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MINIREVIEW

Interwoven Biology of the Tsetse Holobiont

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Microbial symbionts can be instrumental to the evolutionary success of their hosts. Here, we discuss medically significant tsetse flies (Diptera: Glossinidae), a group comprised of over 30 species, and their use as a valuable model system to study the evolution of the holobiont (i.e., the host and associated microbes). We first describe the tsetse microbiota, which, despite its simplicity, harbors a diverse range of associations. The maternally transmitted microbes consistently include two *Gammaproteobacteria*, the obligate mutualists *Wigglesworthia* spp. and the commensal *Sodalis glossinidius*, along with the parasitic *Alphaproteobacteria Wolbachia*. These associations differ in their establishment times, making them unique and distinct from previously characterized symbioses, where multiple microbial partners have associated with their host for a significant portion of its evolution. We then expand into discussing the functional roles and intracommunity dynamics within this holobiont, which enhances our understanding of tsetse biology to encompass the vital functions and interactions of the microbial community. Potential disturbances influencing the tsetse microbiome, including salivary gland hypertrophy virus and trypanosome infections, are highlighted. While previous studies have described evolutionary consequences of host association for symbionts, the initial steps facilitating their incorporation into a holobiont and integration of partner biology have only begun to be explored. Research on the tsetse holobiont will contribute to the understanding of how microbial metabolic integration and interdependency initially may develop within hosts, elucidating mechanisms driving adaptations leading to cooperation and coresidence within the microbial community. Lastly, increased knowledge of the tsetse holobiont may also contribute to generating novel African trypanosomiasis disease control strategies.

Species interactions, across and within the domains of life, are ubiquitous in nature, fundamental in ecology, and pivotal with respect to evolutionary diversification. Throughout history, metazoans have formed intimate partnerships with microorganisms, some of which significantly contribute to the health and development of their host. Due to the important role of microbial symbionts in host fitness, research has increasingly focused on a more holistic examination of the biological system, encompassing the macroscopic host (i.e., animals or plants) and associated microbes, termed the holobiont (1) or the metaorganism (2), with the cumulative genetic material known as the hologenome (3). The hologenome theory of evolution has been used to examine the holobiont as a single unit undergoing evolution, adapting to persist in or expand its niche (3, 4).

Traditionally used in aquatic biology, applications of the holobiont concept have provided insights into marine microbiology such as coral health and the functioning of deep-sea hydrothermal vents (1, 3, 5). Heightened recognition of microbial symbionts as major contributors to host health has spurred the Human Microbiome Project, aimed at characterizing the microbial communities of several distinct spatial sites on the human body to better our understanding of their role in health and disease (6). These communities are highly complex (7) and can be composed of hundreds of species-level phylotypes, determined by ≥97% 16S rRNA sequence identity (8, 9). To fully understand processes occurring within complex holobionts, examination of simpler systems may aid in dissecting intimate interplay among the partners.

THE TSETSE FLY

The tsetse fly (Diptera: Glossinidae) provides a valuable model to study the evolution of a holobiont. The tsetse microbial symbionts range not only in the nature of their association (from mutualistic to parasitic) but also in their establishment times within the host (10). Thus, the system is distinct from other described insect symbioses, including sharpshooters, cicadas, spittlebugs, and mealybugs, where microbial symbionts have coevolved with one another and their host for a significant amount of time (11–16). A holobiont similar to tsetse is the pea aphid, *Acyrthosiphon pisum*, which harbors an obligate, nutrient-provisioning, mutualist *Buchnera aphidicola* (17), in addition to multiple facultative symbionts, such as *Serratia symbiotica*, *Hamiltonella defensa* (itself harboring a toxin-encoding phage protecting against parasitoid attacks), and *Regiella insecticola*, that provide defense against heat stress and parasites and have enabled an expansion of host-plant range (reviewed in reference 18). A notable distinction is that unlike the tsetse holobiont, which has evolved essential metabolic interdependency for both host and symbiont fitness (19, 20), *S. symbiotica* has been shown to partially recover host fitness upon *Buchnera* loss (21, 22), suggesting early steps in symbiont replacement or functional redundancy. Investigations regarding microbial community dynamics within tsetse will greatly contribute to the knowledge of how cooperation may evolve among players with differing establishment times.

In addition to its use as a model system for understanding the evolution of a holobiont, tsetse maintains significance as the sole and obligate vector of protozoan African trypanosomes (*Trypanosoma* spp.). These parasites (*T. brucei rhodesiense* and *T. b. gambiense*) are the causative agents of human African trypanosomiasis (HAT; commonly called "sleeping sickness"), a disease affecting

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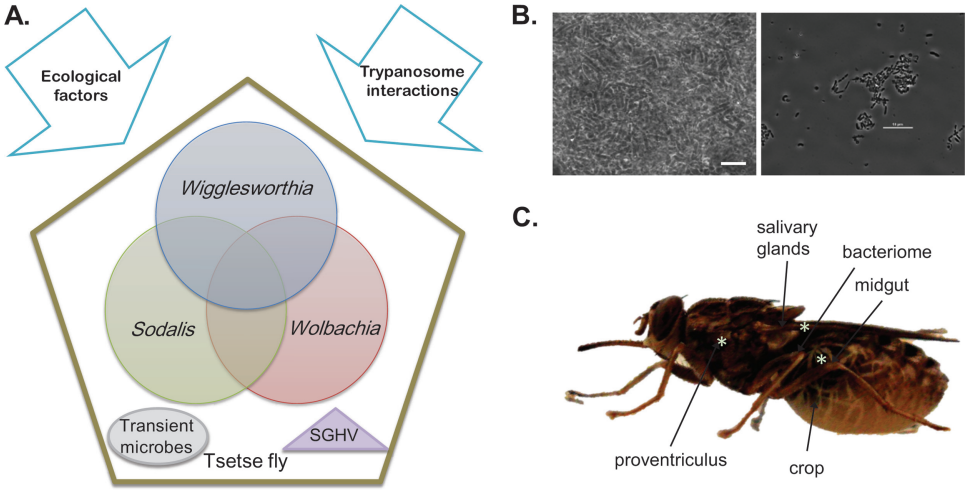

FIG 1 The tsetse holobiont. (A) The holobiont is composed of the tsetse fly and its 3 vertically transmitted bacterial symbionts, *Wigglesworthia*, *Sodalis*, and *Wolbachia*, and may be influenced by intrinsic factors such as other transient microbes, salivary gland hypertrophy virus (SGHV), and trypanosomes as well as by abiotic factors. (B) The enteric microbiota (*Wigglesworthia* and *Sodalis*), whose genomes are both annotated, provide a natural model to examine the early evolution of cooperation and adaptations leading toward microbiome coresidency. Phase-contrast microscopy images of *Wigglesworthia* cells (left) within a *G. morsitans* bacteriome and *Sodalis* cells (right) within culture are shown. Scale bars signify 10 μm. (C) The protozoan parasite *T. brucei* subsp. potentially interacts with microbial symbionts throughout infection. Stages of infection (denoted by asterisks) signify colocalizations of microbes that include the midgut, moving in an anterior manner toward the proventriculus and culminating in the salivary glands.

the central nervous system that is lethal if left untreated. HAT threatens millions of people in approximately 36 countries and has been classified by Doctors Without Borders as a neglected tropical disease, impacting some of the poorest rural areas in Africa (23). Another African trypanosome, *T. b. brucei*, causes Nagana, a similar wasting disease in domesticated animals, particularly cattle, further impeding the economic development of affected areas (24). Disease relief is relatively nonexistent, as there are no vaccines available to prevent African trypanosomiasis, manual trapping is often unreliable due to the social unrest in many affected areas, diagnostics are limited, and the small arsenal of drugs available for treatment are associated with significant toxic side effects. Therefore, vector control is an alternative intervention to break the disease cycle (25), as advocated with other systems (reviewed in reference 26). For example, *Wolbachia* has been shown to inhibit the replication of multiple arboviruses and filarial nematodes within *Aedes* mosquitoes (27–30), as well as to shorten the host life span, not permitting cyclical pathogen development and transmission (31). Additionally, symbionts within the guts of mosquitoes and triatome bugs are being genetically modified to produce antiparasitic molecules, in efforts to block transmission of malaria (32) and Chagas disease (33), respectively. Knowledge of tsetse fly symbiosis not only stands to provide basic insight into how microbial partners adapt and respond to changes in ecological factors and parasite infections but may also be of applied value to generate novel modes of pest biocontrol (26).

Localized exclusively to sub-Saharan Africa, there are approximately 31 species and subspecies of tsetse flies (Diptera: Glossinidae), which can be divided into 3 groups: *Glossina morsitans*, *G. palpalis*, and *G. fusca* (34). The unique biology of tsetse contributes to the maintenance of a simple larval microbiota, consisting of only 3 maternally transmitted bacterial symbionts (35), in comparison to other Diptera, such as mosquitoes and fruit flies, which harbor a greater complexity of bacterial taxa (36, 37). One distinct feature of tsetse biology is that both sexes maintain a strictly hematophagous lifestyle, persisting solely on vertebrate blood, which limits the introduction of additional microbes through an oral/digestive route. Due to their restricted diet, tsetse rely on microbial symbionts for provisioning essential metabolites lacking in blood (38). Another contributing factor to microbiome simplicity is the tsetse reproductive strategy, known as adenotrophic viviparity. This reproductive strategy involves high maternal investment and a low reproductive output of only 6 to 8 offspring in their 3-to-4-month life span (34). Unlike many higher Dipteran, female tsetse have highly modified reproductive tracts (39), enabling the deposition of a single fertilized egg into a muscular uterus, which is connected to highly specialized accessory glands, referred to as milk glands. Milk secretions provide nourishment and a route through which microbial symbionts (40, 41) are transferred during intrauterine larval development. This form of reproduction transmits the microbiota with high fidelity while preventing exposure to transient microbes during early tsetse development (35).

TSETSE MICROBIAL COMMUNITY

The tsetse microbiota consists primarily of three vertically transmitted bacterial species (Fig. 1). These microbes include two enteric *Gammaproteobacteria*, obligate mutualists *Wigglesworthia* spp. (42) and the commensal *Sodalis glossinidius* (43). Tsetse can also harbor the *Alphaproteobacteria Wolbachia* (44), a facultative parasite infecting many different invertebrates (45, 46), which is typically restricted to the reproductive organs (47, 48). Field studies report a more complex diversity in adult flies (49–51), although these microbes are believed to be transient in nature. The tsetse holobiont provides opportunities to examine evolutionary aspects associated with adapting to microbial coresidence, as *Wigglesworthia* and *Sodalis* have drastically different times of establishment (10, 52). Moreover, interactions among microbes with various levels of host dependency and symbiotic roles can be empirically investigated.

Wigglesworthia spp. maintain an obligate mutualism with tsetse and display significant concordant evolution with its specific host species, dating back 50 to 80 million years (10). In both sexes, this symbiont is localized intracellularly in specialized host cells (bacteriocytes) at the anterior midgut, collectively comprising an organ known as the bacteriome (Fig. 1). An additional extracellular population is found in the female milk glands which is maternally transmitted to offspring (40, 41). Described roles of this symbiont include both nutrient provisioning, where *Wigglesworthia* supplements B vitamins lacking in the tsetse blood diet (38, 53–55), and contributions to the maturation of host immunity (35, 56, 57). Insight into these roles has been found by examining tsetse biology upon removal of the symbiont. For example, the bacteriome population is vital for nutrient provisioning during host reproduction, as flies lacking the *Wigglesworthia* bacteriome populations are sterile (19, 58, 59), with fecundity partially restored by B-vitamin or yeast extract supplementation (38, 59). Absence of the milk gland symbiont population does not inhibit reproduction (58), as these populations are believed to be dedicated to vertical transmission and the persistence of the symbiosis through evolutionary time. In addition, the presence of *Wigglesworthia* during larval stages is essential for proper immune development, as larvae that lack this symbiont are significantly compromised in the induction of pathways associated with cellular immunity (35). The immunocompromised phenotype of aposymbiotic larvae can be reversed by feeding their moms a diet supplemented with *Wigglesworthia* cell extracts (57). The presence of the tsetse's larval microbiota also contributes to the proper development of the adult peritrophic matrix, separating epithelial cells from the contents of the lumen, which regulates the timing of immune induction following parasite challenge (60). *Wigglesworthia* also impacts tsetse digestion, temperature sensitivity, and susceptibility to infection with trypanosomes (56, 58, 60). It is important to mention that removing *Wigglesworthia* also causes indirect effects on the host that are not related to the loss of symbiont function. Some examples include additional nutritional deficiencies due to inhibited blood meal digestion (58), transient microbial colonization arising from an altered immune state (35, 56, 57), and perturbations to the remaining tsetse microbiota (20).

The annotation of *Wigglesworthia* genomes, isolated from *Glossina brevipalpis* (Wgb) (53) and *G. morsitans* (Wgm) (54), revealed characteristics similar to those of other ancient insect symbionts (61–63), including a reduced size (~0.7 Mb) and high adenine-thymine bias. Genome adaptations by *Wigglesworthia*, while in association with tsetse, are believed to have resulted in the loss of many capabilities required for a free-living lifestyle through reductive evolution (53, 54). Despite its small genome size, the majority of B-vitamin biosynthesis pathways remain intact, supporting the nutritional mutualism. Comparative analyses revealed that Wgm and Wgb maintain similar genomic repertoires with high synteny. Interestingly, some pockets of unique Wgm genes, potentially contributing to anabolic distinctions, were found (54). As this symbiont has undergone deep codiversification with the tsetse host (10), any unique capabilities of *Wigglesworthia* spp. influencing physiological and phenotypic differences between host species remain largely unknown.

In contrast to the ancient *Wigglesworthia* association, tsetse's commensal partner, *Sodalis*, is believed to have established recently within the tsetse host from a previously free-living progenitor. Evidence lies in its ability to be cultured (64), providing a tremendous benefit for empirical analyses, wide tsetse tissue tropism with both intra- and extracellular localization (41, 48), lack of coevolution with host species (10), and stochastic presence in the field (49, 51, 65). Similar to *Wigglesworthia*, *Sodalis* is vertically transmitted through the maternal milk glands (40, 41). Despite a more recent association, *Sodalis* displays genomic signatures indicating that adaption to the symbiosis has commenced. While the *Sodalis* genome (4.2 Mb) is larger than that of *Wigglesworthia*, it appears to be undergoing massive reduction, as it is composed of >50% pseudogenes (66, 67), indicative of relaxed selection on nonessential genes. Notably, *Sodalis* has modified its outer membrane protein A (OmpA), which represents a molecular adaptation that contributes to host immune tolerance (68). The OmpA protein is also utilized in biofilm production within the tsetse gut, further protecting *Sodalis* from host immune responses (69).

Biotechnological advancements, notably culture-independent techniques, have accelerated the number of described host-associated bacterial species, stimulating interest in examination of the *Enterobacteriaceae Sodalis*-allied clade, as members are present in a diverse array of insect and environmental samples (43, 70–81) (Table 1). While this bacterial group appears to have an enhanced ability to establish within a variety of niches relative to most other characterized symbionts, much of the initial molecular phylogenetic analysis within the *Sodalis*-allied clade has utilized the conserved 16S rRNA gene, resulting in low resolution (70, 75, 82). Examination of surface-encoding loci from *Sodalis* isolates and related symbionts revealed early genomic host-specific modifications, likely aiding in their integration within different insects (82). Moreover, phylogenetic analyses of the internal transcribed spacer regions, which have an accelerated evolutionary rate (83), in conjunction with the adjacent 16S rRNA gene, have provided additional insights into the evolutionary divergence of this clade (84). The *Sodalis* isolates from various tsetse species formed a monophyletic clade, indicative of their divergence from additional known members of this group. Supporting the recent expansion of this clade, the phylogenies also demonstrated that symbionts from additional insect hosts were intertwined, suggesting either horizontal transfer between insects or the acquisition from a common environmental source. A recently described member obtained from an environmental source, known as strain HS, has provided novel insights into the progenitor of this clade (81). Comparative genomic analyses of strain HS, with other members of the *Sodalis*-allied clade, specifically, *Sodalis* and the *Sitophilus oryzae* primary symbiont (SOPE), revealed that both insect symbiont genomes were near-perfect subsets of the strain HS genome and yet each contained a unique set of pseudogenes (81). These results suggest that strain HS may be a representative environmental progenitor of the *Sodalis*-allied clade, which has independently formed symbioses with various insects. Continued examination of this clade will enhance knowledge of potential adaptations aiding in establishment within a broad range of niches, mechanisms facilitating host switching, and the impact of these host jumps on symbiont genome evolution.

The third bacterial member of the tsetse holobiont is an intracellular pathogen within the genus *Wolbachia* (44). While there is a high prevalence of *Wolbachia* infections in laboratory colonies (85), field populations are more stochastic and infection is also not detected in all tsetse species (86). This symbiont is transovarially transmitted through successive host generations and has recently been shown to induce cytoplasmic incompatibility within

TABLE 1 Characterized members of the *Enterobacteriaceae Sodalis*-allied clade exhibiting ≥96% 16S rRNA identity

Member of the *Enterobacteriaceae Sodalis*-allied clade as described in corresponding citation	Source	Insect order: family	Reference
Sodalis glossinidius	Tsetse fly, *Glossina* spp.	Diptera: Glossinidae	43
Symbiont	Hippoboscid fly, *Craterina melbae*	Diptera: Hippoboscidae	70
"*Candidatus* Sodalis melophagi"	Sheep ked, *Melophagus ovinus*	Diptera: Hippoboscidae	71
Sodalis-allied symbiont	Scutellerid stinkbug, *Cantao ocellatus*	Hemiptera: Scutelleridae	72
Sodalis-allied symbiont	Giant jewel stinkbug, *Eucorysses grandis*	Hemiptera: Scutelleridae	73
Symbiont	Long-tailed mealybug, *Pseudococcus longispinus*	Hemiptera: Pseudococcidae	74
Symbiont	Slender pigeon louse, *Columbicola columbae*	Phthiraptera: Philopteridae	75
Symbiont	Longhorn beetle, *Tetropium castaneum*	Coleoptera: Cerambycidae	76
Sitophilus primary symbiont	Grain weevil, *Sitophilus* spp.	Coleoptera: Curculionidae	77
Secondary symbiont	Chestnut weevil, *Curculio sikkimensis*	Coleoptera: Curculionidae	78
Secondary symbiont	Weevil, *Archarius roelofsi*	Coleoptera: Curculionidae	79
Secondary symbiont	Weevil, *Curculio hachijoensis*	Coleoptera: Curculionidae	79
Biostraticola tofi	Environmental, Tufa deposit biofilm isolate	n/a[a]	80
Strain HS	Environmental, hand wound	n/a	81

[a] n/a, not applicable.

the tsetse host (59). The association may also have a long coevolutionary history with some tsetse species, as *Wolbachia* loci were found horizontally transferred into the host genome (86).

IMPACT OF ADDITIONAL MICROBES ON HOLOBIONT SUCCESS

The introduction of additional microbes can influence the fitness of the holobiont. For example, salivary gland hypertrophy virus (SGHV) infection is a well-characterized parasitic disease in tsetse (87). SGHV, a nuclear rod-shaped, enveloped DNA virus (88), has low infection rates in the field and can be both vertically and horizontally transmitted. This infection can quickly spread in laboratory colonies, driven by horizontal transmission through artificial feeding systems, which can harbor concentrated viral numbers in the blood that would otherwise quickly disseminate within a vertebrate host (87). Viral infection is associated with testicular degeneration and ovarian abnormalities (89–91), which can lead to decreased tsetse fertility and longevity (92, 93). This is just one example of how parasitic associations may impact the tsetse host and influence evolutionary adaptations by the bacterial symbionts. The interactions of SGHV and the tsetse microbiota remain largely unknown. SGHV infection, as well as other potential parasitic interactions, within tsetse populations should be considered when examining the success and evolution of the holobiont.

It should also be noted that recent field studies have found an unexpected diversity within the microbial community of tsetse which is dependent on host species and geographic region (49, 51). Differences in abiotic conditions and food sources may influence the composition of these transient microbial communities (49, 94). Although additional microbes have been found in association with tsetse in the field, only *Wigglesworthia*, *Sodalis*, and *Wolbachia* are maternally transmitted, as 16S rRNA clone libraries of 3rd instar larvae contain only these 3 bacterial species (35).

An additional major factor influencing the holobiont is trypanosome presence; once a fly becomes infected with trypanosomes, it remains infected for the duration of its life span (Fig. 1). Tsetse flies play an obligate role in the successful development and transmission of *Trypanosoma* spp. (reviewed in reference 95). A phenotypic characteristic differentiating tsetse species is their vector competency, i.e., their ability to support the development and transmission of trypanosomes to naive hosts (96–103). While in the fly, the parasites are also heavily bombarded by the tsetse immune system, including the synthesis of antimicrobial peptides and the production of reactive oxygen species (104–108). The presence of trypanosomes, as well as the related biological modifications within tsetse, such as heightened immune stimulation and increased competition for space and resources, may also impact their microbial symbionts.

THE TSETSE FLY AS A TOOL TO EXAMINE HOLOBIONT EVOLUTION

The hologenome theory of evolution has been used to describe how variation in the genetic material of symbiotic partners is an important factor in the adaptive evolution of the holobiont (3, 4). This theory is based on four assumptions, the first being that all metazoans are associated with microbial symbionts (4, 109). Second, the fitness of the holobiont requires cooperation among the partners, as conflict may prove detrimental to overall health. Within the tsetse holobiont, synergistic effects of coresidency were recently observed, as clearance of *Wigglesworthia* resulted in the loss of *Sodalis* over generations of the host (20). This phenomenon was possibly due to metabolic dependencies (110), as previously indicated by similar trends in population dynamics through tsetse development (111). Third, the hologenome can change through alterations of the genetic material of any partner. Symbionts can allow the holobiont to adapt more quickly to ecological disturbances, through the acquisition of novel capabilities by horizontal gene transfer or changes in population dynamics or community composition, thereby aiding in the persistence of the holobiont. Within the tsetse fly, an increase in the availability of nutrients, specifically, the supplementation of thiamine (B1) to blood meals, was shown to alter symbiont populations (55, 110) and decreased the transcription of corresponding biosynthetic loci by *Wigglesworthia* (55). Lastly, symbiotic associations must be passed on through generations of the host, maintaining the species composition of the holobiont. By applying the holobiont concept and hologenome theory of evolution to tsetse symbioses, research will elucidate novel mechanisms driving microbial species cooperation and adaptation, which enable successful cooccupancy within a specific niche. Because the *Wigglesworthia*, *Sodalis*, and *T. b.*

brucei genomes are sequenced and annotated and tsetse's (*G. morsitans*) genome is soon to released (S. Aksoy, personal communication), comparative analyses have begun to spur empirical studies sure to advance our knowledge of microbial community evolution within hosts.

METABOLIC INTERACTIONS AMONG THE MICROBIOTA

While the genomic evolution and importance of individual symbiont species within tsetse have been examined, the community dynamics are only beginning to be explored. Although *Wigglesworthia* and *Sodalis* have different evolutionary histories with tsetse, they maintain parallel population dynamics through host development (111, 112), indicative of coordinated activities or a generalized level of host control. Unlike the extensive metabolic complementation observed within ancient coresident symbionts (11–16), comparative genomics reveals that *Sodalis* encodes a majority of *Wigglesworthia* genes (110). This genetic redundancy brings into question the factors contributing to the maintenance of both associations within the tsetse host and how cooperation, rather than competition, occurs among the symbionts. In addition to their colocalization within both the anterior midgut and milk glands, metabolic interplay among the partners has been previously described (55, 110). Specifically, a distinction between *Wigglesworthia* and *Sodalis* lies in their thiamine (vitamin B_1) biosynthesis capabilities. The genome of *Wigglesworthia* encodes the complete pathway, while that of *Sodalis* does not. Thiamine, specifically in the form of thiamine monophosphate (TMP), the derivative putatively produced by *Wigglesworthia*, is required for *Sodalis* proliferation and intracellular infection (110). This nutrient may be imported by *Sodalis* through an ATP-driven thiamine ABC transporter (110). Moreover, *Sodalis* growth was dependent on TMP concentration, suggesting a role for TMP in the maintenance of homeostasis within the tsetse holobiont. TMP supplementation to the tsetse holobiont via blood meals also resulted in decreased transcription of the Wgm thiamine biosynthetic locus *thiC* and in reduced symbiont population density and cell viability (55). These alterations in symbiont dynamics may contribute to the efficiency and stability of the tsetse holobiont, with the reduction of the *Wigglesworthia* population likely representing a lower functional need. Metabolic interactions may be involved in symbiont population control and possibly aid in preventing antagonism among the partners. In support of this theory, a more intricate metabolic complementation has been observed among anciently coevolved microbial symbionts in other insect holobionts (11–16). For example, within the mealybug, essential amino acid synthesis necessitates a medley of gene products arising from both bacterial partners, and possibly the host, for completion (16).

UNDERSTANDING THE TSETSE HOLOBIONT FOR ENHANCED VECTOR CONTROL

Measures to prevent the spread of African trypanosomiasis, such as using mass insecticide spraying during outbreaks and the release of sterile males in restricted areas to reduce field populations, have been historically targeted at controlling the tsetse fly population (95). Such measures have been successful within their targeted locales (113). Nevertheless, the threat of trypanosomiasis remains relevant, as political instability (23) and decreased priority status implemented by local authorities, due to the reduced numbers of reported cases (114), impede the continuous efforts required to prevent the reestablishment and subsequent heightened disease incidence (115). By gaining a more holistic view of tsetse biology, insights into novel strategies for controlling the spread of the disease may be gained. In fact, a recent International Atomic Energy Agency (IAEA)-coordinated research project (CRP) aims to unravel the interactions between the tsetse host, *Wigglesworthia*, *Sodalis*, *Wolbachia*, and SGHV and the development of African trypanosomes to increase knowledge of ways to enhance refractoriness to trypanosome infection (116).

Past studies have demonstrated a positive correlation between *Sodalis* presence and trypanosome infections in field flies (65, 117). *Sodalis* is believed to contribute to the susceptibility of teneral flies (i.e., newly emerged unfed adults) through its endochitinase activity within the midgut which breaks down chitin (118), producing a byproduct of *N*-acetyl-D-glucosamine which inhibits the action of trypanocidal lectins (118, 119).

The role of *Wigglesworthia* in the tsetse fly's susceptibility to trypanosome infection remains largely unknown. A link between *Wigglesworthia* and trypanosome infection was suggested, as the removal of the symbiont resulted in higher susceptibility to midgut infection in older, nonteneral flies—typically a time point of low vector competency (58). Subsequently, the absence of *Wigglesworthia* was found to impair host immune system development (35). Thus, the higher susceptibility to trypanosome infection may be due to compromised immunity, although the effect of an altered nutritional state may also be a contributing factor. Genome comparisons between *Wigglesworthia* spp. revealed potential metabolome differences among the primary symbionts (54). One distinction lies in the complete retention of the chorismate (an intermediate in the production of aromatic compounds, including amino acids and vitamins) and downstream folate (vitamin B9) biosynthetic pathways by Wgm but not Wgb. Interestingly, the parasitic lifestyle of *T. brucei* subsp. has resulted in a highly restricted genomic repertoire, compensating for the absence of biosynthetic pathways by encoding transporters to sequester metabolites (including folate) from the environment (120, 121). This enhanced biosynthetic capability may contribute to the higher reported vector competency of the *G. morsitans* host relative to *G. brevipalpis* (96–98, 100), as trypanosomes necessitate exogenous folate for growth (122). Deeper investigation of this hypothesis is required. Enhanced understanding of the unique capabilities of specific *Wigglesworthia* spp. with respect to holobiont functioning may contribute to a more holistic view of factors that result in different levels of refractoriness between tsetse species.

Tsetse immune tolerance of symbionts may also influence the fly's susceptibility to trypanosome infection. For example, the contribution of the tsetse fly's immune system to the persistence of the *Wigglesworthia* symbiosis may also play a role in the fly's ability to transmit trypanosomes. The host pathogen recognition protein PGRP-LB, which scavenges peptidoglycan, thus preventing immune deficiency (IMD) signaling pathway stimulation, is intimately associated with maintaining the *Wigglesworthia* symbiosis (56, 123). PGRP-LB is maternally transmitted via milk secretions to developing offspring and is produced by adult flies only after their first blood meal (123). This protein also exhibits trypanocidal activity (123). Therefore, higher levels of PGRP-LB may aid in the refractory nature of older, nonteneral flies with respect to trypanosome infection.

Field studies examining the rates of microbial coinfections

within tsetse may also provide insight into symbiont interactions. One study examining the association of coinfections in *Glossina fuscipes fuscipes* in Uganda found a negative correlation between the prevalences of *Wolbachia* and SGHV, while SGHV infection and trypanosome infection were positively correlated (124). This finding highlights the importance of examining the evolutionary and physiological effects of coinfection. One trypanosome control strategy that relies on symbiont interactions is known as paratransgenesis (for more details, see references 25, 26, and 125). Paratransgenesis involves manipulating *Sodalis* to express antitrypanosomal effector molecules and utilizes the cytoplasmic incompatibility properties of *Wolbachia* (59, 126) to drive the genetically modified symbiont into natural populations.

CONCLUSIONS

The low complexity of the tsetse holobiont and the annotated genomes of its members enable investigations into the evolutionary aspects of coresidence and holobiont adaptations when challenged with both intrinsic and ecological disturbances. Comparisons of the tsetse holobiont, in which members are still transitioning into the symbiotic lifestyle, to other anciently coevolved mutualisms will help describe mechanisms contributing to early integration and cooperation within a microbial community. Moreover, a more holistic and comprehensive understanding of the tsetse holobiont may identify additional factors promoting or inhibiting vector competency that may ultimately aid in controlling the spread of African trypanosomiasis.

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