eventually end up in the blood circulation. The tissue spaces are actively drained by suction forces that are based on transient pressure drops of the lymph [59]. The physical connections between interstitium and lymph are characterised by the initial lymphatics, which feature disperse one-way valves, and the collecting lymphatics, which contain regularly spaced pumping valves, so-called lymphangions [60,61]. In the interstitium and the lymph, low Reynolds numbers prevail, that is, flow rates are low and turbulence does not exist. Thus, the lymphatic valves operate in the viscous regime, which is important for cell migration. Ultimately, the lymph (with the contained compounds, particles, and potentially parasites) is transported via many lymph nodes to the subclavian vein, where the fluid is centrally returned into the blood system.

pathways, that is, via the glymphatic system [24,30]. The fact that parasite populations seem to be able to reappear cyclically in the bloodstream, relocating from several organs, including the brain [30,31], reinforces the view of the host body as an optimally exploited continuous network of parasite niches.

A Long and Winding Ride through the Fly

The journey of trypanosomes through the tsetse fly has been studied for many decades. We have a clear picture of the different life cycle stages, and we also know in which parts of the definitive host they mainly reside [32] (Figures 1 and 3). The life cycle in the fly is a series of proliferating and quiescent stages, each one of which is subject to selection processes for further development, often described as bottlenecks that the parasites have to pass on their journey [33,34].

While most work has focused on the parasites in the tsetse, much less attention has been given to the fly itself, and even less to its microanatomy. The first steps of infection remain difficult to observe, as bloodstream form trypanosomes are sucked up into the crop and rapidly pumped into the flys gut. Especially, the apparently chaotic processes during and immediately after the blood meal exemplify what has been generally neglected so far in vector biology: the physical conditions in the insect alimentary tract, such as peristalsis and fluid flow. While the bloodstream form cells develop to procyclic forms in the tsetse gut lumen, they shed their surface coat of variant surface glycoproteins, which cause the production of the **peritrophic matrix** by the **proventriculus** to be compromised [35]. This presumably allows the early procyclic forms to enter the **ectoperitrophic space**, thus circumventing this pathogen defence. Forward swimming does not seem to be necessary for crossing this barrier, but is decisive for further development in the ectoperitrophic space, where active migration is required [36].

Recent work has illuminated the microenvironment of the tsetse digestive tract. Using multi-colour light sheet fluorescence microscopy, the amazing complexity of the fly's interior was detailed as well as the geometry of the host organs related to parasite morphology and swimming capacity [1]. The location of early and late procyclic stages was visualised inside the gut lumen or in the ectoperitrophic space, respectively. At the same time, the procyclic cells were shown to be perfectly adequate swimmers in the fly gut, mystifying us as to why motility is apparently not relevant at this stage of infection. Sparking even more curiosity, the only evidence so far for potential chemotactic behaviour of trypanosomes was found for early procyclic trypanosomes [37].

The first developmental process shown to require forward motility is the migration of mesocylic forms, which develop from the late procyclic forms in the ectoperitrophic space, from the posterior to the anterior midgut [36]. As soon as procyclic cells have crossed the peritrophic matrix they establish an infection in the posterior part of the midgut. Some of these cells develop further to the mesocyclic stage, which navigate all the way to the proventriculus. They are in close contact with the chitinous layer of the peritrophic matrix, which constantly changes its topology due to food uptake and peristaltic movement [1]. It is unclear how the parasites navigate on their journey to the proventriculus, but it has become obvious that there is more to it than swimming directly from A to B. For one, the complex three-dimensional topology of the heavily convoluted peritrophic matrix has been elucidated by light sheet microscopy, together with the localisation of trypanosomes between the intricate folds [5]. The analysis of migration of mesocyclic cells must consider the connectivity of these channels along the fly's midgut and the motile behaviour of the cells in varying degrees of confinement. Then, the degree of collective motion must be considered, as it is clear

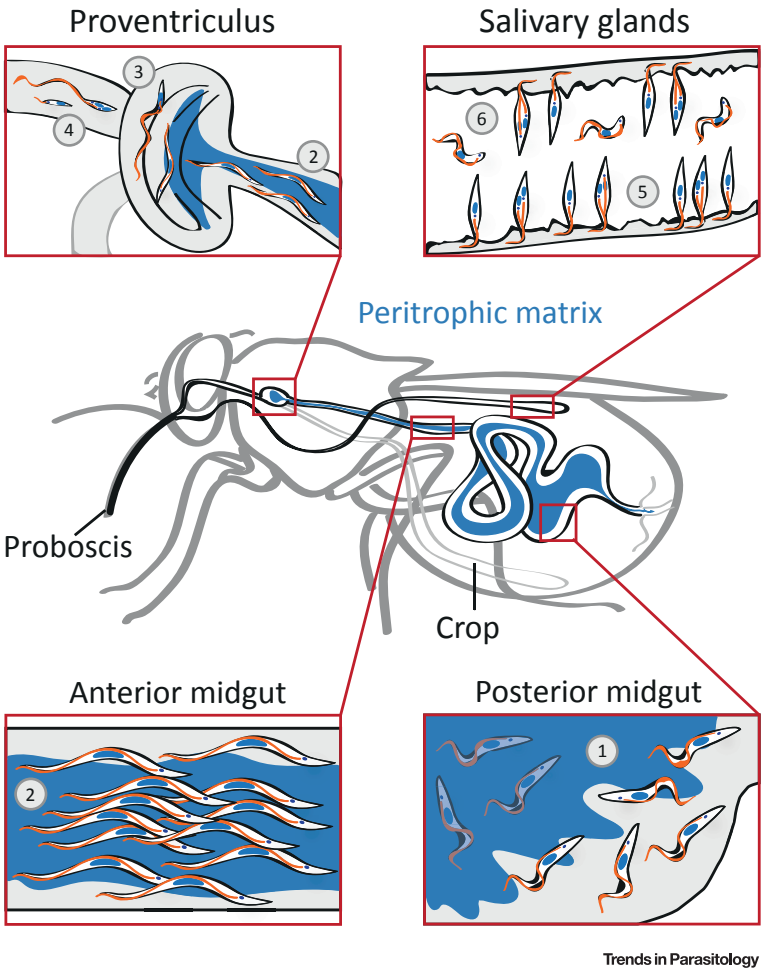

Figure 3. *Trypanosoma brucei* Development Habitats in the Tsetse Fly. Procyclic form cells (1) traverse the tsetse gut after they develop from bloodstream form cells that have been taken up through the crop. They stay motile in the gut lumen while it is filled with blood and throughout the digestive process. They manage to cross the peritrophic matrix and differentiate to the late procyclic stage while infesting the posterior midgut. They become very agile swimmers as they proliferate and concomitantly migrate from the posterior to the anterior midgut, where they develop into the cell-cycle-arrested mesocyclic forms (2). This motile morphotype moves further anterior, aggregating into very dense swarms on the way. The environment of the procyclic and mesocyclic stages is characterised by the heavily convoluted peritrophic matrix and the ectoperitrophic space between gut epithelium and matrix. After actively reaching the proventriculus, the mesocyclic cells transform into long, epimastigote cells (3). Endowed with long free flagella, these highly motile epimastigotes immediately begin to divide asymmetrically and probably transport the arising short epimastigotes (4) to the salivary glands. Here, these slow swimmers attach to the epithelium and elongate once more (5). The cells vigorously continue their flagellar beat at the site of attachment, but are mostly tightly packed in the salivary glands. After another asymmetric cell division, the arising metacyclic-stage cells (6) are released into the narrow salivary duct, where they, although able to swim, sluggishly await injection into the skin of the next mammalian host.

how densely packed large volumes of the ectoperitrophic space can become in an infected midgut, and how procyclic and mesocyclic cells switch between free, directional swimming and synchronised flagellar oscillations in huge swarms [5].

Eventually, the nonproliferative mesocyclic cells migrate to the proventriculus and develop into the epimastigote form. These stages are characterised by a long, free flagellum, a strongly

reduced cell body size, and high swimming capacity. The epimastigote cells re-enter the cell cycle and divide asymmetrically to produce short epimastigote cells, which are relatively ineffective swimmers. The long, effective swimmers are thus thought to function as transporters, carrying the short epimastigotes piggyback during the asymmetric division [38], although this journey has not been directly observed yet. Their destinations are the salivary glands, where the short epimastigote cells attach to the epithelium and develop further to the metacyclic form that is infective for mammals.

What is the function of flagellar beating when attached to the salivary glands? The attachment itself obviously protects the parasites from being prematurely expelled with the saliva. The flagellar forces could become important during cytokinesis, as is probably the case for other proliferative life cycle stages. As the salivary glands are the only place where genetic exchange between *T. brucei* cells can take place, motility might also play an important role for sexual reproduction [39]. Another possibility, likewise difficult to test, is that the beating flagella agitate the surrounding viscous fluids, thereby facilitating diffusion of nutrients within the densely packed array of metabolically active parasites.

The metacyclic cells that are released into the duct of the salivary gland await injection into the skin through the proboscis, another intricate organ and microhabitat that has recently been microscopically re-examined [40]. The exact passage of the parasites through the channels of the proboscis is unclear, but plays a prominent role in the development of several trypanosome species. In any case, the metacyclic cells adopt yet another versatile morphotype that is adapted to both the hydrodynamic environmental parameters in the saliva-transporting ducts, as well as the confined tissue spaces of the skin it is injected into, there to transform again rapidly into the free-swimming, circulating bloodstream form trypanosome.

Concluding Remarks

The apparently simple questions of parasitology, namely those related to life cycles, motile behaviour, or host niches, need some reconsideration (see Outstanding Questions). The precise mode of trypanosome annidation in the mammalian hosts, for example, or the impact of the ever-changing microenvironments on parasite swimming styles and performance, are poorly understood. The methods required to close those gaps, in a statistically sound manner, are available; however, standardised protocols need to be developed and accepted. In addition, more than 100 years of educated observation must be considered. Along these lines, studying the modest problem of how trypanosomes swim in the host may lead to new discoveries, with impact not only in parasitology. Unravelling the exact mode of the dynamics behind trypanosome tropism will guide us beyond blood.

Outstanding Questions

How can we quantitatively standardise the analysis of the connection between trypanosome motion patterns and the environment?

Which life cycle stages move in chemotactic gradients, and/or respond to mechanotactic cues?

How, where, and when do trypanosomes enter and leave the blood circulation?

Does trypanosome motion direct the parasite's path between interstitium, lymph, and blood?

How do brain trypanosomes reach their destination, and how do they develop and swim?

How do the various trypanosome motion patterns contribute to the tsetse passage?

What is the function of collective motion in trypanosomes?

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