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Beyond the Black Box: Reproductive Strategies of the Black Soldier Fly as a Model for Bridging Evolutionary Biology and Applied Entomology

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Short Summary

Despite its global relevance in waste valorization, the adult reproduction of the black soldier fly (BSF; *Hermetia illucens*) remains a "black box" in research. Given the recent surge of studies in behaviour, physiology and genetics, we present a timely synthesis and provide a conceptual model detailing key processes that influence reproductive fitness. From eclosion to senescence, BSF can exhibit sex-specific plasticity induced by larval nutrition, influencing precopulatory mate acquisition and complex postcopulatory ejaculate-female interactions. We identify areas for future research including lekking dynamics, sperm precedence and volatile-guided oviposition. Our review aims to bridge evolutionary ecology and applied entomology in opening the "black box" of BSF reproduction.

Abstract

The black soldier fly (BSF; *Hermetia illucens*) is rapidly emerging as a model for evolutionary biology and insect biotechnology. While larval biology has been extensively characterised, the reproductive biology of adults remains largely underexplored. Here, we present a timely review synthesizing recent empirical findings in physiology, behavioural and chemical ecology to open the "black box" of BSF reproduction, focusing on processes that span eclosion to senescence. We highlight pre- and post-mating mechanisms that underlie overall reproductive fitness: From mating latency, lekking dynamics, courtship and copulation, to sperm transfer, storage and oviposition. We discuss these elements in the context of sexual selection theory and identity notable characteristics of BSF reproduction that differ from traditional insect reproductive models. These include the unique capital breeding life history (non-feeding adults dependent on larval reserves), protandry (early male emergence) that couples with nutritionally influenced sexual maturation and a lek-like mating system. The sex-specific longevity that varies with mating status and morphologically complex reproductive tracts also suggest opportunity for strong postcopulatory sexual selection. Increasingly, environmental factors such as light, substrate volatiles, and rearing design are reported to significantly modulate BSF reproduction in an industrial environment, highlighting the potential for bridging fundamental and applied biology. We propose a novel conceptual framework that interconnects these elements and we outline critical questions that are still unresolved (e.g., mechanisms of sperm precedence, female control of fertilization). This interdisciplinary model supports both fundamental insights into reproductive evolution and provides practical improvements for optimizing industrial mass-rearing.

Keywords: *Hermetia illucens*; behaviour; optimisation

1. Introduction

In the past decade, the black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae) has been gaining interest in both the scientific research and industrial applications. As an efficient decomposer of organic waste, BSF larvae are extensively investigated for the bioconversion of waste into protein and fertilizer, making it a keystone species in sustainable agriculture and circular food systems (Tomberlin & van Huis, 2020; Tomberlin *et al.*, 2022). At the same time, BSF is increasingly recognised as a model organism for fundamental studies in evolutionary biology and behavioural ecology (Tomberlin *et al.*, 2025). Yet, this potential remains largely underexplored: Industry priorities have overwhelmingly driven most of the research towards larval life history traits, rendering the reproductive biology of the adult as a "black box". A recent bio-economic analysis even suggested that improving larval growth and conversion efficiency yields higher economic returns than improving reproductive traits. Yet, reproduction is the 'heartbeat' of any species' life cycle and in the case of a mass-reared insect like BSF, neglecting the reproductive phase constrains both scientific understanding and industrial optimization (Lemke, Dickerson & Tomberlin, 2023; Meneguz *et al.*, 2023; Tomberlin *et al.*, 2025).

Insects vary drastically across lineages in reproductive strategies (Wilson, 1999) and emerging evidence suggests BSF is unique in many ways. For instance, many classical insect models such as *Drosophila melanogaster*, are income breeders. In a strict sense, this means these organisms require feeding as adults to reproduce, and more loosely speaking, foraging can still extend lifespan and increase reproductive fitness for those using a hybrid-strategy (Zhang *et al.*, 2025a). By contrast, capital breeders such as BSF do not need to feed as adults to successfully reproduce (Tomberlin, Sheppard & Joyce, 2002a). Instead, adult reproduction is almost entirely dependent on nutritional reserves accumulated during the larval stage. BSF also exhibit protandry (Tomberlin *et al.*, 2002a) where males emerge several days before females, the timing of whose emergence is modulated by larval nutrition (Zhang *et al.*, 2025a). In nature (Tomberlin & Sheppard, 2001) and captive colonies (Lemke, Rollison & Tomberlin, 2024), adults can form lek-like swarms in which both sexes can mate multiple times in a polygynandrous mating system (Manas *et al.*, 2025b), that impacts both pre- and postcopulatory sexual selection. Moreover, increasing reports from industrial optimization suggests that reproduction in BSF can also be environmentally modulated (Chia *et al.*, 2018; Addeo *et al.*, 2022). Factors such as like UV-light spectrum and intensity (Zhang *et al.*, 2010), the presence of specific substrate volatiles for oviposition (Zheng *et al.*, 2013), and even the physical design of mating cages (Grosso *et al.*, 2025) can significantly influence mating success and fecundity in captivity, further driving shifts in the mating system.

Understanding the features of BSF reproduction has broad implications for both evolutionary biology and applied entomology. For decades, classical model organisms such as *Drosophila melanogaster* Meigen 1830 (Diptera: Drosophilidae) have dominated the fundamental research landscape (Kohler, 1994; Markow, 2015). While it is undeniable that *D. melanogaster* has yielded pivotal insights in science, it also represents only a narrow slice of insect diversity and its utility has often stemmed from an assumption of laboratory tractability (Ankeny & Leonelli, 2011). In fact, comparative studies now suggest that laboratory strains of *D. melanogaster* often differ significantly from wild populations, underscoring the need for broader taxonomic representation of model systems (Markow, 2015). Indeed, laboratory rearing conditions represent an alternative ecological niche by which species evolve and diverge from their wild counterparts (Kohler, 1994). Moreover, recent NIH budget cuts have substantially reduced funding for the *D. melanogaster* bioinformatics repository, FlyBase (flybase.org, May 2025), highlighting the risks of over-reliance on single model species and heralding the need to diversify and develop alternative models. The emergence of BSF as a research model is therefore timely, representing a different lineage (viz., a basal group of the lower Brachycera) with a different reproductive strategy that can also bridge evolutionary biology and applied entomology in ways that few other insect models can. Not only does the BSF lek-like system make it relatable to organisms across the animal tree of life (including lekking crustaceans, insects, fish, amphibians, birds, and mammals) (Lemke, 2025), but BSF is also positioned centrally within the Dipteran lineage between many economically- and culturally-important Nematocera (including mosquitoes, gnats, midges, black flies, drain flies, sand flies, and crane flies) and the Muscomorpha (including hover flies, Tsetse flies, blow flies, bot flies, Tachnid flies, true fruit flies, stalk-eyed flies, and *Drosophila*) (Yeates & Wiegmann, 2005; Lambkin *et al.*, 2013).

Here, we synthesise emerging empirical evidence on BSF reproduction into a comprehensive conceptual framework, identifying key research gaps (Figure 1). We examine processes that govern reproduction from adult emergence and sexual maturation, through mating latency, courtship and copulation, to sperm transfer and storage, as well as oviposition and senescence. Throughout this review, we discuss these elements in the context of sexual selection theory and aim to reposition BSF as a viable model for reproductive evolution and behavioural ecology. We also discuss how external factors, from lighting regimes to substrate cues can influence reproduction and emphasise opportunities to translate fundamental knowledge into improved rearing practices. By comparing BSF with classical models and drawing insights from other taxa, we highlight key areas for future research.

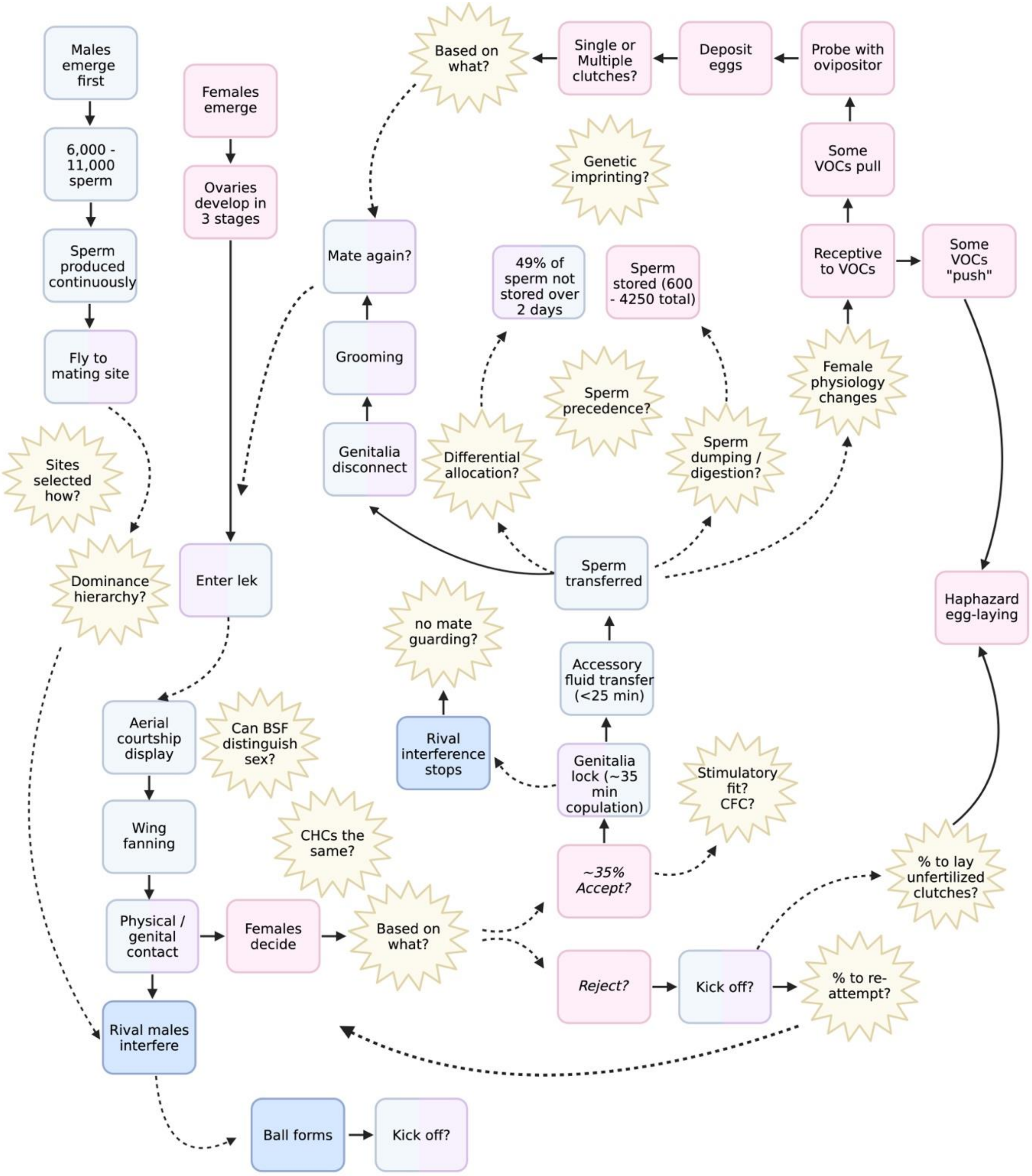

Figure 1. Schematic diagram depicting behavioural and physiological processes in BSF reproduction. Blue boxes represent male processes, whereas pink boxes represent female process. Boxes which are both light blue and pink are processes common to both sexes, while dark blue boxes are male behaviours occurring outside of the mating pair. Yellow, 12-point stars indicate open research questions which need more exploration. Dashed arrows indicate a process is theorised but the link has yet to be demonstrated, whereas a solid arrow is one that necessarily is derived from the previous step.

2. Mechanisms and Dynamics of Reproduction

Reproduction in BSF is a complex process shaped by nutritional history, social context, and environmental cues. From latency to senescence, both males and females exhibit dynamic physiological and behavioural adaptations that challenge earlier depictions of BSF as a monogamous or behaviourally uniform species (Nakamura *et al.*, 2016; Park *et al.*, 2017; Giunti *et al.*, 2018; Freitas Spindola, 2019; Macavei *et al.*, 2020; Malawey *et al.*, 2020; Surendra *et al.*, 2020; Awal *et al.*, 2022). To clarify this issue and others, we summarise how the pre-copulatory, copulatory and post-copulatory phases contribute to overall reproductive success.

2.1. Pre-Copulatory Phase

2.1.1. Latency and Sexual Maturation

Protandry is a defining feature of BSF, with males emerging several days before females (Tomberlin *et al.*, 2002a; Meyermans *et al.*, 2025). In butterflies and other insects, protandry is a common tendency, occurring in ~36% of species (Teder *et al.*, 2021), and is believed to be a strategy used by males to better monopolise females. Protandry may be an adaptive mechanism to facilitate the alignment of male persistence and female receptivity (Zhang *et al.*, 2025b), since by emerging prior to females, this minimises the female's time spent unmated (Fagerström & Wiklund, 1982). The total degree of the separation between males and female emergences, i.e., sexual bimaturism (SBM) in BSF is modulated by larval nutrition and interacts with adult body size and longevity (Generalovic *et al.*, 2025), being also a covariate of size dimorphism (Teder *et al.*, 2021), meaning as females grow in size relative to males, the degree of SBM also increases. In BSF, protandry also seems predicated on the condition that adults do not need to feed or forage prior to mating (Tomberlin *et al.*, 2002a). Instead, they rely almost exclusively on the resource accumulation during the larval stage (Lemke *et al.*, 2023; Harjoko *et al.*, 2023). However, BSF may not be strict capital breeders, and there is some evidence to suggest that supplemental nutrition can benefit adult fitness as well (Thinn & Kainoh, 2022; Klüber *et al.*, 2023; Barrett *et al.*, 2025), though this is not always consistent (Lemke *et al.*, 2023; Coudron *et al.*, 2025a). Certainly, reproduction can proceed without feeding but provisioning adults with water can aid by preventing dehydration (Sheppard *et al.*, 2002) and to also promote welfare (Barrett *et al.*, 2025).

Immediately post-eclosion, male BSF also possess some sperm (ca. 3,000 - 11,000 spermatazoa) (Munsch-Masset *et al.*, 2023), similar to *D. melanogaster.* Males also typically delay initiating mating by one to two days post-eclosion, likely synchronizing with the timeline of female oocyte maturation (Tomberlin *et al.*, 2002a; Meyermans *et al.*, 2025). This timing is also suspected to coincide with wild population dynamics, since BSF males would use this time to establish their position within a lekking site while females emerge and mature (Lemke *et al.*, 2025). Indeed, in true fruit fly species (*Ceratitis capitata*; Tephritidae) and other Diptera where males display in groups, reproductive success is modulated by position within the lek, e.g., a central position or on specific trees (Shelly, 2018). Male BSF continue to produce sperm throughout life but testes size shrinks with age, suggesting that there could be an upper limit on lifetime total sperm production of up to ~50 000 per male (Munsch-Masset *et al.*, 2023; Manas *et al.*, 2024). Recent experimental work also suggests that sperm quantity is influenced by social exposure and housing conditions, which is likely an adaptive response to sperm competition risk (Manas, Labrousse & Bressac, 2025a). Sperm length may also play a role in competitive fertilization success and BSF have relatively long sperm (Malawey *et al.*, 2019, 2020), being 6-times longer than the average length of other animals with internal fertilization (Munsch-

Masset *et al.*, 2023),, with a notable exception of *Drosophila* (Lüpold *et al.*, 2016), e.g., the giant sperm species *Drosophila bifurca* (Pitnick, Markow & Spicer, 1995; Luck *et al.*, 2007). Although inadequate larval nutrition in BSF can generate smaller males with shorter sperm (Zhang *et al.*, 2025b), it is still unclear if there are potential trade-offs in quantity versus quality and whether these would translate to direct fitness effects.

In contrast to males, the ovaries of female BSF develop post-emergence, with synchronous egg maturation from a single ovariole (Munsch-Masset *et al.*, 2023). This distinguishes BSF from many other proovigenic insects where their eggs are all mature at the onset of their adult life, as well as many insects whose fecundity is linked to both the number and variation of ovarioles (Moore, 2014).Synovogenic egg development (i.e., starting adult life with immature eggs) in other insects is thought to be related to stochastic developmental conditions, which aligns with BSF development, given that larvae can subsist on nutrient-poor substrates. Hence, rather than being a fixed number, total female egg number in BSF is instead tightly correlated with adult body size (Spearman's ϱ = 0.73) which in turn depends on larval nutrition (Gobbi, Martínez-Sánchez & Rojo, 2013; Shrestha *et al.*, 2025). As such, BSF exhibit large variations in fertility (i.e., hatching rate) that can range from 30 to 90% and can also be influenced by early development conditions (Laursen *et al.*, 2024; Zhang & Puniamoorthy, 2025). However, it is harder to disentangle what proportion of this variability in fertility is a female versus male mediated effect as the problem could arise with either sperm or eggs.

2.1.2. Navigation and Lekking

The mechanisms by which adult insects locate mating sites can differ. Damselflies seek out riverbanks (*Hetaerina spp.*; Odonata) (Córdoba-Aguilar *et al.*, 2009), neotropical orchid bees target treefalls or decaying wood to collect volatile compounds (Euglossini: Hymenoptera: Apidiae (Kimsey, 1980) whilst coprophagous insects such as dung beetles gather at dung pats that act as competitive mating arenas (Hanski & Cambefort, 2016). To date, the exact mechanisms by which BSF locate mating sites in the wild remain largely unknown. Inferences from industrial observations suggest that flies may be guided by gradients in light, because newly eclosed adults from darkened pupation area will be attracted towards illuminated zones (Dortmans *et al.*, 2017; Coudron *et al.*, 2025b). This is exploited in mass-rearing systems for automated counting, since flies can be funneled through a pinhole (James *et al.*, 2024). This behaviour may mirror patterns of natural emergence in the wild since the larvae are negatively phototropic and often wander off prior to pupation (Giannetti *et al.*, 2022).

While natural lekking sites have been described as occurring hundreds to thousands of meters from emergence zones (Tomberlin & Sheppard, 2001; Lemke *et al.*, 2023), it is unclear whether stochasticity (e.g., updrafts of wind) or specific cues drive BSF navigation to leks in the wild. Other lekking or aggregating insects often follow chemical trails, detect conspecifics swarming frequencies, or seek out certain landmarks (Alcock, 1984). For instance, Neotropical owl butterflies, *Caligo spp.*, (Srygley & Penz, 1999) gather towards forest edges, and a close relative of BSF, *Hermetia comstockii* show preference for Agave plants (Alcock, 1990)). What sparse reports of wild BSF are clearly biased towards the ovipositing females, since these are attracted to the volatiles emanating from anthropogenic wastes. Historically BSF were found in "dense mats" on the sides of confined animal feeding operations (CAFOS) where solid manure from chickens and/or pigs accumulated line (Tomberlin et al. 2002 citing Lorimar 2001 [white paper]), or in the nearby kudzu-infested tree (Tomberlin & Sheppard, 2001). But, since the introduction of feed-through pesticides targeting house flies after ca. 1980 (Sheppard *et al.*, 2002), BSF populations may have been unintentionally affected, since BSF are also highly sensitive to such pesticides (Tomberlin, Sheppard & Joyce, 2002b). In addition, BSF are apparently reluctant to enter enclosed spaces (e.g., homes or CAFO buildings) (Furman, Young & Catts, 1959; Sheppard *et al.*, 2002), making the only obvious link between the mating site to the oviposition site, which can be thousands of meters away (Lemke *et al.*, 2023) is the distance females must fly on limited energy reserves (Tomberlin *et al.*, 2002a; Harjoko *et al.*, 2023).

Other lekking species utilise and respond to semiochemicals, the most famous of which might be male fruit flies which can be attracted with Trimudlure or essential oils, such that they can be easily trapped and leks artificially created (Shelly, Whittier & Kaneshiro, 1993) (Refer to Section 4.4 for further discussion). But unlike these other insects, the mechanism for how BSF assemble in leks is puzzling considering that BSF might not emit any attraction pheromones (Lemke *et al.*, 2023). Within the artificial environment, mating flies often display spatial segregation away from the oviposition sites and towards less-humid microenvironments (Unpublished data). This suggests that in an industrial setting, dedicated mating arenas and oviposition areas within the cage could enhance reproductive success (Salari & De Goede, 2024) (Refer to Box 1 and section 4.3 for further discussion).

2.1.3. Courtship and Mounting

In BSF, male activity and courtship is triggered by female flight or entry into a swarm . In captivity, females have been observed to momentarily leave their perch (on artificial plants or walls) to enter male swarms occurring near artificial light sources (Lemke *et al.*, 2024) before returning to perch or descending *in copula*. This observed behaviour appears to mirror natural history descriptions in which females visit the lek, where, due to protandry, males are already present (Tomberlin & Sheppard, 2001; Lemke *et al.*, 2023). Indeed, similar temporal patterns in perching and flight activity have been described in the lekking *Synpetrum danae* (Sulzer) (Odonata: Anisoptera: Libellulidae), in which reproductive activity peaked at solar noon with male flight activity, compared to females whose activity increased in the early afternoon (Michiels & Dhondt, 1989).

BSF males initiate courtship by performing aerial displays of wing-fanning, and it may be wing-interference patterns (WIPs) that are recognised by conspecifics (Rebora *et al.*, 2024). In addition to these visual displays, male BSF produce vibratory songs (Giunti *et al.*, 2018; Laudani *et al.*, 2024), similar to many other groups of insects (e.g., crickets, grasshoppers, fruit flies) (Baker, Clemens & Murthy, 2019). Wing-buzzing might be a valuable predictor of mating success (Briceño *et al.*, 2002; Aldersley & Cator, 2019), as is the case for in mosquitoes and fruit flies, but in BSF, it could have also evolved as an anti-predator defense, as seen in the blow fly, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) (Sawyer *et al.*, 2021). In *Aedes aegypti* (Diptera: Culicidae), males and females match the harmonics of their buzzing by rapidly modulating the tone of their wingbeats (Aldersley & Cator, 2019). Besides tone, mating songs need to have the proper rhythm (Eberhard & Gelhaus, 2009), as it could provide sex-specific cues about potential mates. In BSF, an earlier study suggested longer duration of wing-buzzing was positively correlated with body size and mating success (Giunti *et al.*, 2018), but more recent work shows an opposite trend with shorter wing-buzzing preferred (Laudani *et al.*, 2024), suggesting the trait is may be plastic.

While air borne, BSF males mount females and attempt genital engagement. Successful pairs *in copula* generally rotate their position 180 degrees resulting in a tail-to-tail posture that is maintained during copulation while perched on a surface, although some remain mounted from behind (Chiabotto *et al.*, 2024). The ability to rotate between the two positions is enabled by flexible genitalia (Rollinson *et al.*, 2025) and may also allow the pair to take flight and relocate in response to disturbance (pers. obs.).

BOX 1: Debates on Mate Discrimination and Lekking

In BSF, detection and discrimination of mates may be facilitated by sexual dimorphism in wing interference patterns created by differences in structural colouration (Butterworth *et al.*, 2021; Rebora *et al.*, 2024). These likely facilitate the differential development of photoreceptive sensitivity of the ommatidia in BSF compound eyes (Oonincx *et al.*, 2016). In fact, evidence suggests that visual neural pathways are more developed in males than females (Barrett *et al.*, 2022). These visual cues may compensate for BSF's limited head mobility and reliance on proprioception while in flight (Paulk & Gilbert, 2006). While some suggest that these may be cues that enable mate recognition in BSF (Rebora *et al.*, 2024), others contend that BSF males cannot discriminate between sexes or kin (Giunti *et al.*, 2018; Laudani *et al.*, 2024) and that these may be artifacts from experimental studies.

Another argument against mate discrimination is the lack of sexual dimorphism in their cuticular hydrocarbon (CHCs) profiles (Lemke *et al.*, 2023), though the same is the case for the New World screw worm fly, *Cochliomyia hominivorax* (Pomonis, 1989). Instead, many sexually reproducing insects communicate via sociochemical signals once they make physical contact (Ingleby, 2015). For instance, the CHCs of *Drosophila* can encode information based on species, sex, reproductive status, age, social rank, which together mediate attractiveness (Holze, Schrader & Buellesbach, 2021). In BSF, however, it is unclear as to how adults differentiate amongst each other and what are the actual cues that signal attractiveness, especially in the context of lekking or mating amidst a swarm. Indeed, females entering swarming leks (associated with flies) have historically been described as exhibiting little if any mate choice (simply allowing males to choose them) (Alcock, 1987); though likely stems from a lack of evidence (and the difficult of obtaining such evidence in field studies) rather than evidence for the contrary.

Lekking is the aggregation of many individuals at a mating site, often in structured hierarchies. It is a complex phenomenon, characterised by several behaviours including a lack of male parental care, sex-based dispersion, territoriality, and the absence of resource monopolization. However, lekking itself is no longer viewed within a strict definition (Alcock, 1987). Each of these criteria can be viewed as a continuum in multidimensional parameter space, like the Hutchinsonian niche (Hutchinson, 1957) to give a more holistic view of lekking systems. Moreover, the lek mating system itself also can be modeled along a gradient of increasing resource dependence and mate-monopolization (Emlen & Oring, 1977; Parker, 1978; Thornhill & Alcock, 1983), and can shift dynamically if these underlying factors change. For instance, when males are unable to control access to females, this leads to scramble competition polygyny (Herberstein, Painting & Holwell, 2017). Conversely, when females become rare, the mating system can also shift to (and between) resource-defense polygyny or female-defense polygyny if males start to monopolise female resources or the females themselves (Buzatto & Machado, 2008). For instance, the males of the Wellington tree weta, *Hemideina crassidens* (Orthoptera: Anostostomatidae) guard tree galleries needed by females as diurnal refugia (Kelly, 2006), whereas territorial male wasps (Hymenoptera) guard the hives from which females emerge or the flowers they visit (Alcock *et al.*, 1978). Similarly, the water used by aquatic species, the fruit used by parasites, or carcasses used by scavengers can be guarded by males, but such is not the case for lekking in which mating occurs independent of such resources. Mating systems that fall shy of these two extremes, but still have some similarity to a true lek, are called 'lek-like'. Lekking has been described in one other congener *Hermetia comstockii* (Diptera: Stratimyidae) which defend perches on agave plants (Alcock, 1990), but not in more distantly related *Merosargus cingulatus* (Diptera: Stratiomyidae), which mate near their oviposition site of decomposing vegetation (Barbosa, 2011), or to *Inopus rubiceps* (Macquart) (Diptera: Stratiomyidae) which engages in scramble competition (Alcock, 1990).

In BSF, there exists debate as to whether lekking as a whole is preserved in captivity (Lemke *et al.*, 2023; Lemke, 2025) and whether large aggregations are even a prerequisite for BSF mating (Laudani *et al.*, 2024), since mating has now been described in lone pairs of BSF (Jensen *et al.*, 2025). Some studies report a clear behavioural segregation of the sexes (Lemke *et al.*, 2024) but other aspects of lekking are often not reported because most detailed behavioural studies in BSF are typically done at small scale (i.e., as few as 2 individuals in a 1m3). However, when BSF are reared at the industrial scale, (i.e., cages housing thousands of individuals per cubic meter), this makes manual observations of individual behaviour impossible. Moreover, a highly competitive breeding environment may facilitate a new evolutionary (sub)optimum whereby satellite leks of lone individuals are favored. Some field and lab observations document stable male aggregations (Tomberlin & Sheppard, 2001; Lemke *et al.*, 2024), whilst other reports mention no aggregation except ephemeral mating balls involving multiple males (Permana, Fitri & Julita, 2020) which might be mistaken for leks. In other words, to date, there exists a lack of evidence (but not necessarily disproving) that BSF colonies exhibit a structured and spatiotemporally mediated hierarchy and whether this is directly linked with variation in reproductive fitness.

2.2. Copulatory Phase

2.2.1. Copulatory Mechanics, Duration and Sperm Transfer

Copulation in BSF is marked by genital engagement lasting approximately 35 minutes [insert +/- SD) (Manas *et al.*, 2025a), the onset of which is defined by a physical 'lock' between the male and female genitalia. Rival males cease interruption attempts and the duration of the genital lock appears mostly unaffected by adult size but varies with age (Manas *et al.*, 2025b, 2025a), suggesting that BSF might not engage in mate guarding. However, overall mating duration is prolonged in inbred cohorts and individuals that experienced poor larval nutrition (Laudani *et al.*, 2024; Zhang *et al.*, 2025a) but it is notable that sperm transfer does not occur continuously throughout this time frame. Dissection of females at different times during mating suggests that sperm is delivered during the latter half of copulation, approximately after the first 25 minutes, following the transmission of seminal fluids (Manas *et al.*, 2025a). Matings might last between 20-42 minutes (Giunti *et al.*, 2018), with an average of 33.32 ± 10.19 min (Manas *et al.*, 2024), but potentially can be as long as 50.2 ± 26.6 min, since mean copulation duration increasing with each subsequent mating (Manas *et al.*, 2025b). At the end, both sexes disengage and typically perform grooming behaviours for 101.15 ± 17.47 s (males) or 296.92 ± 39.22 s (females) (Giunti *et al.*, 2018; Laudani *et al.*, 2024). While at present there is little evidence to suggest mate guarding taking place in BSF, in other species, such as *Parastrachia japonensis* Scott (Hemiptera: Cydinae), mating duration is an alternative mating tactic (AMT) dependent on the density of females (Tsukamoto, Kuki & Tojo, 1994). When females are dense and the species exhibits scramble competition, 93% of matings were 'short', but when females became rare, males monopolised females by increasing mating duration 91-fold from 14.9 ± 10.3 seconds to 22.6 ± 14.7 min (Tsukamoto *et al.*, 1994).

2.2.2. Sperm Storage

After mating, BSF females store sperm in multiple spermathecae, enabling fertilization of several egg clutches over time. Females possess complex sperm storage organs: Three spherical spermathecae, attached to three sclerotised rods that end in hinges, which are connected to three separate fishnet canals (that also contain sperm) (Munsch-Masset *et al.*, 2023; Bruno *et al.*, 2025). The total number of sperm stored in females can vary from the hundreds to the thousands (Manas *et al.*, 2024) and it may take about 48h for approximately 50% of transferred sperm to reach the sperm storage reservoirs. Most critically, recent work has shown that a single mating is enough to fill a female's sperm reservoirs in excess, which challenges previous hypothesis that females were sperm limited (Permana *et al.*, 2020), and instead supports the view that a single ejaculate can provide sufficient sperm for multiple clutches. In addition, recent work also suggests that stored sperm quantity but not viability decreases over time, suggesting that a possibility for sperm dumping or sperm digestion by females. In addition, the social environment or presence of rival males appears to also influence sperm storage because females that mate in the presence of conspecifics also retained more sperm than those isolated post-copulation (Manas *et al.*, 2025a).

Box 2: Reproductive Skews and Re-Mating in BSF

Reproduction in BSF can be dependent on both biotic—e.g., sex ratio, density(Hoc *et al.*, 2019)—and abiotic factors (e.g. light, temperature, stressors(Dearlove *et al.*, 2025)) and experimental studies suggest that mating frequencies can vary dramatically across populations and colonies (Jones & Tomberlin, 2021; Dickerson *et al.*, 2024; Lemke *et al.*, 2024; Meyermans *et al.*, 2025; Lemke *et al.*, 2025). In fact, in populations of 500 M:500 F, only 43-120 mating events were observed over a week, suggesting steep reproductive skews (Meyermans *et al.*, 2025). Indeed a recent experiment has shown that in test populations of 15 M: 15 F, 48% of males did not mate, and of those that did, 50% of BSF mated multiply (Manas *et al.*, 2025b). In addition to the dominance of a few individuals, if some are mating multiple times, but not all flies mate, this suggests a low effective population size, owing potentially to population substructures. For the population, the majority of matings occur on the D1 or D2 of being in mating cages, but continue to occur at a low rate after this, reaching a plateau between D4 and D6 (Tomberlin *et al.*, 2002a; Dickerson *et al.*, 2024; Lemke *et al.*, 2024). Although such reproductive skews are consistent with a lek-based system, in which a minority of males achieve most or all copulations, thus far, no studies have quantified relative reproductive fitness as a function of mating rates (sensu Bateman's gradients (Bateman, 1946)). Behavioural assays have observed male BSF to mate up to four times and females up to twice (Chiabotto *et al.*, 2024; Laudani *et al.*, 2024) but higher female polyandry, up to 5-times, has been supported by parentage assignments (Hoffmann, 2021). These recent studies highlight the potential for strong postcopulatory sexual selection, influencing ejaculate-female interactions, and overall reproductive fitness.

2.3. Post-Copulatory Phase

2.3.1. Oviposition

Following copulation, oviposition for BSF typically occurs between four to six days post emergence and females lay eggs in small, dry crevices near the actual oviposition substrate that is often wet and humid. Indeed, this adaption to thrive in moist substrates is one that must have allowed Diptera to transition away from aquatic development into terrestrial niches. For BSF, female fecundity, i.e. the number of eggs laid, can vary as a function of adult nutrition (Thinn & Kainoh, 2022; Klüber *et al.*, 2023; Barrett *et al.*, 2025), body size (Gobbi *et al.*, 2013), age (Dickerson *et al.*, 2024), and even genetic relatedness (Laudani *et al.*, 2024). Several recent studies suggest female BSF can oviposit multiple times (Hoffmann, 2021; Jones & Tomberlin, 2021; Chiabotto *et al.*, 2024; Laudani *et al.*, 2024), though assertions continue to be made that BSF oviposit only once (Sibonje, 2024). Originally these additional clutches were thought to be completely infertile (Nakamura *et al.*, 2016) but it has now shown that female BSF can potentially lay two to three fertile clutches after only a single mating (Manas *et al.*, 2024).

The timing of oviposition itself can be both genetically and environmentally influenced. Inbred strains exhibit longer pre-oviposition intervals (Laudani *et al.*, 2024), while adequate adult feeding shortens the time between copulation and oviposition (Barrett *et al.*, 2025). Likewise, delaying mating (e.g., as an artifact of experimental set-up) is also suspected to cause females to haphazardly lay eggs when females lay single (or additional) clutches (Dickerson *et al.*, 2024; Muraro *et al.*, 2024; Lemke *et al.*, 2024) far away from the larval substrate. Off-target egg laying can quickly snowball since females are attracted to VOCs released by eggs (Klüber *et al.*, 2024). Fecundity and fertility (i.e. the proportion of larvae that hatch) can trade-off at industrial scale (Hoc *et al.*, 2019) and recent study suggests that larval nutrition can induce phenotypic plasticity in both female fecundity as well as fertility (Zhang & Puniamoorthy, 2025).

2.3.2. Aging & Senescence

Aging in adult BSF is often marked by visible changes in the physical condition of flies including wing damage, limb loss, and desiccation. These likely occur from repeated attempts to mate or oviposit despite declining energy reserves, since in other mass-reared Diptera, harassment is associated with injury to females (Meza *et al.*, 2025). Observations from both laboratory and industrial settings report older BSF frequently crashing to cage floor during flight or even spinning erratically after having broken one of their wings. In BSF, aging can be estimated based on the decreasing opacity of the abdominal 'windows' (Harjoko *et al.*, 2023) though which one can observe changes in the stored nutrient reserves. These translucent windows are thought to have evolved to mimic the petiole of *Polistes* paper wasps (James, 1935; Alcock, 1990). Observing changes in it can serve as a non-invasive indicator of physiological decline and has been successfully adopted for population management in industrial settings (Salari & De Goede, 2024).

Experimental studies suggest that longevity in both sexes can be extended by provisioning adults with water (Tomberlin & Sheppard, 2002) and food (Lemke *et al.*, 2023; Barrett *et al.*, 2025). Interestingly, the seminal components transferred by males to females may also contribute to female longevity, potentially acting as nuptial gifts (Harjoko *et al.*, 2023) for females to digest (Manas *et al.*, 2024) by supplying amino acids and/or lipids that may support egg development and/or metabolic function. Older males exhibit reduced mating frequency (Dickerson *et al.*, 2024) and fertility, due to declining sperm viability (Malawey *et al.*, 2020). This means that housing mixed ages of BSF adults can reduce the efficiency of reproduction at the industrial scale (Lemke *et al.*, 2025). For instance, older, less competitive males may interfere with females mating with younger mates, as observed in Tephritid flies (Papanastasiou *et al.*, 2011), which has necessitated habitat design that features distinct areas for each sex. Similarly, female clutch size and egg viability also declines with age and egg collections from mixed age cohorts will undoubtedly exhibit a greater variability in survival and overall fitness.

➔ Box 3: Dynamics of oviposition site selection

In natural settings, mating and oviposition often occur at spatially distant and distinct sites (Tomberlin & Sheppard, 2001; Lemke *et al.*, 2023; Lemke, 2024), which one hypothesis states could have evolved to dilute predation pressure (Rathore, Isvaran & Guttal, 2023). BSF larvae are polyphagous and are able to thrive on a wide range of substrates but most accounts of wild-trapped females are typically associated with anthropogenic waste (Sripontan *et al.*, 2017; Nyakeri *et al.*, 2017; Ewusie *et al.*, 2019; Ferdousi *et al.*, 2022; Purkayastha & Sarkar, 2023; Sable & Chavan, 2024; Yaseen *et al.*, 2025) Controlled experiments have revealed that BSF prefer to oviposit in vegetable/fruit waste (Kotzé & Tomberlin, 2020; Laksanawimol, Singsa & Thancharoen, 2023; Zim *et al.*, 2023), though may also utilise the medium near aged carrion (Kotzé & Tomberlin, 2020), or manure (Zim *et al.*, 2023). However, experiments have also shown that female BSF do not necessarily select the oviposition site which coincides with highest fitness (Boafo *et al.*, 2023; Tekaat, 2024), meaning there are other ecological factors at play, and possibly maternal-offspring conflicts. The selection of oviposition sites (ergo larval foraging sites) is undoubtably driven by volatised cues emitted by microbial communities and conspecifics (Zheng *et al.*, 2013; Klüber *et al.*, 2024; Thomas *et al.*, 2024; Zhang *et al.*, 2025b). Key attractants included tetradecanoic acid, sulcatone and acetophone that could increase oviposition. Such olfactory actions have been confirmed by amputating BSF antennae for choice test experiments (Klüber *et al.*, 2024). Importantly, there could be a physiological switch that is triggered post-mating for females (Lemke *et al.*, 2025), as has been found in Tephritidae (Jang *et al.*, 1999), because unmated/virgin female BSF showed no clear behavioural preferences for oviposition cues, whilst gravid females exhibited a clear response to the tetradecanoic acid (Klüber *et al.*, 2024), or various plant-based attractants (Laksanawimol *et al.*, 2023). However, electroantennographic recordings confirm the olfactory abilities of BSF in response to various volatiles by both sexes with substantial overlap in their responses (Piersanti *et al.*, 2024), despite female antennae having longer flagellum than males, which would suggest increased function (Pezzi *et al.*, 2021). Moreover, it is unclear whether oviposition behaviour is modulated by seminal fluid proteins; molecules similar to sex peptide in *Drosophila* that are transferred in the male ejaculate, triggering a cascade of behavioural and physiological changes in females (Chapman *et al.*, 2003). Interestingly, they evolved as a way to increase male fitness often at a cost to their fitness (Wigby & Chapman, 2005), which has engendered a great deal of study by evolutionary biologists.

3. Implications for Evolutionary Biology

The black soldier fly (BSF) offers a distinctive combination of traits that make it a promising model for reproductive evolution. As a capital breeder (Stephens *et al.*, 2009), adult reproduction is fueled almost entirely by larval-derived reserves, decoupling gametogenesis and courtship investment from adult foraging. This life-history mode shifts sexual selection dynamics relative to other income-breeding insects (Stephens *et al.*, 2009) as well as all the anautogenous blood-feeding Diptera (which require a blood meal to produce eggs), and some potential trade-offs include those between larval resource allocation, somatic growth, longevity, and reproductive output (Miller, 2005). Multiple mating by females influences sperm competition among males, a ubiquitous force in insect reproduction (Parker, 1970). Comparative studies also show how sperm traits evolve under such competition: *Drosophila bifurca* produces the longest sperm in the animal kingdom, while lepidopterans package sperm into nutrient-rich spermatophores (Pitnick *et al.*, 1995; Vahed, 1998). Mating system studies in BSF now document polygynandry, steep reproductive skews, extended sperm storage, and possible cryptic female choice, including ejaculate digestion and selective sperm retention (Muraro *et al.*, 2024; Manas *et al.*, 2024, 2025a). These patterns provide a framework to quantify pre- vs post-copulatory sexual selection gradients under varying sex ratios, densities, and age structures. Because BSF lek-like aggregations can be labile in captivity (Lemke, 2025), they also allow experimental manipulation of mating system architecture.

Phenotypic plasticity in response to larval diet affects key reproductive traits, including body size, fecundity and sperm length, that also interacts with heritable variation to shape evolutionary potential (Gobbi *et al.*, 2013; Shrestha *et al.*, 2025; Zhang & Puniamoorthy, 2025). Selection for adult body weight or oviposition output can yield rapid responses but also risks inbreeding depression (Meyermans *et al.*, 2025). Laboratory studies show BSF can adapt within generations to suboptimal diets (Jiggins, 2024), supporting their use in experimental evolution. Recent genomic surveys indicate substantial diversity among global BSF populations, including behavioural and reproductive incompatibilities between some strains (Silvaraju *et al.*, 2025). This raises the possibility of a cryptic species complex (Ståhls *et al.*, 2020; Generalovic *et al.*, 2023; Athanassiou *et al.*, 2024) and provides an avenue to examine potential mechanisms governing reproductive isolation under domestication. The interaction between industrial selection pressures and ancestral reproductive behaviours such as mate-site separation from oviposition sites (Tomberlin & Sheppard, 2001), also enables tests of how evolutionary “holdovers” persist or decay in closed systems (Lemke *et al.*, 2024).

3.1. Cryptic Female Choice, Sperm Competition and Sexual Conflict

The complex interactions between males and females, both pre- and post-copulation reveal a potential for sex-specific strategies that can influence fitness. Insects such as *Drosophila*, crickets, and butterflies can bias sperm use through differential storage or ejection of sperm from less-preferred males (Eberhard, 1991). The highly specialised sperm storage organs of BSF females are capable of retaining more sperm than required to fertilise a single clutch (Manas *et al.*, 2024). Retention duration, sperm viability, and selective sperm use suggest potential for cryptic female choice as described in other Stratiomyidae (Barbosa, 2009, 2011, 2012, 2015). Females may regulate sperm uptake or storage, discard rival sperm, or bias fertilization toward preferred males, but such mechanisms remain largely untested in BSF.

Males exhibit strategic ejaculate allocation, and can adjust their sperm investment in response to perceived competition (Manas *et al.*, 2025a). Sperm length in BSF is unusually long for internally fertilizing animals (Malawey *et al.*, 2019, 2020) and is sensitive to larval diet quality (Zhang & Puniamoorthy, 2025), suggesting potential trade-offs between sperm quality and quantity that could shape competitive fertilization outcomes. However, the degree to which sperm precedence follows “last male wins” patterns, as seen in other Diptera, is unknown, nor how this pattern may subsequently break down with high amounts of remating (Zeh & Zeh, 1997; Laturney, van Eijk & Billeter, 2018) or as flies senesce (Mack, Priest & Promislow, 2003). Indeed this phenomena is quite complex, since last-male sperm precedence may arise, not just males remove or displace their rivals’ sperm, but also as alluded to if the female herself digests or ejects the first males’ sperm from her genital tract prior to a subsequent mating (Luck *et al.*, 2007; Schnakenberg, Siegal & Bloch Qazi, 2012).

Although not formally tested, the mating system of BSF may also be subject to sexual antagonism or sexual conflict, and vice-versa (Bedhomme *et al.*, 2009; Candolin, 2019; Plesnar-Bielak & Łukasiewicz, 2021). Specifically, sexual conflict refers to interactions between males and females that can impose fitness costs on one sex while benefiting the other, potentially driving coevolutionary dynamics between male manipulation and female resistance (Chapman & Partridge, 1996). Often, the number of matings that optimises fitness for males is higher than it is for the females (Wigby & Chapman, 2005). As mentioned before, BSF males have been seen to mate up to 4 times, whereas females can mate twice or more (Chiabotto *et al.*, 2024). Male harassment to secure these extra matings may interfere with female oviposition or even induce injury (Pitnick & García–González, 2002), such that increased exposure to males may be negatively associated with female lifetime fitness (Fowler & Partridge, 1989; Morrow & Gage, 2001). In addition, males may transfer seminal fluid proteins (e.g. sex peptides) that influence female re-mating latency or stimulate reproductive development (Kubli, 1992). These act to increase the male's fitness at the cost of female's (Wigby & Chapman, 2005). In fact, mating in BSF has been shown to reduce male longevity whilst increasing females' suggesting a potential trade-off between mating frequency and fitness for each sex (Harjoko *et al.*, 2023).

Together, these processes place BSF among the minority of capital-breeding insects (e.g., Lepidoptera of the families Notodontidate, Arctiidae, Lymantriidae, Saturniidae and Lasiocampidae (Tammaru & Haukioja, 1996), and others) in which both sperm competition and cryptic female choice can be studied under controlled conditions. The combination of tractable rearing, measurable reproductive traits, and manipulable social context makes BSF a suitable system for testing predictions of post-copulatory sexual selection theory, from sperm allocation models to sexual conflict evolution.

4. Implications for Industrial Applications

Industrial BSF production depends on predictable, high-output reproduction, yet adult reproductive biology remains a major bottleneck. While larval traits dominate economic models (Zaalberg *et al.*, 2024), adult reproduction is the rate-limiting step in closed-cycle rearing (Boller, 1972). Optimizing adult phase management requires translating evolutionary and behavioural insights into practical protocols.

4.1. Genetics and Population Structure

High genetic diversity among global BSF stocks (Ståhls *et al.*, 2020; Sandrock *et al.*, 2021; Kaya *et al.*, 2021; Generalovic *et al.*, 2023) underlines the need for controlled breeding strategies to prevent inbreeding depression and maintain adaptive capacity. Selective breeding regimes for increased body size, fecundity, or other traits must thus consider potential correlated (or antagonistic) trade-offs in mating behaviour or sperm traits Recent work suggests that within populations, age (Dickerson *et al.*, 2024; Lemke *et al.*, 2025) and genetic heterogeneity (Laudani *et al.*, 2024; Meyermans *et al.*, 2025) influence mating rates, sperm competition intensity, and egg viability, As for. Overrepresentation of old or non-competitive males appears to depress productivity (Dickerson *et al.*, 2024; Lemke *et al.*, 2025), thus managing the instantaneous operational sex ratios (iOSR) by rotating breeding cohorts is predicted to reduce skewed mating success and maintain effective population size.

4.2. Nutritional Management

Studies have shown that for BSF, adult access to water and nutrient supplements (viz., honey, pollen, or sugar water) can extend lifespan, shorten pre-oviposition intervals, and improve egg output under some conditions (Thinn & Kainoh, 2022; Klüber *et al.*, 2023; Barrett *et al.*, 2025). So, although BSF are typically considered capital breeders, providing adults access to nutrition will shift their position within the capital-income breeding continuum (Davis *et al.*, 2016), and many sympatric sister species have been shown to occupy slightly different ecological niches in this way, such as in *Curculio* weevils (Coleoptera: Curculionidae) (Pélisson *et al.*, 2013). Interestingly, in Hymenoptera and Lepidoptera income breeding is negatively associated with ovigeny index (the initial egg load divided by the potential lifetime fecundity) but allows for individuals to engage in mixed strategies to compensate for deficient nutrition acquired as larvae. The opposite is true too, with capital breeding and strict proovigeny (the emergence of a female's entire potential lifetime complement of eggs) is associated with stable and predictable larval conditions (Pélisson *et al.*, 2012). For BSF, since larval diet can influence adult reproductive strategies, supplementing the nutrition of adult breeding stocks may be necessary to sustain high fecundity, especially to compensate for the fact that waste remediation efforts by industry will necessarily require larvae to be fed on low quality feed stocks. However, such changes could extend generation times and overall gains should be balanced against longer production cycles and increased labor (Lemke *et al.*, 2023).

4.3. Sex Ratios and Cage Design

Because some lek-like behaviours appear to persist in captive BSF (Tomberlin & Sheppard, 2001; Lemke *et al.*, 2023, 2024) it is theorised that physical separation of the mating and oviposition zones within cages, proper provisioning of microclimates, and a precise control of light gradients can enhance reproductive success (Lemke *et al.*, 2025). Such has been done for mass-reared fruit flies (Diptera: Tephritidae), which require specialised habitat design with separate environments for male and female premating development (Liedo *et al.*, 2007; Meza *et al.*, 2025). A recent report indicates that BSF microhabitat preference is related to their age, and so different cohorts of flies may be physically isolated through cage compartmentalization to (Salari & De Goede, 2024) which ultimately should help to maintain a proper iOSR and age-structure of the breeding zone, increasing fertile egg production.

In addition, cage designs should consider enhanced structural complexity by including artificial or live plants. For BSF, the increased perching area provided by the plants has been shown to increase mating rates, although the magnitude of the effects varies with the density of the plants provided (Lemke *et al.*, 2024; Grosso *et al.*, 2025). In the dragonfly species *S. danae*, females preferred higher perches and overgrown regions of their outdoor cage and their preferred height increased along with higher temperatures (Michiels & Dhondt, 1989), further suggesting the importance of habitat design and abiotic interactions for optimizing BSF production. Although not necessary for BSF, live plants can help modulate ambient humidity via transpiration of water vapor, as well as to ameliorate air quality by assimilating ammonia (NH3) into their leaf tissues (Zayed *et al.*, 2023). By mimicking natural conditions, the effects of providing plants can potentially improve insect welfare by reducing stress, as is the case in captive cockroaches (Blattodea) (Free & Wolfensohn, 2023).

4.4. Chemical Ecology and Oviposition Control

The interplay between VOC-mediated oviposition cues and deterrents opens opportunities for chemical manipulation of oviposition behaviour in industrial mass-rearing. In addition to attractants, some compounds (e.g., decanoic acid) have been shown to delay oviposition behaviour in BSF, while others increase off-target laying. Because synthetic blends combining multiple VOCs elicit stronger responses than individual components alone (Thomas *et al.*, 2024), this means that a precise blend can be engineered as part of a push–pull strategy (Menger *et al.*, 2015; Cui *et al.*, 2022) to more precisely direct egg-laying both away from cage materials and towards the trap. Once realised, this will improve collection efficiency, reduce off-target oviposition, and facilitate automated egg harvesting.

In addition to directing oviposition, aromatherapy using both plant essential oils (e.g., α-copaene derived from sweet orange, grapefruit, guava, papaya, and mango; methyl eugenol; raspberry keytone; α-Pinene; Zingerone) (Zeni *et al.*, 2021) and synthetic compounds (e.g., Trimedlure) (Shelly *et al.*, 1993) has been shown to artificially stimulate lek formation and mating in many species of Tephridae. Of course, because Tephritids have co-opted secondary plant metabolites as a rendezvous-signal for their mating aggregations (often in fruit trees), a similar effect of a secondary plant metabolite on BSF is mere speculation until such a plant-insect interaction can be uncovered.

5. Conceptual Model

The previous sections highlight many important research gaps still yet to be unveiled. Here we have condensed them into a single process flow diagram depicting the BSF mating system (Figure 1), described as follows:

As adults, male BSF emerge first, followed by females due to protandry. From this point males fly to a lekking site, but it is unknown what drives site selection or the mechanisms that determine the dominance hierarchy therein. Females then fly to visit the lek. Aerial courtship commences and males will fan and buzz their wings. Whether or not BSF can recognise conspecifics or distinguish between sexes based on WIPs is unknown, nor what features a successful BSF courtship song has, or whether gentilic stridulation is necessary for females to accept mates. The characteristics that a female might select or reject a mate based on are also unknown, as are whether alternative mating tactics emerge from a result of these preferences. During mating rivals could interfere but can also be rebuffed by the females who ‘kicks’ them off. Successful mating seems to occur once BSF lock genitalia for ~30 minutes (but potentially up to 1 hr), though this total duration might be plastic, and it is not known whether BSF utilise mate guarding as an AMT. For ~25 minutes, seminal fluid is transferred to the female, of which might include something like sex peptide found in *Drosophila*. How mating or non-gametic material transferred during mating acts to influence female physiological changes is also unknown but speculated to occur. During the last 5 minutes of mating sperm is transferred to females. After mating flies groom themselves. During the next few days, sperm compete with one another within the genital tract of the female, and may either be stored in her spermathecae, digested, or discarded. What enables BSF sperm to outcompete their rivals such as sperm precedence is not fully understood, nor the potential mechanisms of post-reproductive (cryptic) choice in females such as genetic imprinting. After mating females become receptive to volatile organic compounds which can then direct them towards or away from an oviposition site. Once there, the female extends her ovipositor which has gustatory and hygroreceptors and probes the area. If abiotic conditions are suboptimal at the oviposition site, this may drive the female to haphazardly lay her eggs outside of the target area. Of course, both males and females have the option to remate, but it is not fully understood what leads an individual to mate multiply (or not at all), nor what drives females remate prior to or after oviposition. Moreover, many of these interactions might have male and/or female components driving them, leaving many opportunities for future research.

6. Conclusions and Future Directions

Studying BSF reproduction aids in addressing both fundamental evolutionary questions and practical constraints for industry. As a capital-breeding, lek-like species with plastic reproductive traits, BSF offer a rare opportunity to experimentally test pre- and post-copulatory processes. In addition, as a non-pest species that is easily culturable, BSF provides an avenue to study reproductive processes that are happening in other insect species with similar life history and reproductive traits.

Future research priorities include: (i) Identifying the visual, chemical, and acoustic cues used for mate recognition and lek formation in wild and captive context; (ii) Quantifying sperm precedence patterns and female control over fertilization; (iii) Investigating the potential trade-offs between production efficiency (larval size and nutrition), supplemental adult nutrition, and breed stock resilience (adult reproduction); (v) Documenting the variation in BSF phenome with respect to differing genetic provenances and nutritional legacies; and (v) Developing management strategies to balance genetic diversity with targeted selection.

Achieving these will require controlled experiments, comparative fieldwork, and molecular tools to track paternity and reproductive investment. In addition, standardizing methodologies such as population densities, light regimes, and cage designs, will improve reproducibility and comparisons across studies. We propose that future research leverage this model to bridge gaps between behavioural ecology, reproductive physiology, and insect biotechnology.

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