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Big Baby, Little Mother: Tsetse Flies Are Exceptions to the Juvenile Small Size Principle

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While across the animal kingdom offspring are born smaller than their parents, notable exceptions exist. Several dipteran species belonging to the Hippoboscoidea superfamily can produce offspring larger than themselves. In this essay, the blood-feeding tsetse is focused on. It is suggested that the extreme reproductive strategy of this fly is enabled by feeding solely on highly nutritious blood, and producing larval offspring that are soft and malleable. This immense reproductive expenditure may have evolved to avoid competition with other biting flies. Tsetse also transmit blood-borne parasites that cause the fatal diseases called African trypanosomiases. It is discussed how tsetse life history and reproductive strategy profoundly influence the type of vector control interventions used to reduce fly populations. In closing, it is argued that the unusual life history of tsetse warrants their preservation in the areas where human and animal health is not threatened.

"Go away, stop, turnaround, come back".[1]

1. Introduction

Science strives to identify basic principles, especially those that fit together in a causative chain, but some of the first principles in such chains are so obvious that it might seem absurd to dwell on them. In biology, the most fundamental principle is that all creatures die, leading to the next principle that reproduction is essential. Vegetative reproduction is common in many taxa but, for most, sexual reproduction is optional or obligatory, increasing adaptive potential by promoting genetic variation or modulating genomic architecture.[2] Sexual reproduction means that populations usually involve three types of members: mothers, fathers, and offspring.

The next principle—which follows from sexual reproduction—is that offspring are always born smaller than their mothers, as highlighted satirically by Ellstrand.[3] He named this the juvenile small size (JSS) principle and offered six supposedly serious reasons why it should hold. These included plausible considerations of population dispersal, predation, and parent–offspring competition. Ellstrand described only one case of juveniles being larger than their mothers—the sexual reproduction of diatoms—but this case is contentious because the new diatom is not "born" from its mother. Rather, the mother is destroyed upon creation of the zygote, which then expands independently to offset the size reductions associated with the diatom's mode of vegetative reproduction.[4] Ellstrand mischievously concluded his article by stating that the JSS principle is as fundamental a principle as offspring being born younger than their parents.

In the four decades since it was published, Ellstrand's article has been cited only 13 times, yet this does not reflect its quiet appreciation among the community of evolutionary ecologists.[5] Aside from its parodic nature, citing JSS is unnecessary because juveniles being smaller than their parents is indeed a general phenomenon. Among herbivores, the average birth weight of most ungulate offspring remains below ≈17% of the maternal weight,[6] and the baby of a two-toed sloth (*Choloepus didactylus*) weighs up to 15% of its mother.[7] The principles evident for herbivores also apply to creatures with other feeding habits. For example, the carnivorous female blue whale (*Balaenoptera musculus*), weighing on average 180 000 kilograms, produces a baby that is only 1.5% of her weight.[8] Furthermore,

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even in the extreme case of maternal allocation by the omnivorous kiwi, where females often lay only a single egg per year, the massive egg produced is merely a quarter the size of the female.[9]

The precise extent to which babies should be smaller than their mothers in order to maximize fitness is the subject of numerous arguments and modelling studies (reviewed by Rollinson and Rowe[10]). Moreover, the JSS principle has intuitive appeal, perhaps because our science tends to be subjective.[11] We are familiar with human babies being only ≈6% of the mother's mass,[12] and cannot easily imagine a mother birthing a newborn the size of a young adult.

There is sufficient reason for the JSS principle to apply to the vast majority of species, but equally sound reason there are some exceptions that turn this principle on its head. To the best of our knowledge, all of the exceptions are insects, having anatomies and life histories radically different from those of humans and other vertebrates. Moreover, all exceptions of supersized offspring belong to the superfamily Hippoboscoidea within the order of true flies (Diptera).[13] Four families are placed in this group and all are obligate blood-feeders: ked flies, two families of bat flies, and tsetse flies. Within this superfamily, females do not lay eggs, but instead retain the developing embryos within a modified uterus and nourish them on milk-like secretions until the offspring are ready to be born (adenotrophic viviparity). The Hippoboscoidea[13] differ from other viviparous invertebrates in that they can develop and give birth to a single, very large offspring at a time.

More than 700 species of louse, ked, and bat flies are geographically distributed across all continents except Antarctica. These creatures are ectoparasites of many species of mammals and birds[13,14] and can transmit diseases of veterinary importance such as *Bartonella*[15] and Blue tongue virus.[16] In general, these insects have a limited capacity for flight and often a strong host specificity. In contrast, there are 22 species of tsetse fly (*Glossina* spp.) inhabiting and restricted to 10 million square kilometers of sub-Saharan Africa.[17] Tsetse flies, however, differ notably from the other hippoboscids in several ways. They are strong flyers that maintain autonomy from their vertebrate hosts and each tsetse fly species commonly feeds from several host species, in particular those having fixed home-ranges, such as pigs and lizards. Tsetse flies also give birth to a single live offspring (larva) that can weigh as much as the mother—or be even heavier.[18,19]

It is intriguing, therefore, that a reviewer who rejected Ellstrand's first submission of his JSS essay to another journal wrote "... it is difficult to see how any organism with a nutritionally dependent juvenile could produce a juvenile of larger mass, unless the adult acts like a nutrient pump over a long period of time, slowly inflating the ballooning infant". How ironic that this sentence, written in deep scepticism, describes the very process of prenatal juvenile development in tsetse flies, keds, and bat flies. In this essay, we focus on the causes and consequences of producing relatively large offspring in tsetse flies, as there are extensive data on their ecology and life history and because of their capacity to transmit the fatal diseases known as the African trypanosomiases.

Figure 1. A recent blood-fed tsetse fly (*Glossina morsitans morsitans*) seen through the plexiglass sheet on which it is resting. Within a few minutes, the fly must rapidly excrete 80% of the water and salts from the blood-meal using a process called diuresis. A drop of excreted fluid has already fallen onto the plexiglass cage wall and is acting as a magnifying glass to accentuate the next droplet forming.

2. How Tsetse Flies Defy the Juvenile Small Size (JSS) Principle

Since tsetse flies defy the JSS principle, we can now ask how natural selection could produce such an extreme form of reproduction. This evolution seems to have been driven—or facilitated—by a combination of two main factors: first, the protein-rich diet (blood) that allows the production of relatively enormous offspring, and second, a lack of the physiological constraints faced by vertebrates.

2.1. Diet

Tsetse flies have been described as "little lions on wings" as they are free-living, voracious predators that hunt widely for rich vertebrate protein.[20] Every few days, tsetse flies can consume up to twice their body weight in blood (**Figure 1**).[21,22] Both sexes, as adults, feed solely on blood, in contrast to most other blood-sucking flies (such as mosquitoes, blackflies, and sand flies), where only the females ingest blood—and not exclusively, because they supplement their diet with nectar.[20]

The challenge of ingesting a large quantity of blood is that tsetse flies must excrete much of the nitrogen that the blood-meal contains, because nitrogen accumulation is toxic to the fly. Alongside the excretion of the nitrogen-rich amino acids arginine and histidine, uric acid is also excreted. The process of forming uric acid is energetically costly as this procedure can burn half of the total energy available from the meal.[23] There is thus the opportunity for the female to invest a large part of her protein surfeit into producing protein-rich secretions to meet the high nutritional demands of her offspring. In taking this opportunity, the female tsetse fly matures one egg at a time, retaining it in her uterus, where it hatches into a larva. The unborn larva feeds on ≈30 mg (wet weight) of a milk-like secretion consisting of 50% protein and 50% lipids that is produced by an intrauterine milk

Figure 2. A female tsetse fly (*Glossina morsitans morsitans*) is giving birth to a third instar larva. The larva often weighs more than the female fly.

gland.[24] All three in utero larval instar stages are completed in ≈9 days (reviewed by Benoit et al.[25]). Shortly after birth, the larva quickly burrows into the soil, where it is relatively well protected from most predators and parasites during pupation.[26] The larva has all the energy and raw material reserves needed to develop into an adult and to survive the search for its first bloodmeal. In contrast to tsetse flies, keds, and bat flies, other blood-sucking female flies produce batches of many eggs. These eggs are then abandoned to an uncertain fate, leaving the small, newly hatched larvae to find their own food in water bodies or damp habitats that are essential for completing development.[27]

2.2. Physiology

The short gestation period of tsetse flies (relative to their lifespan) approximates to that of small, viviparous vertebrates such as mammals,[28] when, in comparison, larger vertebrates have longer relative gestation periods. The shorter gestation in small vertebrates is explained by the cube-square law,[29] which holds that the placental area of absorption relative to the volume of the fetus increases as linear dimensions decline, facilitating quicker growth in smaller mammals. Tsetse flies go even further, however, and sidestep placental limitations altogether by having no placenta at all. They instead rely on the direct and continuous feeding of "milk," which is a phenomenon observed in other arthropods.[30] This continual flow of milk to the larva enables tsetse flies, in just a few days, to complete the development of offspring weighing more than the mother.

Moreover, like all members of the Cyclorrhapha, tsetse flies produce a larva that, being soft and malleable in form, can be readily squeezed out of the birth canal, despite the larva being as large as its mother (**Figure 2**).[22] We see in this mechanical consideration why it is that the reproductive strategy of tsetse flies can be so different from that of species with physiological restrictions. To compare this feature with more familiar creatures, even dedicated carnivores feeding on a protein-rich diet could not deliver a giant baby through the pelvic girdle. This difficulty seems so great that there appears to be little evolutionary pressure to develop ways of overcoming it. There is only a weak correlation between birth size and the channel width of any pelvic girdle,[10] although adaptations to viviparity include uniquely modified reproductive morphologies such as a vagina that is more muscular and tracheated (ridged).[13]

Yet another problem, especially for larger-bodied creatures, is that Galileo's cube-square principle plays a role. A creature's strength depends on the cross-sectional area of its muscles, which changes with the square of its dimensions, whereas the mass that the muscles must move generally increases proportionally with the cube of bodily dimensions.[29] Hence, tsetse flies are strong enough to maneuver effectively when carrying either a bloodmeal or baby weighing as much as themselves, but most vertebrates are not. This principle is not as important with smaller blood-feeders like vampire bats, because they are not so many times larger than tsetse flies. Yet, even an adult female vampire bat, weighing roughly 40 g, can only produce a single baby weighing ≈25% of the mother's weight (**Figure 3**).[31,32]

3. What are the Immediate Consequences of Giving Birth to Large Offspring?

While breeding rates are greater for tsetse flies than for most mammals (2–4 offspring per month),[17] the tsetse flies' strategy of birthing one enormous offspring (third instar larva) at a time means that, overall, tsetse flies produce much fewer offspring during their lifetime than most egg-laying flies. This results in a slow life history where female tsetse flies must be long-lived and able to mature their larvae as continuously as possible if the number of female offspring produced is to be sufficient for population replacement.[33,34] Indeed, once a female tsetse has become old enough to ovulate (i.e., a few days into adult life), it mates and indefinitely stores the sperm, thus guaranteeing continual pregnancy for the remainder of its life. Given that mothers produce approximately equal numbers of male and female offspring, and that adults and pupae are subject to many causes of death,[35] the average life span of adult females must cover several larval cycles, that is, about 50 days. This means that the maximum life span must be much longer and, indeed, tsetse flies can survive in the field for about 6–8 months[36–38] thanks to adaptive behaviors to avoid predation, to tolerate temperature extremes, and to select safer resting sites.[39]

By evolving a strategy where larvae do not need to feed, tsetse flies are able to reproduce in a variety of environmental conditions, and are not constrained by seasonal variability in rainfall or plant phenology.[40] Moreover, by feeding solely on host animals, tsetse flies have food available all year round, and are not limited by the cyclic absence of flowers and nectar. Unlike many other flies, tsetse flies do not need to aestivate to avoid dry conditions; adults or pupae in a resting phase would be subject to an accumulating daily mortality and significant selective disadvantage anyway. Tsetse flies thus reproduce throughout the year,[35] hence facilitating a long and steady reproductive life.

The all-year activity of tsetse flies is advantageous because it allows them to feed during the hot dry season in the absence of other biting flies, such as stable flies, mosquitoes, and many tabanids, which need aquatic environments for breeding.[40] During the wetter seasons, there can be huge numbers of these other flies, which cause visible distress and reduced complacency of animal hosts, hence ultimately leading to decreased feeding success in tsetse flies.[41] It is likely therefore, that the weak competition from other biting flies in the dry season is important in allowing tsetse flies to offset the temperature stress occurring at that time.

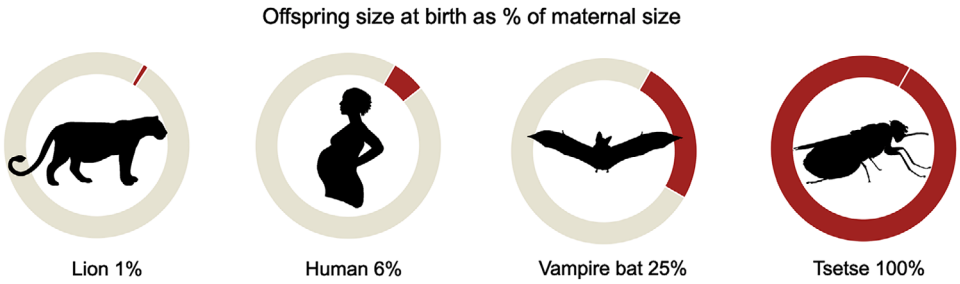

Figure 3. A schematic representing the relative mother and offspring sizes across select taxa. Offspring weight (red) is shown as a percentage of the mother's pre-pregnancy weight (grey).

However, tsetse populations cannot cope if they are exposed to temperature regimes that prevent breeding at certain seasons. Abnormally high and low temperatures can kill pupae directly, but the more subtle and common problem is how temperature affects the way that pupae expend the fat reserves provided by their mothers. This matter governs the distribution of tsetse flies, and threatens to make them particularly sensitive to climate change.[42] The key limitation seems to be the effects of temperature on the survival of pupae and newly emerged adults.[43,44] At low temperatures, pupae take so long to develop that fat stores are exhausted before development is complete. High temperatures can kill pupae directly, and also critically deplete the fat reserves: for that reason, emerging flies have insufficient energy to find a meal before starving to death. The consequence of such temperature sensitivity is that the free-living life of a tsetse fly limits its habitat range to parts of the tropics that do not experience marked temperature extremes. By contrast, keds and bat flies are more widely distributed because they benefit from continual and stable homothermy beneath the insulating wool, hair, or feathers of their hosts.

4. Tsetse Reproductive Strategy has Implications for Vector Control

The blood-feeding habit of tsetse is not just a problem of direct damage and irritation to humans and livestock; these flies can also spread parasites that cause deadly diseases in mammalian hosts.[17] Tsetse flies are excellent disease vectors for two reasons: first, they live long and range far, and second, males and females feed regularly, which together, ensures effective parasite transmission. Moreover, the total dependence on bloodmeals, and the associated and distinctive strategy of reproduction, profoundly influence the most effective measures used to control tsetse fly populations and, thereby, reduce disease transmission. Below we discuss some links between reproductive biology and vector control in tsetse.

4.1. Targeted Killing of Adults

In most disease-transmitting, biting flies such as malaria-causing mosquitoes, it is possible to target both immature and adult stages to reduce or eradicate populations.[45,46] Since tsetse larvae are protected within the mother, and the pupae are hidden in the soil, controlling tsetse fly populations is unfortunately limited to attacking the adults. On the brighter side, the slow breeding of tsetse flies means that killing about 4% of the adult female population per day will be sufficient to eradicate any tsetse population.[38,47,48] Such mortality rates are achieved relatively economically by using insecticide-treated cattle[49] or artificial host-like baits[50] to capitalize on the fact that tsetse flies rely on frequent bloodmeals.

4.2. Sterile Insect Technique

Female tsetse flies are naturally long lived and can breed throughout their lives once they have mated as young adults. This means that controlling tsetse flies using the sterile insect technique[51] requires the mass rearing, effective sex-sorting, sterilization, and release of large numbers of sterile males in a protracted steady stream. This is problematic since the special feeding habits (sterile blood) and slow rearing of large tsetse fly populations make the sterile males costly to produce and, furthermore, they are difficult to rear in conventional insectaries. Moreover, male releases are ethically questionable since male tsetse flies, unlike the males of most other biting flies, also feed on blood and are thus as effective as females in spreading the African trypanosomiases. If tsetse flies also laid eggs, it would be easier to use genetic techniques to engineer parasite-resistant (disease refractory) flies for release as has been done for other vector populations.[52]

4.3. Microbiome-Mediated Control

Alongside strategies to reduce female fecundity, exploiting the tsetse fly microbiome for disease control has potential,[53] especially since slow breeding means that only slight disruption of microbial dependency can have significant effects. Tsetse flies rely on symbiotic bacteria to provide crucial vitamins and cofactors that are essential for fly immunity, fecundity, and longevity.[25,54] For symbiont-specific tsetse control, an insect control strategy called paratransgenesis[55] genetically exploits symbiotic bacteria to express molecules that reduce fly fitness or disease competence, or directly target and kill the disease-causing parasites arriving in an infected bloodmeal. Given that the enormous tsetse larva acquires substantial quantities of milk and symbiotic bacteria from the mother to survive as a reproductive adult,[25] understanding how to disrupt, suppress, or interfere with this microbial dependency emphasizes how important it is to understand

tsetse life history within the greater context of controlling tsetse-borne diseases.

4.4. Other Vector Control Strategies

Given that the breeding strategy of tsetse flies confines them to the tropics, their habitats are restricted to specific geographical regions in Africa. Much of the literature on insect vector control has focused on aerial spraying of pesticides and sterile insect technique developed outside tsetse endemic countries. As a result, these methods will not be as successful in controlling tsetse as other insect pests. For instance, aerial spraying was originally developed in 1921 to treat cash crops on a relatively small but financially justified scale[56] and the sterile insect technique was first used successfully against New World screwworms in the 1950s. Unlike tsetse flies, egg-laying screwworms breed faster, can be reared relatively simply, and involve only females in the direct spread of disease.[57] More recently, new locally inspired techniques have been specifically designed to suit the unique tsetse life history and the special context in which tsetse control is performed. These techniques, which capitalize on visual and olfactory attractants for the flies,[58–60] offer low-technology baits that can control resident populations and also provide relatively cheap, year-round barriers to tsetse invasion. Of note, the strong economic pressure to use a single control measure at any one place has created a debate about the relative merits of high and low technology, which curiously tends to be more animated with tsetse flies than with other insects.[61]

5. Conclusions

This essay describes how several species within the blood-feeding Hippoboscoidea superfamily are clear exceptions to the JSS principle, which is best illustrated by tsetse flies birthing offspring larger than themselves. We are also focusing on tsetse flies because these long-lived, free-living flies are restricted to the African continent and are vectors of a parasite causing harm to humans, livestock, and wildlife. This magnifies their economic importance because they can destroy human health, constrain agricultural development (livestock and land), and deter tourism.

Although effective management of tsetse fly populations to control disease transmission is the priority, we must also recognize that tsetse flies are biologically fascinating organisms. It is important to maintain biodiversity and species richness in tsetse-endemic regions[62,63] so as to understand how their peculiar reproductive strategy evolved within its natural environment. We still have much to learn from this extraordinary fly. For example, do different tsetse fly species vary their maternal investment according to different ecologies? Do female tsetse flies alter resource allocation to offspring according to their age or access to blood? Is disease susceptibility linked to how much the female invests in her offspring? Can tsetse reproductive strategy and life history be incorporated into models to predict the dynamics of populations and disease transmission, and ultimately help improve current vector control and surveillance?[64]

As a final reflection, tsetse fly control must be a priority for crucial interfaces where humans and livestock risk exposure to these flies and the diseases that they spread. Answering outstanding questions about tsetse life history, such as their unusual reproduction, could ultimately lead to more effective vector control strategies and interventions. Where tsetse flies exist outside of human landscapes, however, in particular, in areas rich in wildlife, support should be given to local stakeholders to conserve biodiversity and understand the value of tsetse flies as contributing to species richness and habitat preservation. Disease elimination does not justify pushing these species to extinction.[65]

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Conflict of Interest

The author declares no conflict of interest.

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Glossinidae, life history traits, maternal allocation, reproductive strategy, vector control, vector ecology, viviparity

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