



Published in final edited form as:

Curr Opin Insect Sci. 2025 June ; 69: 101369. doi:10.1016/j.cois.2025.101369.

Viviparity and obligate blood feeding: tsetse flies as a unique research system to study climate change

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Abstract

Tsetse flies (*Glossina* species) are unique organisms that combine several remarkable traits: they are obligate blood feeders, serve as critical vectors for African trypanosomes, and reproduce through adenotrophic viviparity—a process in which offspring are nourished with milk-like secretions before being born live. Here, we explore how climate change will impact the physiological processes associated with live birth in tsetse. This includes considerations of impacts on how blood feeding, host-pathogen interactions, and host-symbiont dynamics are likely to be impacted by thermal shifts. The highly specialized biology of tsetse flies suggests that this system is likely to have a distinctive response to climate change. Thus, detailed empirical research into these unique features is paramount for predicting tsetse population dynamics under climate change, and cautions against generalising from other well-studied vectors with contrasting ecology and life histories such as mosquitoes and ticks. At the same time, the reproductive biology of tsetse, as well as microbiome and feeding dynamics, allow for a powerful model to investigate climate change through the lens of pregnancy and associated physiological adaptations in an extensively researched invertebrate.

Keywords

viviparity; heat stress; trypanosome; metabolism; climate change

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Introduction

Tsetse flies are distributed across sub-Saharan Africa [1], where climate change is likely to occur in the form of increases in mean temperature, increased frequency of extreme events such as heatwaves and droughts, and changes in land use which impact the availability of vertebrate hosts for tsetse. Tsetse flies (*Glossina* spp.) are extensively researched due to their role in transmitting trypanosome parasites, the causative agent of sleeping sickness (Human African Trypanosomiasis) in humans or nagana (Animal African Trypanosomiasis) in animals. These flies, as part of the Hippoboscoidea superfamily, are characterized both by the fact that both males and females are obligate blood feeders, and by their distinct mode of matrotrophic reproduction (adenotrophic viviparity, gland-fed followed by live birth [4]), producing a single maternally nourished larva similar in mass to the mother each gonotrophic cycle [9]. There are three ecotypes among *Glossina* spp., Savannah flies (*Morsitans* sub-genus), Riverine flies (*Palpalis* sub-genus), and Forest flies (*Fusca* sub-genus) [1] grouped according to climatic, habitat preferences, host associations, and, geographic distribution [2,3]. Importantly, there are substantial differences in the baseline thermal tolerance between each species with those species with the smallest thermal tolerance margins most likely at risk [4].

The primary goals of this review are, first, to highlight how considering how climate change affects tsetse populations through the lens of their viviparous reproduction requires detailed understanding of their unique biology (including obligate blood feeding and microbial symbionts), which is facilitated by extensive field, laboratory and modelling work in these flies [2,3,5]. Second, as well-studied viviparous flies at the forefront of climate change effects, findings on tsetse can help consider how climate change will impact other, viviparous invertebrates which are less well researched.

Diversity and distribution of viviparity across animals

There are a multitude of reproductive strategies utilized across the animal kingdom [6]. However, most fit into one of three categories: oviparity (development and deposition of eggs in early embryonic development); ovoviviparity (deposition of eggs at later stages of embryonic development or live offspring early in their development); or true viviparity. True viviparous reproduction is characterized by the obligate retention and nourishment of developing offspring in the maternal reproductive tract. This is often associated with reproductive tract modifications that facilitate gestation for immature development and birth. An important distinction between true viviparity and ovoviviparity is the general inability of offspring of viviparous species to survive premature birth or egg deposition. There exist many diverse implementations of viviparity for animals, where the provision of extra-vitelline nutrition to developing progeny usually represents the most advanced states [6–8].

While pregnancy is most commonly associated with mammals, viviparous reproduction represents a striking example of convergent evolution in the animal kingdom [6]. This type of reproduction is well represented in arthropods and even organisms as basal as sea sponges (Porifera). Independent evolutionary origins of viviparous reproduction have been identified in over 150 invertebrate lineages alone [6–9]. Present in at least 13 orders of

insects, viviparity has at least 62 independent origins within the 22 families of Dipterans [6,9].

Matrotrophic viviparity, whereby mothers provision developing offspring with nutrients, is also widespread across the animal kingdom (21 of the 34 animal phyla). It is estimated that 213 chordate and 45 arthropod families have member species that are matrotrophic viviparous [8]. Despite the widespread incidence of diverse forms of viviparous reproduction, the current understanding of matrotrophic viviparity in insects is sparse outside a few systems [7,8,10,11].

The lack of information on viviparous reproduction outside of mammals, specifically in ectotherms, hinders our ability to predict how these species will respond to climate change. The dramatic physiological differences between oviparous and viviparous species mean that broad comparisons between these groups to predict the impacts of climate on viviparous species could be misleading due to incorrect assumptions about the underlying biology [11].

Viviparity, blood feeding, and parasite transmission in tsetse flies

Tsetse fly viviparity

Tsetse flies have three significant morphological adaptations that facilitate adenotrophic viviparity. Tsetse ovaries only produce a single oocyte per gonotrophic cycle; the reproductive tract is expanded and lined with tracheated muscle-lined epithelial cells that form the uterus [7,13]; and the female accessory gland is expanded into a large, branched organ [7,10,13] attached directly to the uterus. The tsetse uterus can accommodate a single larva each gonotrophic cycle [7,13]. A unique aspect of tsetse biology is the provisioning of nutritive secretions (fly milk) to the developing larvae that allow a 100-fold (dry mass) growth *in utero* [14,15]. Milk protein components consist of a lipocalin, Hippoboscoidea-specific milk proteins, transferrin, acid sphingomyelinase, and other minor constituents [1,16]. In addition to essential proteins, the milk secretions contain bacterial symbionts transmitted from the mother to the larva [1,7,17].

These adaptations to viviparity come with significant nutritional and physiological costs. An oviparous organism under nutritional stress can modulate its reproductive output via oocyte resorption to balance the output relative to acquired nutrients. *Glossina* do not have this option, and if adequate nutrients are not obtained during pregnancy, they must abort the developing larvae and start the cycle again [7,16].

Tsetse fly blood feeding and symbiosis

The intense nutritional demands of pregnancy have driven distinct adaptations for diet and nutrient processing. Across all tsetse species, both sexes feed exclusively on vertebrate blood, which contains abundant proteins and lipids but few carbohydrates and specific micronutrients. After locating a host, feeding requires a complex suite of salivary proteins unique to *Glossina* species [18,19]. Immediately after feeding, blood moves into the crop and passes through the proventriculus, where the peritrophic matrix (PM) envelops the blood. Rapid secretion of the excess water in the blood through diuresis is critical to digestion [20]. A suite of specific enzymes digest the lipids and proteins [21–23]. The

digestion process involves the secretion of multiple peptidases, proteinases, and lipases, which induce rapid breakdown of blood components. Free amino acids and lipids generated from the breakdown of blood are metabolized into lipid reserves stored in the fat body tissue [7,24]. The digestion process is temperature-dependent, where the process of the blood meal occurs more rapidly at higher temperatures [25]. Due to their dependence on a high protein diet, *Glossina* relies on protein-based metabolism. As a result, tsetse have likely evolved to use proline as an energy source to fuel their biological processes [26,27].

Key for metabolism, tsetse flies harbor the obligate mutualist *Wigglesworthia*, a symbiotic association that dates 50–80 million years [28]. This bacteria is found both intracellularly within bacteriocytes that form tsetse's bacteriome organ, located adjacent to the anterior midgut, and extracellularly within maternal milk. *Wigglesworthia* is capable of B vitamin synthesis, which is critical to supplement tsetse diet of vertebrate blood [27,29]. Fly metabolism is drastically impaired without B vitamin provisioning from *Wigglesworthia* [1,30]. Tsetse flies have other known bacterial associates, primarily *Sodalis* and *Wolbachia*, that impact their biology. However, these bacteria are not found in all species and populations [1,17,30]. Notably, the elimination of the symbionts leads to suppression of reproduction that can be recovered by supplementing a cocktail of B vitamins [27,29].

Tsetse fly parasite transmission

African trypanosomes must complete 14–28 days of developing in tsetse flies to be transmitted to vertebrate hosts [31]. At the ingestion of infectious blood, the parasites must undergo multiple stages of differentiation and replication to move from the midgut through the cardia organ (proventriculus) and then into the foregut, salivary glands, and mouthparts, where they undergo the last differentiation into vertebrate-infectious metacyclics. Despite likely ingesting many trypanosome-infected blood meals, only a low percentage of challenged tsetse (1–10%) become capable of onward transmission [32]. This low infection prevalence is reflective of robust passive and active gut-associated innate immune mechanisms, which include reactive oxygen intermediates (ROIs) [33,34], antimicrobial factors [34–36] and their type II peritrophic matrix (PM). The PM is a sleeve-like structure secreted by the cardia organ [37] lines the midgut and separates epithelial cells from ingested products and pathogens [38]. Young, unfed ('teneral') flies have an immature PM structure – which does not fully line the midgut – and this is one potential mechanism explaining the higher susceptibility of becoming infected among young flies taking their first blood meal [39]. Mature adult tsetse, in contrast, have a fully formed PM and are more refractory to trypanosome infection than young adults [39,40].

Impact of climate changes on tsetse fly biology

Climate change and tsetse fly blood feeding, parasite transmission, and interactions with their symbionts

Being ectotherms, tsetse body temperatures are primarily dictated by environmental conditions [41]. Behaviorally, tsetse flies can buffer changes by moving into protected microhabitats, such as warthog dens, that remain cooler and more humid, protecting both the mother and deposited larvae [42]. Warmer ambient environmental temperature

will exponentially increase resting and active metabolism, resulting in more rapid blood meal digestion [25], and likely leading to increased blood feeding frequency (summarized in Figure 1). These physiological and behavioral changes may cause increased disease transmission risk, given that blood meals are tsetse's only source of nutrients, and thus increase R_0 in classic disease transmission equations. However, transmission risk may be lower as vertebrate host populations become scarce or patchy (due to direct climatic factors or changes in suitable habitat), negatively impacting tsetse population sizes.

The effects of thermal stress on the interactions between tsetse flies and their symbionts have been limited. However, continual exposure to temperatures above 37°C will lead to death in *Sodalis* [43]. While direct studies are lacking on *Wigglesworthia*, prolonged exposure to high temperatures will likely lead to symbiont elimination resulting in reduced fly health and female sterility [44]. There are few studies on thermal stress and parasite dynamics in tsetse [45], but both direct and indirect impacts of climate change are anticipated. The parasites are impacted by several factors, including environmental stress, tsetse nutrition, and host biology (e.g., reproductive status and age) interactions. Moreover, parasite dynamics within tsetse are impacted by symbionts, as these influence tsetse immune and nutritional state. Thus, climate change directly and indirectly impacts all facets of the host-microbe-parasite system and the interactions therein.

Tsetse fly viviparity and how climate change will impact this process.

Given that climatic factors likely played a significant role in the evolution of tsetse viviparity, climate change conditions will impact tsetse viviparity in myriad ways (summarized in Figure 2). On the one hand, producing so few offspring (~10 per lifetime), each requiring high maternal investment, renders tsetse particularly vulnerable to climate change. On the other hand, the benefits of offspring retention, allowing females to reproduce across the year and thermoregulate via behavior and without the need to select suitable larval habitats, may provide some buffer. Most likely, tsetse evolved this costly reproductive strategy under optimal thermal conditions with abundant hosts [7,46], suggesting that climate change is likely to have a substantial negative impact on tsetse viviparous processes. Detailed population dynamics modeling is required to manage this complexity (e.g. [2,3,5]), incorporating how aspects of tsetse reproduction vary with climatic factors, not all pathways of which are fully understood.

We anticipate females will produce smaller and fewer offspring under warmer temperatures. This is because warmer temperature increases the developmental rate [47], with less time to develop into a larger pupa. These smaller offspring, in turn, will have reduced survival in hotter months due to their limited reserves for completion of metamorphosis and locating their first blood meal [48]. Moreover, females have higher rates of abortion when it is warmer [49], a potential mechanism being ovarian damage due to heat stress that prevents subsequent investment in nonviable progeny [50]. Recent experimental work simulating heatwaves has shown that tsetse females take longer to produce their first offspring after a heatwave, possibly due to early egg-stage abortion or delayed ovulation [51].

Tsetse survival and reproduction could be significantly impacted by nighttime temperatures, which appear to be rising disproportionately [52]. Tests focusing on the effects of nighttime

temperatures on tsetse have yet to be conducted, representing a promising avenue of research to better understand potential outcomes. Importantly, as tsetse flies show very little activity during the night [53] behavioral regulation of thermal status may be reduced. While slight shifts in timing of activity may be possible under strong selection (as per *Anopheles* mosquitoes shifting feeding behaviour in response to selection pressure of widespread use of bednets [54]). It is unlikely that tsetse – with a visual system highly adapted to being diurnal – would shift to being more active when there is not sufficient light for movement. Direct heating may also lead to cumulative cellular heat injury, which, once critical environmental temperature thresholds are surpassed, can result in exponential organismal failure and inability to recover from high temperatures [55].

Climate change produces increased environmental temperatures and more frequent and unpredictable droughts [56]. Drought-induced changes in vertebrate host behavior and abundance may indirectly impact tsetse flies, given their reliance on blood for water requirements [20]. Locating vertebrates has been suggested as a more reliable water source during scarcity of free water or water from plants, potentially driving increased blood feeding during drought, as in other vectors [57]. Furthermore, as blood is the sole water source for tsetse, teneral flies may be highly susceptible to dehydration-induced mortality if emergence occurs under dry conditions with few hosts available [58]. There are species-specific differences in water balance parameters among tsetse flies [59], suggesting that different ecotypes will vary in drought tolerance. As such, reduced host availability coupled with warmer and dry conditions reducing suitable flight time for tsetse and increasing metabolic rate can impose substantial nutritional and water balance stress. Such stress produces smaller offspring [60] and may lead to increased starvation and dehydration of teneral flies if they run out of lipid or water reserves before their first blood meal.

Conclusions

Here, we focus on the different physiological adaptations of tsetse related to their unique reproduction and feeding biology that are relevant to consider climate change impacts. The unique facets of tsetse biology and microbial partnerships suggest that tsetse flies will respond to climate change in ways that do not occur in other insects. Their viviparous reproductive strategy may allow mothers to protect their intrauterine progeny for short periods. Still, increasing temperatures for short periods can eliminate the symbionts (preventing reproduction) and lead to high mortality [51]. Further studies on behavioral adaptations that include the use of protected microhabitats, such as warthog burrows [42] choice of pupal deposition sites, along with more extensive field studies on ecophysiology, are necessary to fill critical gaps in the understanding of tsetse thermal biology.

Based on current observations, temperature, climate shifts, and other environmental changes (e.g. habitat fragmentation [5,61]) are leading to a decline in tsetse levels, which may be related to weak plasticity in thermal tolerance and adverse cross-generational effects [4]. These combined factors suggest that tsetse flies will significantly decline due to shifting thermal effects as temperatures increase, in line with previous and more recent population models [2,3,5].

While understanding these patterns is important for predicting tsetse population responses and associated tsetse-borne disease risk, there is also a unique opportunity to generalise from tsetse to other live-bearing ectotherms (such as cockroaches [11], keds [62], and aphids [63].

Acknowledgments

The development of this review was partially supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number R01AI148551 and R21AI166633 (to J.B.B.). Support was received by a UKRI Future Leaders Fellowship MR/W007711/1 (to S.E.), and a Royal Society Dorothy Hodgkin Fellowship DH140236 and Enhancement Award (to S.E. and C.M.). J.S.T. is supported by ICE2009 and Stellenbosch University and G.M.A. is supported by the Agricultural Experiment Station Grant (NIFA)—CA-D-ENM-2477-H.

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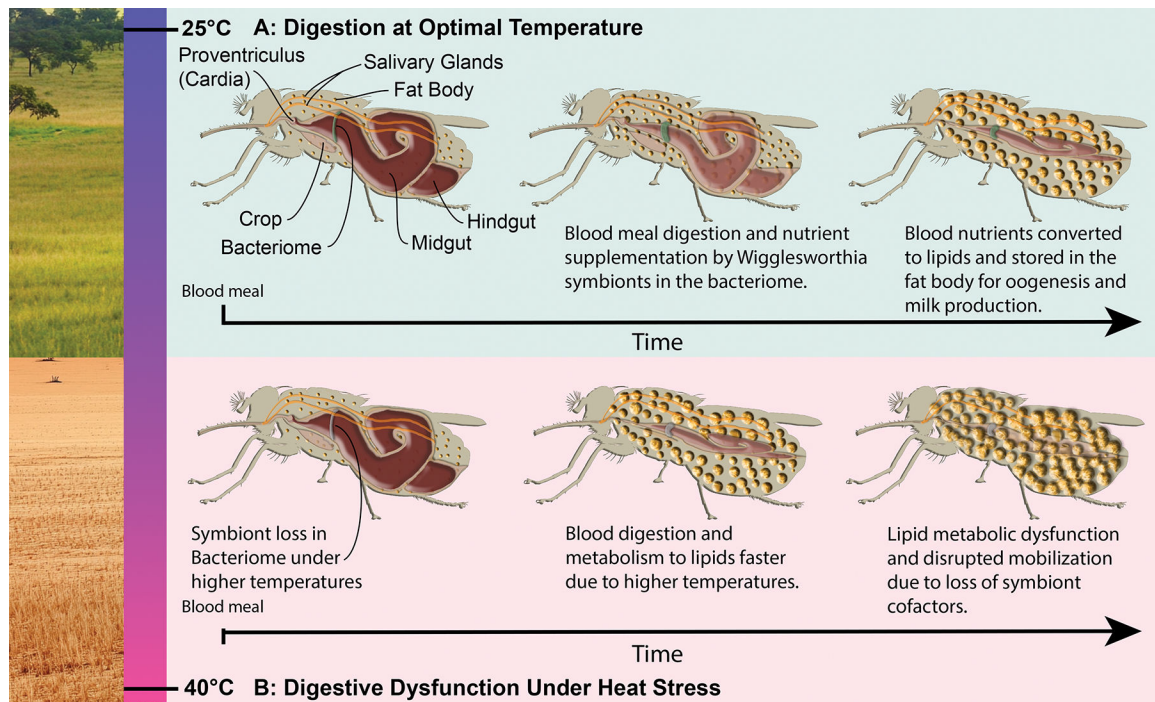
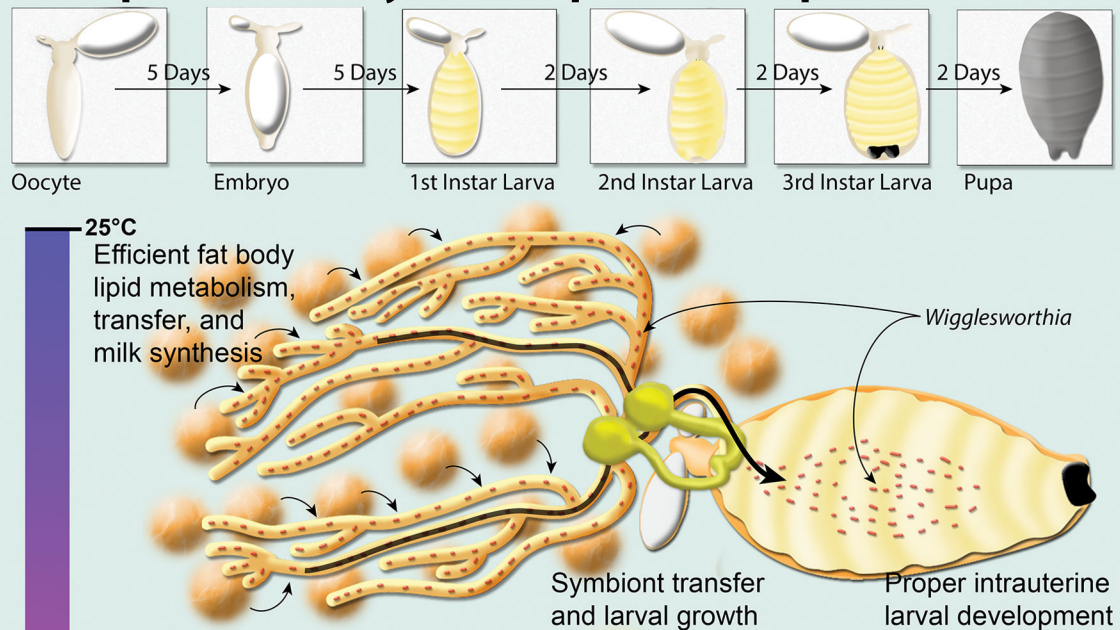


Figure 1 - Summary of the impact that high thermal stress will have on tsetse fly digestion.

A: The general digestion process (under optimal temperatures) requires symbiont-derived nutrients, mainly B vitamins from *Wigglesworthia*, to allow for lipid and amino acid metabolism [27,44]. This allows for lipid accumulation that is necessary for providing the resources for oogenesis and milk production. B: Higher temperature exposure will increase blood digestion rate [25], but prolonged exposure to higher temperatures could eliminate symbionts, leading to loss of metabolic homeostasis, including dysfunction in lipid metabolism [44].

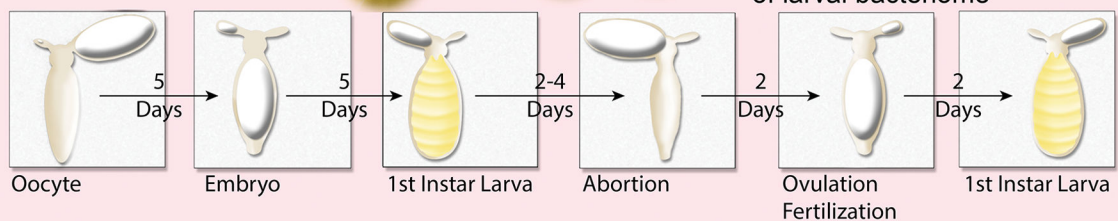
A: Reproductive Cycle at Optimal Temperature



Disruption of lipid metabolism and nutrient transfer from fat body to milk gland due to lack of symbiont cofactors

Delayed or aborted larval development

Symbiont death/disruption of larval bacteriome



B: Reproductive Dysfunction Under Heat Stress

Figure 2 - Summary of the impact that high thermal stress will have on tsetse fly reproduction. A balance between metabolism and nutrient allocation is necessary for the progression of tsetse pregnancy. A: At optimal temperatures, metabolism and milk production match those to allow larval development, including symbiont transfer. This includes an accumulation of lipid reserves between pregnancy cycles [7]. B: Higher temperatures will lead to a restriction of nutrient transfer to the developing progeny, which can lead to whole-organism failure [55]. At extreme temperatures, symbiont elimination will yield progeny unable to complete

development. These effects could explain increased abortion during hotter periods, where a lack of symbionts could yield an inability to produce milk [49].

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