BRILL
WAGENINGEN
ACADEMIC

JOURNAL OF INSECTS AS FOOD AND FEED (2025) DOI:10.1163/23524588-BJA10342

Journal of Insects as Food and Feed
brill.com/jiff

RESEARCH ARTICLE

Influence of diet on initial mating behaviour in the black soldier fly (*Hermetia illucens*)

Yvonne Kortsmit, Margarita Sklavounou, Marcel Dicke and Joop J.A. van Loon*

Laboratory of Entomology, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; *Marcel.Dicke@wur.nl

Received 19 September 2025 | Accepted 5 December 2025 | Published online 11 December 2025

Abstract

The black soldier fly (BSF; *Hermetia illucens*) is widely used as a waste converter and novel protein source in feed. Its larvae can convert a wide range of organic feed substrates that vary in nutritional quality, into biomass. Our aim was to investigate whether larval and/or adult diet impact BSF body weight, pupal duration and reproductive behaviour. We focussed on comparing low- and high-nutrient larval diets as well as the presence or absence of food in the adult stage. Experimental cages were specifically designed to accommodate lekking behaviour. To promote BSF reproductive behaviour, UV, green and blue LED lights were provided, in addition to fluorescent strip lights. We quantified the time to first mating attempt and first mating, the number of mating attempts and mating events, mating success and the influence of wing fanning on mating success by analysing one-hour video recordings. Courtship duration, copulation duration and male–male interactions were quantified. Our results show that both male and female flies reared on a high-nutrient larval diet had a greater body weight and longer pupation time. Among BSF reared on the low-nutrient diet, longer duration of pupation was associated with increased adult body weight. Mating behaviour, especially copulation time, was influenced by both larval-diet nutrient content and the availability of food during the adult stage. Flies reared on a low-nutrient larval diet exhibited longer copulation duration than those on a high-nutrient diet. Availability of food in the adult stage had the most pronounced influence on mating behaviour: the absence of food resulted in earlier and more frequent mating attempts, a shorter wing-fanning duration and a longer copulation time. These results highlight the importance of diet for body weight, pupal duration and reproductive behaviour. Understanding these effects is relevant for optimising mass-rearing conditions and improving BSF welfare in commercial production systems.

Keywords

black soldier fly – lekking behaviour – nutrient content – pupal duration – reproductive behaviour

1 Introduction

The black soldier fly (BSF; *Hermetia illucens* Linnaeus; Diptera: Stratiomyidae) is a saprophytic fly species whose larvae naturally occur on a variety of decomposing organic materials (Tomberlin and Sheppard, 2001). This fly species is increasingly used as an organic waste converter and alternative protein source in livestock feed (Barragan-Fonseca *et al.*, 2017; Dörper *et al.*, 2021). Black soldier flies can successfully reproduce in the absence of nutrients during the adult stage (Sheppard *et al.*, 2002; Tomberlin *et al.*, 2002). Hence, they likely

Published with license by Koninklijke Brill BV | DOI: 10.1163/23524588-BJA10342

rely on their reserves from the larval stage which suggests that variation in larval diet may lead to differences in reproduction (Savola *et al.*, 2022). Nutritional differences in larval diet affect BSF life-history traits such as development time, pupation time, time until adult emergence, adult body weight, ovary development and survival in BSF (Barragan-Fonseca *et al.*, 2018; Gobbi *et al.*, 2013; Laursen *et al.*, 2024). The variation in larval development time and survival are also dependent on larval density and frequency of feed provision, via for example providing all feed substrates at once or in several batches (Barragan-Fonseca *et al.*, 2018). Larval diet can be important for adult emergence of male and female flies. For instance, brewer's spent grain resulted in the fastest emergence, highest body weight and the highest longevity compared to other diets (Laursen *et al.*, 2024). Females with large bodies can have larger ovaries and faster developing eggs depending on the food quality and quantity (Gobbi *et al.*, 2013).

Larval diet may also influence adult behaviour; for example, larval diet can influence foraging choices in adult *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Davies *et al.*, 2018). While the influence of larval diet on the adult phenotype is widely accepted, feeding by adult BSF has not been considered relevant for reproduction until recently (Sheppard *et al.* 2002; Bertinetti *et al.* 2019). This has resulted in a main focus on larval diets to improve life-history traits of BSF (Tettamanti *et al.*, 2022). Usually, adult flies in mass-rearing systems are only provided with water via misting or fountain systems (Barrett *et al.*, 2023). However, BSF adults do engage in feeding behaviour and have a functional digestive tract (Bruno *et al.*, 2019). Stratiomyid flies can even be considered typical nectar feeders as they are seen feeding on honeydew (Beuk, 1990) and flowers (James, 1981). Feeding by adults can also lead to benefits such as increased longevity (Nakamura *et al.*, 2016), which can increase the oviposition period resulting in higher total egg production and a higher number of fertilised eggs (Bertinetti *et al.*, 2019).

Although BSF is widely accepted as a species of great economic importance and is reared on a large scale, relatively little is known about the effects that diet may have on BSF mating behaviour. Such knowledge is important for a stable colony as the understanding of reproductive behaviour can lead to an increase in reproduction. BSF are known to have complex mating behaviour and are known to exhibit lekking behaviour (Tomberlin and Sheppard, 2001) similar to other stratiomyid flies (Alcock, 1990; Barbosa, 2009). During this lekking behaviour same-sex interactions are common and sometimes described as aggressive behaviour (Jones and Tomberlin, 2021) or as male–male courtship behaviour (Giunti *et al.*, 2018). Male–male interactions occur when a courting male grabs a conspecific male in flight, with the interaction ending when the courted male escapes (Giunti *et al.*, 2018). The initiation of the female–male courtship sequence starts in a similar manner, with the courting male grabbing a passing female in mid-air and spiralling to the ground while wing fanning (Giunti *et al.*, 2018; Tomberlin and Sheppard, 2001). After landing, the male continues wing fanning while tapping the female's abdomen and attempting to make genital contact (Giunti *et al.*, 2018; Jones and Tomberlin, 2021). Receptive females will stop moving and spread the wings (Julita *et al.*, 2020), the courtship sequence then often ends with the typical end-to-end mating position with heads facing in opposite directions (Giunti *et al.*, 2018; Lemke *et al.*, 2023). Upon mating, the female stores the sperm in her spermathecae (Manas *et al.* 2024). Unsuccessful mating attempts can be identified when the latter position is not achieved and the female performs wing fanning and tries to dismount the male during the courtship sequence (Giunti *et al.*, 2018; Julita *et al.*, 2020). No matings are observed in flies within the first two days since emergence (Bertinetti *et al.*, 2019).

Even though diet effects on mating behaviour are not yet known in BSF, size-related differences have been reported in the literature. Populations with mixed-sized adults showed an increased mating success and larger males showed more male–male interactions (Jones and Tomberlin, 2021). Larger males, when paired with smaller females, achieve more successful matings compared to smaller males (Jones and Tomberlin, 2021). The high mating success of larger males may result from increased wing size, a good indicator of body size in BSF (Gobbi *et al.*, 2013), as wing fanning is found to be an important component of the courtship sequence (Giunti *et al.*, 2018), possibly linking shorter wing-fanning duration to higher mating success (Giunti *et al.*, 2018; Jones and Tomberlin, 2021).

BSF larvae use ephemeral resources in nature. On resources of decomposing organic material, a fast reproductive output may have evolutionary benefits, suggesting that adults that are exposed to limited resources may show energy trade-offs to increase reproductive output (Harjoko *et al.*, 2023). For example, male *Derocephalus angusticollis* Enderlein (Diptera: Neriidae) reared on poor larval diet invest more in some aspects of reproduction, such as a longer mating duration and a higher subsequent egg-hatching rate (Fricke *et al.*, 2015). Mating latency may also be influenced by diet history. *Drosophila melanogaster* mating latency was influenced by environmental factors, such as housing with rivalling males (Filice *et al.*, 2020). Mate quality may also influence female acceptance when diets differ. Females fed on a high-nutrient diet may be more receptive to male courtship, similar to what has been observed in *Anastrepha obliqua* Macquart (Diptera: Tephritidae) (Medeiros-Santana and Zucoloto, 2016). Based on the structure of the sperm tract in BSF, these flies may control sperm transfer based on copulation duration (Munsch-Masset *et al.*, 2023) and therefore may be able to alter the number of spermatozoa transferred based on mate quality. Thus, with poor mate quality we would expect a shorter copulation duration similar to what has been reported for *Musca domestica* Linnaeus (Diptera: Muscidae) (Baldwin and Bryant, 1981).

This study aims to investigate the influence of larval and adult diets on BSF reproductive output. The effect of larval diets from waste streams on life-history traits such as emergence time and adult body weight is already known in BSF (Laursen *et al.*, 2024). However, whereas these studies mainly compared different waste streams, here we investigate the effects of a difference in nutrient content of larval diet on life-history traits and behaviour.

We expect that BSF reared on diets of different nutritional content differ in reproductive behaviours, such as time until first mating or number of mating attempts. Specifically, we expect an increased courtship duration in BSF adults when reared as larvae on a low-nutrient diet, to obtain a mate as fast as possible. We expect a shorter duration of mating latency when the diet is nutritionally poor which then may lead to an increased investment in reproduction. A high-nutrient larval diet is expected to result in shorter pupal duration and higher adult body weight when compared to low-nutrient larval diet. The influence of larval and adult diets on reproduction is mostly studied with a focus on egg yield (Bertinetti *et al.*, 2019; Laursen *et al.*, 2024), ovary development (Gobbi *et al.*, 2013) and longevity (Nakamura *et al.*, 2016). Differences in reproductive behaviour have been investigated in relation to adult body size (Jones and Tomberlin, 2021). The present study aims to further explore the effect of larval and adult diets on important variables of BSF reproductive behaviour. These behavioural variables are: time until the first mating attempt, mating latency, mating frequency, mating success, courtship duration and copulation duration. We focussed on the first mating to avoid the effects of previous experiences on mating behaviour. We expect insects reared on low-nutrient diet to be less selective in accepting a mating partner due to their shorter longevity, smaller body size and therefore lower fat reserves when compared to insects reared on high-nutrient diet. Similarly, we expect adult flies without access to nutrition to be less selective when compared to adult flies that have access to diet in the adult stage. Thus, we expect that BSF reared on low-nutrient diet and adults that have no access to additional diet to exhibit earlier mating attempts, a shorter mating latency, higher mating frequency, higher mating success, longer courtship duration and shorter copulation time compared to conspecifics reared as larvae on a high-nutrient diet or conspecifics with access to nutrition in the adult stage.

2 Materials and methods

Insects

Black soldier fly eggs were sourced from the BSF central rearing colony at the Laboratory of Entomology, Wageningen University. The colony was maintained in a climate chamber (27 ± 1 °C, RH 70 ± 10%, L:D 16 h:8 h) with a standard rearing diet of one part chickenfeed (Kuikenopfokmeel 1, Kasper Faunafood, Woerden, The Netherlands) and two parts tap water. Adult flies had access to milk powder (10033868, MAAS, Eindhoven, The Netherlands), ucrose (10033867, MAAS) and tap water *ad libitum* (Bertinetti *et al.* 2019). Female BSF were allowed to oviposit for 6 h in corrugated cardboard strips (±3.5 × 10 cm) placed in an oviposition box with sawdust and some mouse faeces as odour source.

Dietary treatments

Eggs were collected from the oviposition box after 6 h and removed from the corrugated cardboard strips with a soft brush. Five mg of eggs were weighed and placed in a Petri dish (∅ = 60 mm) with moist filter paper. After 48 h, the Petri dish with eggs was placed without lid on top of the larval diet in the rearing container (BugDorm Pint-Sized rearing pots 480 ml, MegaView Science, Taichung, Taiwan). Larvae were reared on either of two diets, a low-nutrient and a high-nutrient diet (Table 1), generated by mixing different amounts of cellulose (Alphacel Non-Nutritive Bulk, MP Biomedicals, Solon, OH, USA) with a standard chickenfeed (Kuikenopfokmeel 1, Kasper Faunafood) (Barragan-Fonseca *et al.*, 2018). Tap water was added to the diet based on the % dry matter (DM) of the feed (88% DM chickenfeed, 93% DM cellulose) until the water content of the feed substrate was *ca.* 70%. The BSF larvae were reared on these

TABLE 1 Composition of the two larval diets used for the experiments based on chickenfeed

	Low nutrient	High nutrient
Ingredient (g/100 g diet)		
Chickenfeed	43	85
Cellulose	57	15
Nutrient (g/100 g diet)		
Crude protein	9	18
Non-cellulose carbohydrates	17	34
Protein:carbohydrate ratio	1:1.9	1:1.9

Nutrient analysis derived from Shah *et al.* (2024).

diets for 20 days and the position of the rearing containers was randomised every two days to avoid an effect of temperature differences within the climate chamber. The dark-coloured wandering larvae close to pupation were counted, weighed and transferred to a new rearing container (henceforth pupae box; BugDorm Pint-Sized rearing pots 360 ml) with 8.0 g sawdust and kept in a dark environment (27 ± 1 °C, RH 70 ± 10%). Insects were given 40 days after the pupal transfer to emerge and pupae boxes were randomised every two days to avoid temperature differences within the climate chamber.

Experimental treatments

Larval diet treatments: This experiment focussed on the differences in behaviour of flies resulting from larvae that had been reared on either of the two diets (Table 1). Emerged flies were collected from the pupae box once every 24 h. After weighing, the flies were separated per sex and corresponding dietary treatment in mesh cages (BugDorm-1 insect rearing cage 30 × 30 × 30 cm) in a climate chamber (27 ± 1 °C, RH 70 ± 10%, L:D 16 h:8 h). Per mesh cage a diet consisting of milk powder (±12 g, 10033868, MAAS), sucrose (±12 g, 10033867, MAAS) and additional tap water was provided *ad libitum*. The flies were kept in these mesh cages for 1–2 days. When enough flies were collected for behavioural experiments, flies were weighed and divided into groups of 11 individuals per mesh cage (BugDorm-1 insect rearing cage 30 × 30 × 30 cm), separated by dietary treatment and sex. There were 6–8 biological replicates per dietary treatment cage: 3–4 cages with 11 males and 3–4 cages with 11 females. In each mesh cage a fresh diet of milk powder, sugar and water was added *ad libitum*. Flies were kept in these mesh cages at 27 ± 1 °C, RH 70 ± 10%, L:D 16 h:8 h, for an additional day until the behavioural experiments started.

Adult diet treatments: In a separate experiment we investigated the effect of the availability of food to adult flies on mating behaviour. For this experiment BSF larvae were reared on the low-nutrient diet (Table 1). Emerged flies were collected from the pupae box once every 24 h and the flies were separated per sex and corresponding dietary treatment in mesh cages (BugDorm-1 insect rearing cage 30 × 30 × 30 cm) in a climate chamber (27 ± 1 °C, RH 70 ± 10%, L:D 16 h:8 h). Upon emergence, adults were either provided with a diet of milk powder (10033868, MAAS), sugar (10033867, MAAS) and additional water *ad libitum* or only water *ad libitum*. The flies were kept in these mesh cages for one day after being weighed again and divided into groups of 11 individuals per mesh cage (BugDorm-1 insect rearing cage 30 × 30 × 30 cm) at 27 ± 1 °C, RH 70 ± 10%, L:D 16 h:8 h, separated by diet treatment and sex, with 8 dietary replicates per experimental cage: four mesh cages with 11 males and four mesh cages with 11 females per dietary treatment. In each mesh cage, dependent on the adult diet treatment, either a fresh diet of milk powder, sugar and water was added *ad libitum* or fresh water was added *ad libitum*.

Behavioural experiments

On the experimental day, 10 female flies and 10 male flies of the same treatment group were randomly collected from the mesh cages in releasing tubes (Ø 3 cm, length 32 cm) at room temperature and transferred to the climate chamber for behavioural experiments.

Experimental set-up

All behavioural experiments were conducted in a climate chamber (27 ± 1 °C, RH 70 ± 10%, L:D 16 h:8 h) and started at ca. 11:00 am which is 5 hours after the initiation of the photophase. Experimental cages were glass terrariums (40 × 10 × 40 cm, glass thickness 5 mm, Terraglas, Nieuwendijk, The Netherlands) (Fig. 1) with a cylinder-shaped hole 5 cm underneath the rim (Ø 3 cm) for connection of a release tube. The experimental cages included a wooden lid with 1 mm mesh size to promote airflow (PE-gaas 5016, Top Zeven, Haarlem, The Netherlands).

Per experimental cage, a panel with three cameras (one located at the bottom and two at the top, PD20K, Bascom® Camera's, Nieuwegein, The Netherlands; Fig. 1) was placed at 25 cm distance of the experimental cage. The cameras were full HD cameras with a resolution of 1920 × 1080 pixels, a frame rate of 25 frames per second, and a wide-angle lens of 112°. For recording, one 8-channel recorder (R8XK, Bascom®) and one 16-

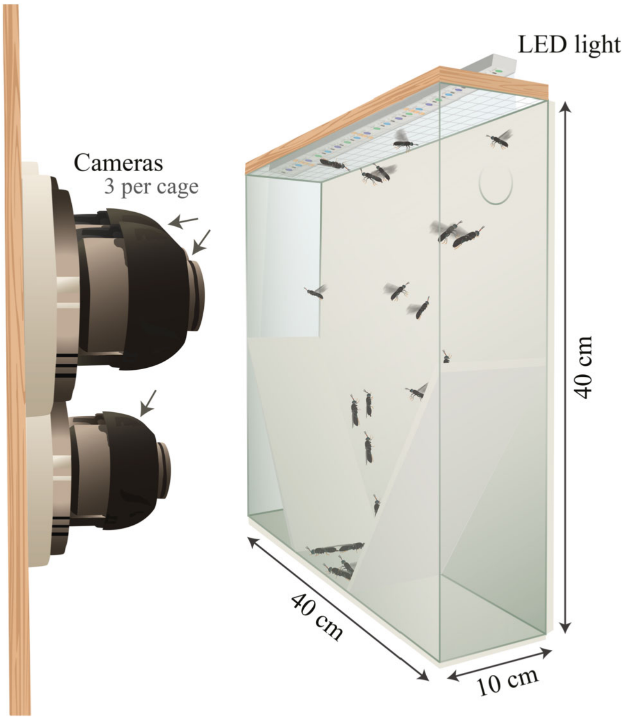

FIGURE 1 Experimental behaviour cage used during the experiments. Three cameras were connected per cage (indicated by the three arrows) and an additional LED light was added (for light spectrum and intensity see Fig. S1 in the Supplementary Material).

channel recorder (R16XK, Bascom®) were used. This allowed the behaviour of the flies to be recorded in all locations in the cage. Fly behaviour was recorded for 1 h per experiment; 3–4 cages were simultaneously recorded per treatment per day. The difference in body shape and genital differences made it easy to distinguish males from females. The video recordings could also be analysed frame by frame, ensuring a correct distinction between the sexes.

To promote mating behaviour, favourable light conditions were created by LED lights (Oonincx *et al.*, 2016). LED strips were placed above the cage with green (510–525 nm), blue (455–470 nm) (LS24RGB96X5050 PLX, Luxalight, Eindhoven, The Netherlands) and UV LED lights (365 nm, LS24UV240X2835PLX, Luxalight) as an addition to the standard fluorescent strip lights mounted at the room ceiling above the cages (for light spectrum and intensity see Fig. S1 in the Supplementary Material). These LED lights were switched on for the duration of the experiment. White foamboards (920492, Interoffice Kantoorinrichting, Winschoten, The Netherlands) were used at the back of the experimental cage to increase contrast. A funnel shape was created in the bottom of the cage with white foamboards (25.0 × 9.1 cm, 920492, Interoffice Kantoorinrichting) to guide falling insects into the view of the bottom camera (Fig. 1).

Life-history traits

After spending 20 days on either high- or low-nutrient diet, pupae were weighed (Mettler Toledo NewClassic MF, model ML54/01) in six groups of 10 pupae per rearing container. The average pupal weight was calculated per rearing container. The pupal weight of 32 high-nutrient and 32 low-nutrient rearing containers was recorded.

The number of days until fly emergence after larvae had been reared on high- or low-nutrient diet was recorded daily for the full 40 days for three different egg batches collected over three weeks. This resulted into 12 different rearing containers per dietary treatment. Data were collected per treatment and sex.

Newly emerged flies were randomly selected and transferred into 1.5 ml Eppendorf tubes (Eppendorf Safe-Lock tubes 72690001, Eppendorf Nederland, Nijmegen, The Netherlands) for weighing (Mettler Toledo NewClassic MF, model ML54/01). After weighing, the number of days the specific fly spent in the pupal stage was recorded. The aim was to collect 6 newly emerged females and 6 newly emerged males per treatment over the course of 33 days, however, this depended on the number of flies emerging on the days of data collection. In total, this resulted in weight data of 195 low-nutrient reared females, 196 low-nutrient reared males, 200 high-nutrient reared females and 196 high-nutrient reared males.

The 1–2-day-old flies that were to be used for the behavioural experiments were weighed one day before these experiments. This time was also used to determine whether enough flies had emerged for behavioural experiments to take place (11 females and 11 males per treatment for each behavioural cage). The target number of weighed flies was 44 females and 44 males per treatment per experimental day (four behavioural cages per treatment per day). The total number of flies weighed was: 264 high-nutrition reared females, 264 high-nutrition reared males, 265 low-nutrition reared males, 253 low-nutrition reared females, 264 females with diet, 264 males with diet, 264 females without diet and 264 males without diet.

Behavioural variables

Videos of the left and right overview cameras were combined into one overview video using Adobe Premiere Pro (v24.1). BORIS (v8.22.6; Friard and Gamba, 2016) software was used to extract behavioural sequences relevant to BSF mating behaviour. The order of treatments being analysed was random and the person analysing the videorecordings did not know the treatment related

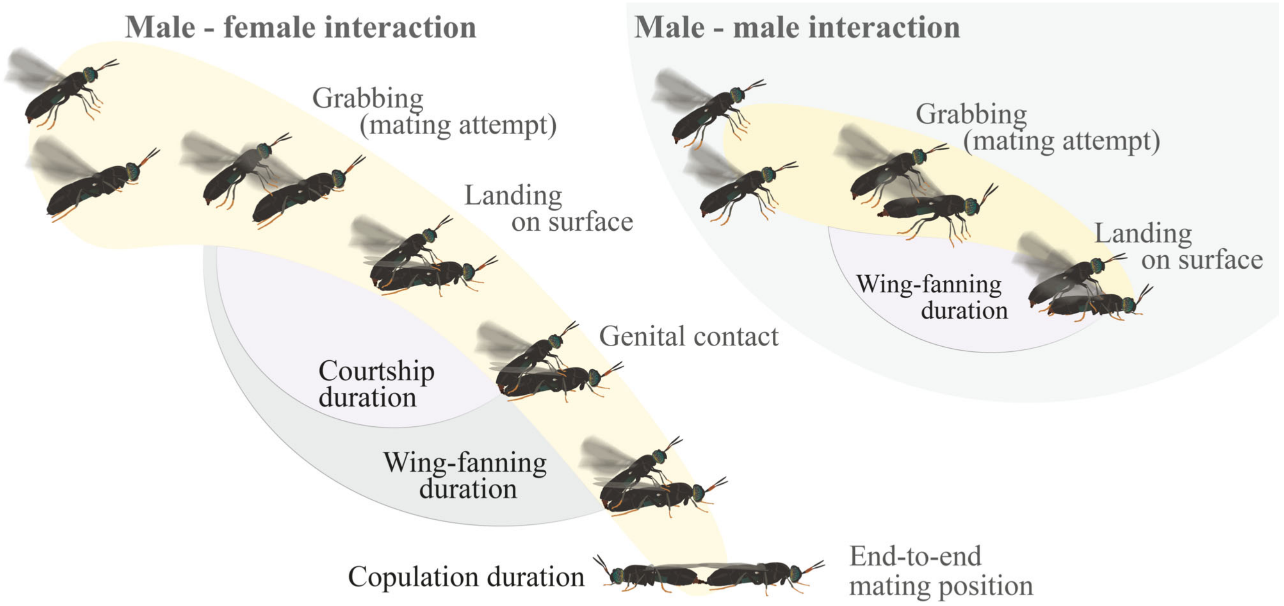

FIGURE 2 Behavioural sequence of BSF male–female and male–male interactions. In most cases interaction pairs land on the cage surface and female–male interactions result in genital contact.

to the recordings to avoid any observer bias. These sequences consisted of: grabbing, landing on cage surface, genital contact, courtship duration, wing fanning duration and mating duration (Fig. 2). Some of these parameters were only found in female–male interaction pairs (Fig. 2). Based on these behavioural sequences, mating behaviour was quantified. Mating behaviour was analysed based on seven behavioural parameters; time until first mating attempt, mating latency, number of mating attempts, proportion of successful mating attempts, wing-fanning duration, courtship duration and copulation (Table 2). The variables time until first mating attempt and mating latency were added to identify possible differences in mating motivation of the experimental flies. Since this variable was to identify the motivation to mate directly after release, without possible learning possibilities of other flies in the experimental cage and to prevent repeated measures of the same individuals, only the first mating attempt by a female–male and the first mating attempt by a male–male pair was recorded per experimental cage. The sample size of most of the behavioural variables was dependent on the frequency at which these behaviours occurred.

Ethical note

This research is conducted on BSF which is not legally protected under Dutch legislation. However, regular hygienic measures were taken place to provide clean housing during experiments. After behavioural experiments, flies were euthanised by freezing at −20 °C.

Statistical analysis

All statistical analyses were done with R (v 4.3.1.R Core Team, 2023). For all constructed models assumptions were visually inspected via the DHARMa package (Hartig, 2022). All models were fitted based on Akaike's Information Criterion (AIC) (Bertrand *et al.*, 1988) and visually inspected for meeting model assumptions.

Life-history traits

Fly emergence was analysed as a time-to-event variable and flies from the two different larval diets were compared based on Kaplan–Meier curves (survival package; Therneau, 2023). Fly emergence time expressed as the number of days spent in the pupal stage was analysed with a Kruskal-Wallis rank sum test (R Core Team, 2023) per sex. The average pupal weight and 1–2-day-old fly weight were compared separately for larval diets and adult diets using a Generalized Linear Mixed Model (GLMM) using Template Model Builder (TMB) (glmmTMB package; Brooks *et al.*, 2017) with a Gaussian distribution. The model accounted for heterogeneity in the dispersion across the two diet treatments. In data of 1–2-day-old flies there was a random intercept of day fitted based on AIC (Bertrand *et al.*, 1988).

After model selection, the effect of larval diet or adult diet on bodyweight was tested with the best fitting GLMMTMB via a type-II Wald Chi-square test (car package; Fox and Weisberg, 2019). For the weight of flies that were less than 24 h old a similar GLMMTMB was fitted and a similar model selection took place. Significance of this weight variable was tested per larval diet,

TABLE 2 Behavioural variables measured during experiments

Behavioural variable	Definition
Time until first mating attempt (min)	The time from release of the experimental flies (start of the experiment) within the experimental cage until a first male grabbing a conspecific took place. Mating attempts were observed for male–male and female–male pairs. Only experimental cages with one or more mating attempts were included in the analysis. The sample size was the first pair (female–male or male–male) attempting a mating.
Mating latency (min)	The time from release of the experimental flies (start of the experiment) within the experimental cage until a first mating took place. Only the first mating was recorded per experimental cage. The sample size was the first pair with a successful mating.
Number of mating attempts	Grabbing by a male of another individual, either male or female. The male grabs the other individual with its legs. Mating attempts mostly occur in mid-flight and are the starting point of courtship and wing fanning. The sample size was the total number of mating attempts in all behavioural cages.
Proportion of successful mating attempts	The proportion of only female–male attempts, calculated as the number of successful attempts divided by the number of total attempts. The sample size was the number of behavioural cages that had at least one female–male mating attempt.
Wing fanning duration	From the moment of grabbing, the male starts fanning its wings. With female–male interaction pairs wing fanning stops most often before copulation. These interactions are recorded as successful or unsuccessful. With male–male interaction pairs, the grabbing male starts fanning the wings until one of the males moves away and the wing fanning stops, these interactions are always unsuccessful. All individual pairs exhibiting wing fanning were a unit of replication (male–male, female–male unsuccessful or female–male successful).
Courtship duration	The time from beginning of the courtship sequence (grabbing) until first genital contact. Measurements were only reported when the complete courtship sequence occurred and resulted in copulation. All individual pairs were the unit of replication All individual pairs that achieved a successful mating included with a courtship sequence were a unit of replication.
Copulation duration	From the start of the end-to-end mating position until genital contact ended. All individual successful mating pairs were a unit of replication.

sex and 10 day-time class via a type-II Wald Chi-square test. When the interaction between larval diet and time was significant, a post-hoc test for pairwise comparisons with Tukey adjustment was used to identify differences between diet and time (emmeans package; Lenth, 2021). An overview of all fitted models can be found in Table S3 in the Supplementary Material.

Behavioural traits

The time until the first mating attempt was analysed via a Linear Mixed-Effects Model (Bates *et al.*, 2015). The experimental date and cage ID were used as a random intercept. Where needed, data were log-transformed to fit a Gaussian distribution. After model selection, the significance of the time until first mating on the pair type (male–male or female–male), experimental diet and the interaction between the two was tested via a type-II Wald Chi-square test (car package; Fox and Weisberg, 2019). Larval diet and adult diet experiments were analysed separately. Mating latency was analysed in a similar manner, with only diet treatment being analysed for significance after model selection.

The number of mating attempts was analysed via a Generalized Linear Mixed-Effects Model (GLMM) (Bates *et al.*, 2015) with Poisson log-link function. To account for the repeated measures and day effect, experimental date and cage ID were used as a random intercepts. The pair type, experimental diet and the interaction of the two was tested via a type-II Wald Chi-square test (car package; Fox and Weisberg, 2019). Larval diet and adult diet experiments were analysed separately. To analyse mating success, the proportion of successful attempts based on the total female–male attempts was calculated. The proportion of mating success was analysed via a Generalized Linear Mixed-Effects Model (GLMM) (Bates *et al.*, 2015) with a binomial distribution. Experimental date was added as random intercept. After model selection, the significance of the experimental diet was tested via a type-II Wald Chi-square test (car package; Fox and Weisberg, 2019). Larval diet and adult diet experiments were analysed separately.

Wing-fanning duration was fitted to a GLMMTMB. Data were log-transformed to fit a Gaussian distribution. The model had experimental diet and attempt type as fixed effects. The model accounted for heterogeneity in the dispersion across attempt type. To account for the repeated measures and day effect, experimental date and cage ID were used as random intercept. When the interaction of attempt type and diet was significant, a post-hoc test for pairwise comparisons with Tukey adjustment was used to identify differences between mating attempt type (emmeans package; Lenth, 2021).

Courtship duration and copulation duration were fitted via a Linear Mixed-Effects Model. To account for the repeated measures and day effect, experimental date and cage ID were used as random intercept. An overview of all fitted models is presented in Table S4 in the Supplementary Material.

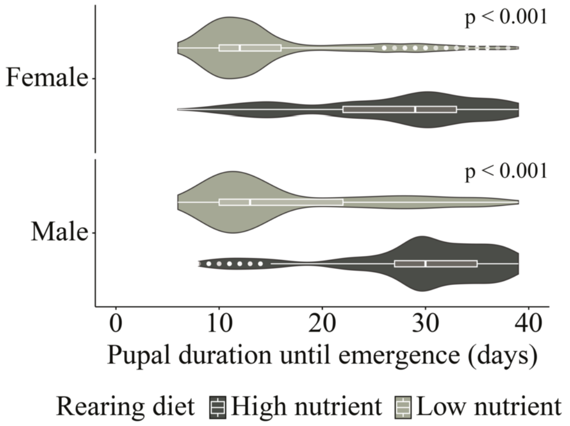

FIGURE 3 Pupal duration of female flies reared on high-nutrient diet ($N = 844$), female flies reared on low- nutrient diet ($N = 732$), male flies reared on high-nutrient diet ($N = 909$) and male flies reared on low-nutrient diet ($N = 890$). The influence of diet was analysed separately for female and male flies. The bold vertical line in the horizontal box plot shows the median. The left and right box boundaries show the third and first quartiles. The whiskers extend to the most extreme values within 1.5 times the interquartile range. Dots indicate outliers. The greyish shapes around the box plots represent density plots in which the width of the shape corresponds with the proportion of individuals emerging.

3 Results

Fly emergence based on larval diet

Larval diet influenced proportion of adult emergence which was earlier for insects reared on the low-nutrient diet compared to insects reared on the high-nutrient diet ($p < 0.001$) (Fig. S2 in the Supplementary Material), even though the survival of flies reared on both low-nutrient (80.9%) and high-nutrient (81%) diets are quite similar after 40 days of pupation time (Fig. S2 in the Supplementary Material). Diet treatment influenced pupal duration until emergence significantly for both female flies ($\chi^2 = 582, p < 0.001$; Fig. 3) and male flies ($\chi^2 = 624, p < 0.001$; Fig. 3).

Adult body-weight differences when insects are exposed to different experimental diets

Effects of larval diet and pupal duration on weight of newly emerged flies

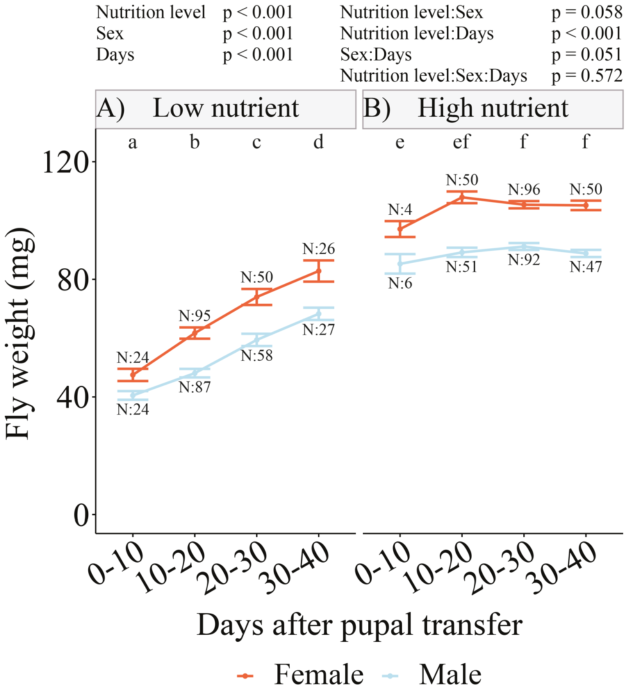

FIGURE 4 Body weight (mean ± SEM) of newly emerged flies (<24 h old) reared on (A) low nutrient or (B) high nutrient diet. Flies are separated by sex and emergence time class (divided over four 10-day classes). Values having no letters in common differ significantly (Tukey HSD, $p < 0.05$). N represents number of female or male insects weighed within the four time classes.

The number of surviving pupae present in the substrate after 20 days was not significantly different for the two larval diets (Table S1). However, pupal weight (Tables S1 and S3 in the Supplementary Material) and the weight of freshly emerged flies (<24 h old; Table S3 in the Supplementary Material) was significantly affected by larval diet (Fig. 4, Table S3 in the Supplementary Material). The high-nutrient larval diet resulted in heavier pupae and heavier flies (Fig. 4, Table S1 in the Supplementary Material).

Body weight of the newly emerged flies differed between the sexes: females were heavier than males (Fig. 4; Table S3 in the Supplementary Material). The duration of the pupal stage also influenced the weight of freshly emerged flies (Fig. 4; Table S3 in the Supplementary Material). This was especially the case for flies that developed from larvae reared on low-nutrient diet: those that spent more time in the pupal stage had the highest weight on this diet (Fig. 4A). In the high-nutrient treatments, this effect was less pronounced, as only flies that had emerged between 0–10 days after pupal transfer were significantly lighter when compared to the flies that emerged between 20–40 days after pupal transfer (Fig. 4B).

Effects of larval and adult diet on weight of 1–2-day-old flies

Both larval diet and adult diet affected the weight of 1–2 day-old flies (Tables S2 and S3 in the Supplementary Material). Body weight differed between males and females (Table S2 and S3 in the Supplementary Material). The high-nutrient larval diet resulted in the heaviest females and the low-nutrient diet yielded the lightest males (Table S2 in the Supplementary Material). Similar results were found for the adult diet for which females provided with diet showed the highest weight and males that did not receive diet had the lowest weight (Table S2 in the Supplementary Material in the Supplementary Material). For both larval diets and adult diets an interaction between sex and diet type and was found (Table S3 in the Supplementary Material).

Mating behaviour

Time until the first mating attempt and mating latency

Larval diet did not influence time until first mating attempt (Fig. 5A, Table S4 in the Supplementary Material). First male–male and female–male mating attempts took place at similar times after fly release (Fig. 5A, Table S4 in the Supplementary Material). Exposing adult flies to diet resulted in later mating attempts, compared to adult flies that did not have access to diet (Fig. 5A, Table S4 in the Supplementary Material). The interaction between diet and the interaction type (female–male or male–male) was similar for both larval and adult diets (Fig. 5A, Table S4 in the Supplementary Material). Even though later mating attempts were observed in adults provided with diet, mating latency (time from release of the experimental flies into the cage until a first mating took place) was similar (Fig. 5B, Table S4 in the Supplementary Material). Mating latency was also similar for flies reared as larvae on a high-nutrient or low-nutrient diet (Fig. 5B, Table S4 in the Supplementary Material).

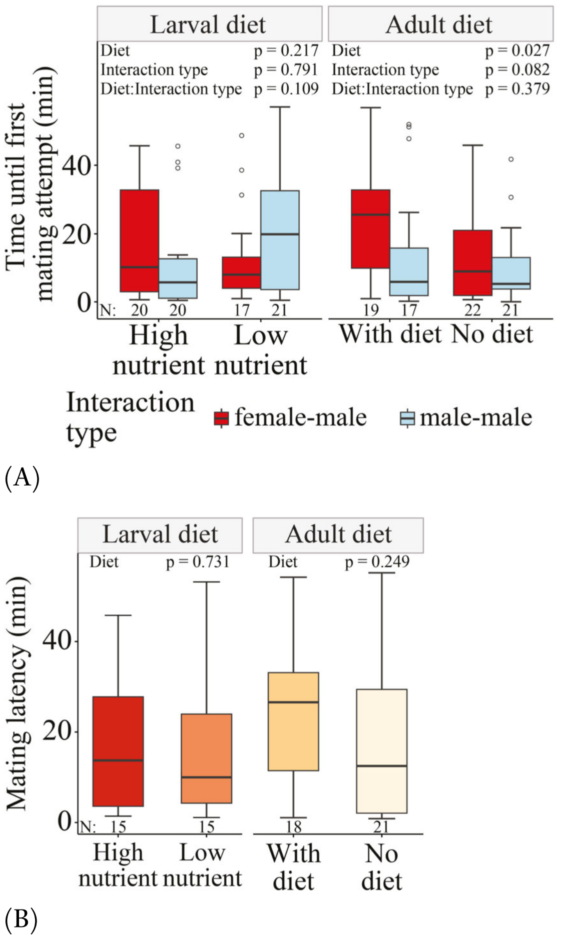

FIGURE 5 (A) Time until the first mating attempt took place (min); high- or low-nutrient larval diets and flies with or without adult diet. Time until the first mating attempt is presented per interaction type: female–male attempt (red) and male–male attempt (blue). (B) Mating latency (time from release of the experimental flies into the cage until a first mating took place) (min). The bold horizontal line shows the median. The upper and lower box boundaries show the third and first quartile. The whiskers extend to the most extreme values within 1.5 times the interquartile range. The dots indicate the outliers. *N* represents the number of times a particular interaction type took place.

Number of male–male and female–male mating attempts depending on diet treatment

The number of mating attempts within the first hour of observation was not influenced by larval diet (Fig. 6A, Table S4 in the Supplementary Material). Male–male interactions occurred with higher frequency than female–male interactions (Fig. 6A, Table S4 in the Supplementary Material). Adult diet influenced the overall number

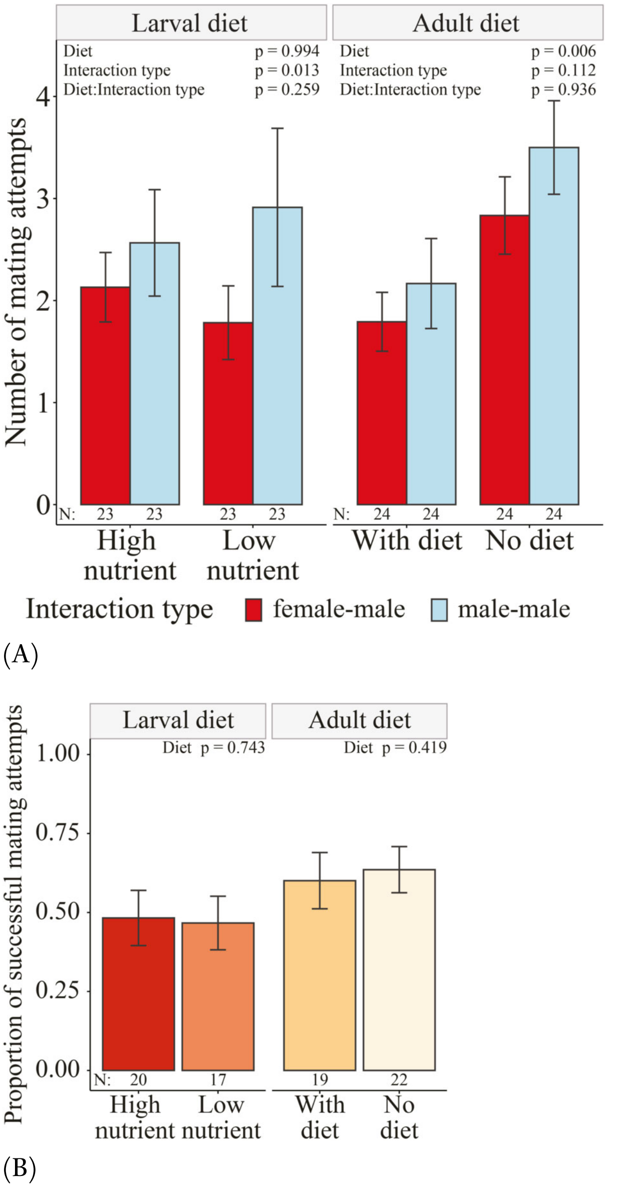

FIGURE 6 (A) Number of mating attempts (mean ± SEM) during the first hour of observation per interaction type (female–male or male–male). Red colour indicates female–male mating attempts and blue colour indicates male–male mating attempts. N represents the number of experimental cages. (B) Proportion of female–male mating attempts (mean ± SEM) that resulted into a successful mating. *N* represents the number of experimental cages that had at least one female–male mating attempt.

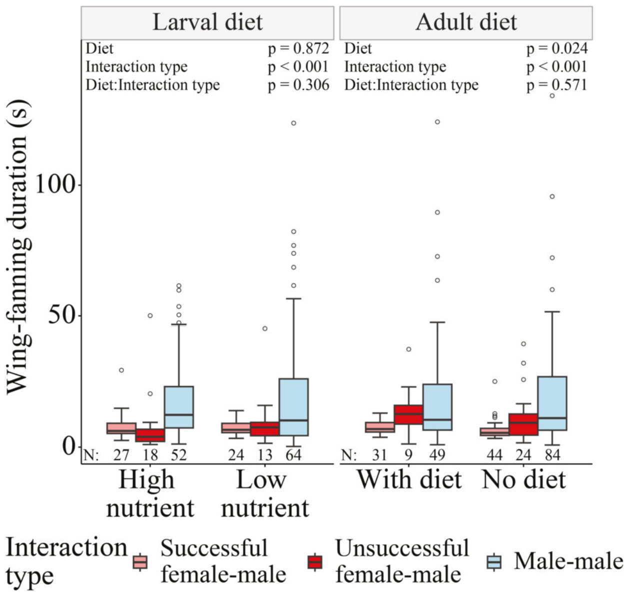

FIGURE 7 Wing-fanning duration (s) for flies reared as larvae on either of two diets and for adult flies with or without diet. Wing-fanning duration is presented per interaction type: successful female–male attempt (light red), unsuccessful female–male attempt (red) and male–male attempt (blue). The bold horizontal line shows the median. The upper and lower box boundaries show the third and first quartile. The whiskers extend to the most extreme values within 1.5 times the interquartile range. The dots indicate the outliers. N represents the number of times an interaction type took place among flies exposed to the diet treatments indicated.

of mating attempts (Fig. 6A, Table S4 in the Supplementary Material). More mating attempts occurred among flies that did not receive nutrition in the adult stage (Fig. 6A). The frequency of male–male and female–male interactions was similar among the two adult diets (Fig. 6A, Table S4 in the Supplementary Material). There was no significant interaction between interaction type (female–male or male–male) and dietary treatment for either larval or adult diet (Table S4 in the Supplementary Material).

Mating success

Proportion of successful mating attempts

The female–male mating attempts resulted either in a successful attempt, followed by mating, or an unsuccessful attempt that did not result in mating. Neither larval nor adult diet influenced the proportion successful mating attempts (Fig. 6B, Table S4 in the Supplementary Material).

Differences in wing-fanning duration

Wing fanning in female–male pairs resulted either in mating or in a rejection (Fig. 7). Wing-fanning duration differed between interaction types in flies reared on different larval diets (Fig. 7; Table S4 in the Supplementary Material). Male–male wing fanning duration was significantly longer when compared to unsuccessful female–male interactions ($p < 0.001$) and successful female–male interactions ($p < 0.001$). However, wing-fanning duration of successful female–male and unsuccessful female–male attempts was similar ($p = 0.375$). Larval diet did not affect wing-fanning duration (Fig. 7, Table S4 in the Supplementary Material).

Wing-fanning duration differed between interaction types in flies exposed to different adult diets (Fig. 7, Table S4 in the Supplementary Material). Male–male pairs showed a longer wing-fanning duration when compared to the wing-fanning duration of successful female–male pairs ($p < 0.001$). Male–male wing-fanning duration was similar to the wing-fanning duration of unsuccessful female–male pairs ($p = 0.270$) and unsuccessful female–male pairs displayed a similar wing-fanning duration as successful female–male pairs ($p = 0.173$). Adults fed with diet had a longer wing-fanning duration compared to adults that did not receive additional diet (Fig. 7, Table S4 in the Supplementary Material).

Courtship duration and copulation duration

Flies that were fed on different diets in the larval stage and flies that were exposed to different diets in the adult stage exhibited similar courtship duration (Fig. 8A; Table S4 in the Supplementary Material). However, flies reared as larvae on low-nutrient diet had a longer copulation time compared to flies reared as larvae on high-nutrient diet (Fig. 8B; Table S4 in the Supplementary Material). Flies that did not receive diet as adults had a longer copulation time than flies that had access to diet in the adult stage (Fig. 8B; Table S4 in the Supplementary Material).

4 Discussion

Our results show that pupation time is longer when the nutrient content of the larval diet quality was high than when nutrient content was low. However, BSF reared on a low-nutrient diet gained a significant weight benefit from a relatively longer pupation time. Not only did we find that diet can influence body weight but also mating behaviour. Low-nutrient diet in the larval stage resulted in a longer copulation time compared with flies that had developed on a high-nutrient larval diet. Food availability in the adult stage had the largest influence on mating behaviour: the absence of food in the adult stage resulted in earlier mating and more frequent mating attempts, shorter wing fanning and a longer copulation time. Such effects on behaviour are relevant in the context of welfare, when considering that insects should be able to express natural behaviour (Barrett *et al.*, 2023).

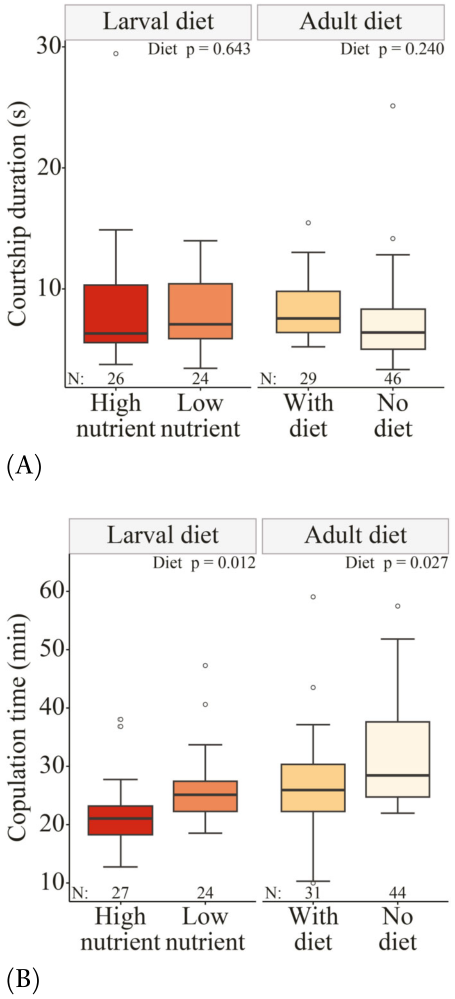

FIGURE 8 (A) Courtship duration (s) of BSF for each dietary treatment group. (B) Copulation time (min) for each dietary treatment. Bold horizontal line shows the median, upper and lower box boundaries show the third and first quartile. Whiskers extend to the most extreme values within 1.5 times the interquartile range. Dots indicate the outliers.

Dietary influences on life-history traits

BSF is sexually dimorphic; females were found to be heavier than males, consistent with the fact that BSF females have a larger body than males (Gobbi *et al.*, 2013). Larval diet did not affect pupal survival at 20 days, which is similar to the findings by Barragan-Fonseca *et al.* (2018) who tested similar dietary treatments.

Prolonged pupation time is associated with increased fly body weight

Our results showed that larvae reared on high-nutrient diet spent significantly more days in the pupal stage. The increased pupation time of BSF reared on high-nutrient diet when compared to BSF reared on low-nutrient diet was not expected due to the similar development time until the pupal stage observed by

Barragan-Fonseca *et al.* (2018) who investigated similar dietary treatments. Even though prolonged pupation time may be accompanied with dehydration effects in non-diapausing insects (Hadley, 1994), we did not see such an effect for BSF as the body weight of emerging flies was higher when spending more time in the pupal stage. Flies reared on high-nutrient diet showed similar body weight when emerging between 10 and 40 days after pupal transfer. The earlier adult emergence of individuals reared on low-nutrient diet was associated with an increase in body weight with every 10 days of additional pupation time. While previous research showed a prolonged pupation time in BSF due to dietary differences, this was associated with low-quality diets resulting in smaller flies (Gobbi *et al.*, 2013), our findings show the opposite effect. The increased body weight may offer advantages, especially because larger females have larger ovaries (Gobbi *et al.*, 2013) and populations of larger females produce more eggs (Jones and Tomberlin, 2021). Increased body weight can also lead to higher reproductive success, as large males achieve a higher number of successful matings compared with smaller males and are considered more aggressive (Jones and Tomberlin, 2021). However, whether such an effect on mating behaviour is present in flies with prolonged pupation time remains to be studied. Even with possible reproductive advantages, most of the flies reared on low-nutrient diet emerged early and with a lower body weight. This increased developmental rate could be an evolutionary strategy to increase reproductive success.

Diet influences pupal and adult body weight

Pupal weight and 1–2-day-old fly weight were highest when the insects had been reared on high-nutrient diet. When provided with access to diet, 1–2-day-old flies were heavier when compared with flies that were deprived of diet. This indicates that feeding adults is advantageous to the flies. Not only can females benefit from nutrient acquisition in the adult stage leading to increased oviposition (Bertinetti *et al.*, 2019), also males may benefit. As spermatogenesis in virgin BSF males is continuous (Munsch-Masset *et al.*, 2023) they may need the extra nutrition to increase mating success over time. This result can be especially interesting in the context of insect welfare, as stratiomyid flies can be considered typical nectar feeders as they are seen feeding on flowers (James, 1981) and also on honeydew (Beuk, 1990) even though feeding by adult BSF is generally considered unnecessary for successful reproduction (Tettamanti *et al.*, 2022).

Dietary influences on mating behaviour

Duration of behavioural recording

We aimed at analysing the first mating attempts, because subsequent mating attempts may be affected by prior experiences. We had recorded the behaviour for longer periods but the first matings generally took place within the first hour of recording and analysing the videos for longer time did not change the results.

Dietary influences on mating attempts

Adult diet influenced BSF mating behaviour in our experiments. Flies that did not have access to food in the adult stage showed earlier and more frequent mating attempts than adults that had food available. This may indicate that starved males invest more energy in reproduction than well-fed males. Starved, sexually mature *Nicrophorus vespilloides* Herbst (Coleoptera: Silphidae) males also showed a higher reproductive investment in alternative mating tactics such as increasing the proportion of time spent in signalling females (Richardson and Smiseth, 2019). Interestingly, such a potential increase in reproductive investment did not result in a higher proportion of mating success in food-deprived BSF adults. In our study, the mating latency was not influenced by diet. This differs from what was observed in *A. obliqua* Macquart (Diptera: Tephritidae) in which fly pairs fed on high-quality diet demonstrated their first mating earlier than pairs fed on low-quality diet (Aluja *et al.*, 2009). However, these differences can be due to the differences between the species, as BSF and *A. obliqua* feed and reproduce on different substrates. The terminal investment hypothesis predicts that under threat on survival, such as starvation, individuals invest in current reproduction (Foo *et al.* 2023). In female–male interactions adult starvation resulted in faster and more mating attempts which meets with the predictions of the terminal investment hypothesis.

Wing-fanning duration and the connection to mate choice

We rarely observed copulation without prior wing fanning, in line with the results of Giunti *et al.* (2018). Even though overall wing-fanning duration was longer in fed adults, we found a similar wing-fanning duration for successful and unsuccessful female–male mating attempts for flies reared on different larval or adult diets. When larvae were subjected to different dietary treatments, male–male pairs had a longer wing-fanning duration compared to successful and unsuccessful female–male attempts. When only adults were exposed to different dietary treatments, male–male wing-fanning duration was only longer when compared with successful female–male mating attempts. However, the wing-fanning duration of successful and unsuccessful female–male pairs was similar. This suggests that, even though wing fanning is important in the courtship sequence (Giunti *et al.* 2018), in our study it did not seem to be linked to mating success of female–male pairs.

If wing fanning does not regulate mate choice, there may be other mechanisms of mate choice operating in BSF, for example chemical cues, vibration frequencies, sperm competition and cryptic female choice that occur after copulation (Kotzé *et al.*, 2019; Terzidou *et al.* 2022; Piersanti *et al.* 2024; Manas *et al.* 2025). The BSF sperm tract is favourable for continuous sperm transfer during copulation, therefore the regulation of sperm quantity may be influenced by the male or female, potentially influenced by mate choice (Munsch-Masset *et al.*, 2023).

The investment in reproductive behaviour

Copulation duration is dependent on diet, with a prolonged copulation duration in flies reared as larvae on low-nutrient diet and a prolonged copulation duration in flies that did not receive diet in the dietary treatments in the adult stage. Even though copulation duration of smaller *M. domestica* was shorter (Baldwin and Bryant, 1981), some other insects such as protein-deprived male *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) (Blay and Yuval, 1997) and *Derocephalus angusticollis* Enderlein (Diptera: Neriidae) males reared on poor larval diet were found to invest more in reproductive output by increasing mating duration (Fricke *et al.*, 2015).

Sperm competition theory proposes that prolonged mating increases a male's reproductive success because it increases sperm transfer (Parker, 1970). BSF that did not have access to food in the adult stage may have some form of evolutionary adaptation to ensure reproductive success, by which increased copulation time would give a higher probability of reproduction. This remains to be tested by investigating fitness outcome. Smaller males of *Scathophaga stercoraria* L. (Diptera: Scathophagidae) show a similar prolonged copulation time. This is the result of the decreased sperm output that smaller males have in comparison with larger males (Simmons *et al.*, 2020). A prolonged copulation time in smaller males can result in a similar number of spermatozoa being transferred and therefore a similar probability of paternity is expected when compared with larger males (Charnov and Parker, 1995). Since BSF, as previously mentioned, may be able to influence the number of spermatozoa transferred via modulation of copulation time (Munsch-Masset *et al.*, 2023), a similar effect as in *S. stercoraria* is expected.

Additionally, an increase in spermatophore quality may be a way to ensure reproductive success, for example via an increased mating effort that occurs due to immediate survival threats such as infection in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Reyes-Ramírez *et al.*, 2021) and the combination of old age and infection in *Nicrophorus marginatus* Fabricius (Coleoptera: Silphidae) (Farchmin *et al.*, 2020). The reproductive investment may extend to providing seminal fluid proteins (SFP) during mating similar to what has been found for *D. melanogaster* (Avila *et al.*, 2010). BSF may transfer SFPs together with the seminal fluid during mating, as mating increases longevity of BSF females (Harjoko *et al.*, 2023). In our case, insects reared on low-nutrient diet or without food in the adult stage in combination with the age of the tested flies may result in an increased mating effort, as a reproductive investment in proteins may be very costly for these individuals. Furthermore, longevity can decrease due to the lack of nutrients in the adult stage (Macavei *et al.*, 2020) and due to mating status, as mated BSF males show a decreased longevity (Harjoko *et al.*, 2023).

This increased investment may not be relevant if BSF exhibits some sort of re-mating strategy. In other Diptera, females mated with males exposed to low-protein diets are more likely to re-mate when the opportunity presents itself (Blay and Yuval, 1997, Costa *et al.*, 2012). In wild *Anastrepha fraterculus* Wiedemann (Diptera: Tephritidae) a similar pattern is present, when females mated with males fed on lower nutrient diet in the adult stage a higher proportion of females remated within 48 hours (Abraham *et al.*, 2011). BSF may have a similar re-mating strategy, as females have spermathecae (Munsch-Masset *et al.*, 2023), and both re-mating and multiple parentage has been reported in this species (Hoffmann *et al.*, 2021; Jones and Tomberlin, 2021). Females seem to be unable to select sperm via differential filling of the sperm storage reservoirs (Munsch-Masset *et al.*, 2023) and the increased chances of parentage for the male may be due to the last male sperm precedence, the phenomenon by which the last male that mated fertilises a larger number of eggs (Birkhead and Hunter, 1990; Ridley, 1989).

Male–male interactions

In our study we frequently observed male–male interactions, similar to what has been reported in other studies (Gobbi *et al.*, 2013; Jones and Tomberlin, 2021).

Male–male interactions are not uncommon in dipteran species. In *Drosophila*, males that have limited courtship experience were found to court a wide range of conspecifics when sexually mature, and based on the rejection experience, alter courtship choice in future attempts (Dukas, 2010). The long wing-fanning duration in male–male courtship attempts in BSF may be one of these behavioural experiences that could alter subsequent male courtship choices. In BSF, the connection with female choice based on male–male interactions and courtship experience still remains to be studied.

5 Conclusion

In conclusion, both larval and adult diet have an effect on mating behaviour in BSF. However, the differences in mating behaviour variables were more strongly influenced by the provision of adult diet. Still very little is understood about the dynamics of mate choice, and increased insight may help with creating more beneficial mating conditions in mass-rearing facilities. The dietary effects on BSF reproductive behaviour are relevant for maintaining a central rearing colony and also to ensure insect welfare in the adult stage. Insect welfare is a subject increasingly discussed especially in the context of insects reared for food and feed (Barrett *et al.*, 2023; Kortsmit *et al.*, 2023; van Huis, 2019; Voulgari-Kokota *et al.*, 2023). Our study suggests that the access of adults to nutrients such as protein and sugar is one of the aspects that needs to be taken into account in the context of insect welfare (Voulgari-Kokota *et al.*, 2023). Because freedom from hunger is considered an important welfare aspect and adults are typically only provided with water (Barrett *et al.*, 2023), our data suggest that there is potential for improvement of welfare of flies in mass-rearing systems.

Supplementary materials

Data is available on https://doi.org/10.1163/23524588-bja10342 under Supplementary Materials.

Acknowledgements

We thank Hans Smid for help with designing the experimental camera set-up, Ton van der Zalm of Tupola for the help with completing the experimental cages with the construction of LED strips and Davy Meijer for helping with the light measurements. We thank our colleagues Anna Dörper, Kelly Niermans, Sevasti Maistrou and Parth Shah for their help with these experiments. Furthermore, we would like to thank Luuk Croijmans and Gerrit Gort for statistical advice. Finally, we thank Pieter Rouweler, André Gidding and Kimmy Reijngoudt for maintaining the BSF colony. This research has been supported by the Dutch Research Council (NWO; NWA programme, InsectFeed project, NWA.1160.18.144).

Authorship contribution

Yvonne Kortsmit: conceptualisation, methodology, project administration, investigation, data curation, software, validation, formal analysis, visualization, writing–original draft, writing (review and editing). Margarita Sklavounou: conceptualisation, investigation, data curation, software, writing (review and editing). Marcel Dicke: conceptualisation, funding acquisition, resources, supervision, writing (review and editing). Joop J.A. van Loon: conceptualisation, funding acquisition, methodology, resources, supervision, writing (review and editing).

Data availability

Data are available via: https://doi.org/10.4121/4d34a6ec-b683-4166-acca-d156d5addc19. Full length video recordings are available upon request.

Declaration of interest

The authors declare no conflicts of interest.

References

Abraham, S., Goane, L., Cladera, J. and Vera, M.T., 2011. Effects of male nutrition on sperm storage and remating behavior in wild and laboratory *Anastrepha fraterculus* (Diptera: Tephritidae) females. Journal of Insect Physiology 57: 1501-1509. https://doi.org/10.1016/j.jinsphys.2011.08.004

Alcock, J., 1990. A large male competitive advantage in a lekking fly, *Hermetia comstocki* Williston (Diptera: Stratiomyidae). Psyche 97: 267-279. https://doi.org/10.1155/1990/72328

Aluja, M., Rull, J., Sivinski, J., Trujillo, G. and Pérez-Staples, D., 2009. Male and female condition influence mating performance and sexual receptivity in two tropical fruit flies (Diptera: Tephritidae) with contrasting life histories. Journal of Insect Physiology 55: 1091-1098. https://doi.org/10.1016/j.jinsphys.2009.07.012

Avila, F.W., Ravi Ram, K., Bloch Qazi, M.C. and Wolfner, M.F., 2010. Sex peptide is required for the efficient release of stored sperm in mated *Drosophila* females. Genetics 186: 595-600. https://doi.org/10.1534/genetics.110.119735

Baldwin, F.T. and Bryant, E.H., 1981. Effect of size upon mating performance within geographic strains of the housefly, *Musca domestica* L. Evolution 35: 1134. https://doi.org/10.2307/2408127

Barbosa, F., 2009. Cryptic female choice by female control of oviposition timing in a soldier fly. Behavioral Ecology 20: 957-960. https://doi.org/10.1093/beheco/arp083

Barragan-Fonseca, K.B., Dicke, M. and Van Loon, J.J.A., 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed-a review. Journal of Insects as Food and Feed 3: 105-120. https://doi.org/10.3920/JIFF2016.0055

Barragan-Fonseca, K.B., Dicke, M. and Van Loon, J.J.A., 2018. Influence of larval density and dietary nutrient concentration on performance, body protein and fat contents of black soldier fly larvae (*Hermetia illucens*). Entomologia Experimentalis et Applicata 166: 761-770. https://doi.org/10.1111/eea.12716

Barrett, M., Chia, S.Y., Fischer, B. and Tomberlin, J.K., 2023. Welfare considerations for farming black soldier flies, *Hermetia illucens* (Diptera: Stratiomyidae): a model for the insects as food and feed industry. Journal of Insects as Food and Feed 9: 119-148. https://doi.org/10.3920/JIFF2022.0041

Bates, D., Mächler, M., Bolker, B. and Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1-48. https://doi.org/10.18637/jss.v067.i01

Bertinetti, C., Samayoa, A.C. and Hwang, S.-Y., 2019. Effects of feeding adults of *Hermetia illucens* (Diptera: Stratiomyidae) on longevity, oviposition and egg hatchability: insights into optimizing egg production. Journal of Insect Science 19: 1-7. https://doi.org/10.1093/jisesa/iez001

Bertrand, P.V., Sakamoto, Y., Ishiguro, M. and Kitagawa, G., 1988. Akaike Information Criterion statistics. Journal of the Royal Statistical Society Series A 151: 567. https://doi.org/10.2307/2983028

Beuk, P.L.T., 1990. Honeydew as a food source for insects and in particular for soldierflies (Diptera: Stratiomyidae). Phegea 18: 137-140.

Birkhead, T.R. and Hunter, F.M., 1990. Mechanisms of sperm competition. Trends in Ecology and Evolution 5: 48-52. https://doi.org/10.1016/0169-5347(90)90047-H

Blay, S. and Yuval, B., 1997. Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae. Animal Behaviour 54: 59-66. https://doi.org/10.1006/ANBE.1996.0445)

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M. and Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated Generalized Linear Mixed Modeling. The R Journal 9: 378. https://doi.org/10.32614/RJ-2017-066

Bruno, D., Bonelli, M., Cadamuro, A.G., Reguzzoni, M., Grimaldi, A., Casartelli, M. and Tettamanti, G., 2019. The digestive system of the adult *Hermetia illucens* (Diptera: Stratiomyidae): morphological features and functional properties. Cell and Tissue Research 378: 221-238. https://doi.org/10.1007/s00441-019-03025-7

Charnov, E.L. and Parker, G.A., 1995. Dimensionless invariants from foraging theory's marginal value theorem. Proceedings of the National Academy of Sciences of the United States of America 92: 1446-1450. https://doi.org/10.1073/pnas.92.5.1446

Costa, A.M., Anjos-Duarte, C.S., Roriz, A.K.P., Dias, V.S. and Joachim-Bravo, I.S., 2012. Male diet and age influence to inhibit female remating in *Ceratitis capitata* (Diptera: Tephritidae). Journal of Applied Entomology 136: 456-463. https://doi.org/10.1111/J.1439-0418.2011.01669.X

Davies, L.R., Schou, M.F., Kristensen, T.N. and Loeschcke, V., 2018. Linking developmental diet to adult foraging choice in *Drosophila melanogaster*. Journal of Experimental Biology 221: jeb175554. https://doi.org/10.1242/jeb.175554

Dörper, A., Veldkamp, T. and Dicke, M., 2021. Use of black soldier fly and house fly in feed to promote sustainable poultry production. Journal of Insects as Food and Feed 7: 761-780, https://doi.org/10.3920/jiff2020.0064

Dukas, R., 2010. Causes and consequences of male-male courtship in fruit flies. Animal Behaviour 80: 913-919. https://doi.org/10.1016/j.anbehav.2010.08.017

Farchmin, P.A., Eggert, A.-K., Duffield, K.R. and Sakaluk, S.K., 2020. Dynamic terminal investment in male burying beetles. Animal Behaviour 163: 1-7. https://doi.org/10.1016/j.anbehav.2020.02.015

Filice, D.C.S., Bhargava, R. and Dukas, R., 2020. Plasticity in male mating behavior modulates female life history in fruit flies. Evolution 74: 365-376. https://doi.org/10.1111/EVO.13926

Foo, Y.Z., Lagisz, M., O'Dea, R.E. and Nakagawa, S., 2023. The influence of immune challenges on the mean and variance in reproductive investment: a meta-analysis of the terminal investment hypothesis. BMC Biology 21: 24. https://doi.org/10.1186/s12915-023-01603-4

Fox, J. and Weisberg, S., 2019. An R companion to applied regression. 3rd ed. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Friard, O. and Gamba, M., 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods in Ecology and Evolution 7: 1325-1330. https://doi.org/10.1111/2041-210X.12584

Fricke, C., Adler, M.I., Brooks, R.C. and Bonduriansky, R., 2015. The complexity of male reproductive success: effects of nutrition, morphology and experience. Behavioral Ecology 26: 617-624. https://doi.org/10.1093/beheco/aru240

Giunti, G., Campolo, O., Laudani, F. and Palmeri, V., 2018. Male courtship behaviour and potential for female mate choice in the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae). Entomologia Generalis 38: 29-46. https://doi.org/10.1127/entomologia/2018/0657

Gobbi, P., Martínez-Sánchez, A. and Rojo, S., 2013. The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). European Journal of Entomology 110: 461-468. https://doi.org/10.14411/EJE.2013.061

Hadley, N.F., 1994. Water relations of terrestrial arthropods. Academic Press, San Diego, CA.

Harjoko, D.N., Hua, Q.Q.H., Toh, E.M.C., Goh, C.Y.J. and Puniamoorthy, N., 2023. A window into fly sex: mating increases female but reduces male longevity in black soldier flies. Animal Behaviour 200: 25-36. https://doi.org/10.1016/j.anbehav.2023.03.007

Hartig, F., 2022. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models (version 0.4.6). R package. R Foundation for Statistical Computing, Vienna.

Hoffmann, L., Hull, K.L., Bierman, A., Badenhorst, R., van der Merwe, A.E.B. and Rhode, C., 2021. Patterns of genetic diversity and mating systems in a mass-reared black soldier fly colony. Insects 12: 480. https://doi.org/10.3390/INSECTS12060480

James, M.T., 1981. Stratiomyidae. In J.F. McAlpine, B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth and D.M. Wood (Eds.), Manual of Nearctic Diptera, Vol. 1. Research Branch Agriculture Canada. Available online at https://publications.gc.ca/site/eng/9.817747/publication.html

Jones, B.M. and Tomberlin, J.K., 2021. Effects of adult body size on mating success of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae). Journal of Insects as Food and Feed 7: 5-20. https://doi.org/10.3920/JIFF2020.0001

Julita, U., Lusianti, F.L., Eka Putra, R. and Dana Perma, A., 2020. Mating success and reproductive behavior of black soldier fly *Hermetia illucens* L. (Diptera, Stratiomyidae) in tropics. Journal of Entomology 17: 117-127. https://doi.org/10.3923/je.2020.117.127

Kortsmit, Y., van der Bruggen, M., Wertheim, B., Dicke, M., Beukeboom, L.W. and Van Loon, J.J.A., 2023. Behaviour of two fly species reared for livestock feed: optimising production and insect welfare. Journal of Insects as Food and Feed 9: 149-169. https://doi.org/10.3920/JIFF2021.0214

Kotzé, R.C.M., Muller, N., du Plessis, L. and van der Horst, G., 2019. The importance of insect sperm: Sperm ultrastructure of *Hermetia illucens* (black soldier fly). Tissue and Cell 59: 44-50. https://doi.org/10.1016/j.tice.2019.06.002

Laursen, S.F., Flint, C.A., Bahrndorff, S., Tomberlin, J.K. and Kristensen, T.N., 2024. Reproductive output and other adult life-history traits of black soldier flies grown on different organic waste and by-products. Waste Management 181: 136-144. https://doi.org/10.1016/j.wasman.2024.04.010

Lemke, N.B., Dickerson, A.J. and Tomberlin, J.K., 2023. No neonates without adults. BioEssays 45: 2200162. https://doi.org/10.1002/bies.202200162

Lenth, R.V., 2021. emmeans: estimated marginal means, aka least-squares means (R package version 1.6.2-1). Available online at https://cran.r-project.org/package=emmeans

Macavei, L.I., Benassi, G., Stoian, V. and Maistrello, L., 2020. Optimization of *Hermetia illucens* (L.) egg laying under different nutrition and light conditions. PLoS ONE 15: e0232144. https://doi.org/10.1371/journal.pone.0232144

Manas, F., Piterois, H., Labrousse, C., Beaugeard, L., Uzbekov, R. and Bressac, C., 2024. Gone but not forgotten: dynamics of sperm storage and potential ejaculate digestion in the black soldier fly *Hermetia illucens*. Royal Society Open Science 11: 241205. https://doi.org/10.1098/rsos.241205

Manas, F., Laboursse, C. and Bressac, C., 2025. Plastic responses in sperm expenditure to sperm competition risk in black soldier fly (*Hermetia illucens*, Diptera) males. Journal of Insect Physiology 161: 104751. https://doi.org/10.1016/j.jinsphys.2025.104751

Medeiros-Santana, L. and Zucoloto, F.S., 2016. Sexual receptivity of *Anastrepha obliqua* (Diptera: Tephritidae) wild females affected by nutrition and egg load. Entomological Science 19: 233-238. https://doi.org/10.1111/ens.12186

Munsch-Masset, P., Labrousse, C., Beaugeard, L. and Bressac, C., 2023. The reproductive tract of the black soldier fly (*Hermetia illucens*) is highly differentiated and suggests adaptations to sexual selection. Entomologia Experimentalis et Applicata 171: 857-866. https://doi.org/10.1111/EEA.13358

Nakamura, S., Ichiki, R.T., Shimoda, M. and Morioka, S., 2016. Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: low-cost and year-round rearing. Applied Entomology and Zoology 51: 161-166. https://doi.org/10.1007/s13355-015-0376-1

Oonincx, D.G.A.B., Volk, N., Diehl, J.J.E., Van Loon, J.J.A. and Belušič, G., 2016. Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. Journal of Insect Physiology 95: 133-139. https://doi.org/10.1016/j.jinsphys.2016.10.006

Parker, G.A., 1970. Sperm competition and its evolutionary consequences in the insects. Biological Reviews 45: 525-567. https://doi.org/10.1111/j.1469-185X.1970.tb01176.x

Pellegrino, A.C., Peñaflor, M.F.G.V., Nardi, C., Bezner-Kerr, W., Guglielmo, C.G., Bento, J.M.S. and McNeil, J.N., 2013. Weather forecasting by insects: modified sexual behaviour in response to atmospheric pressure changes. PLoS ONE 8: e75004. https://doi.org/10.1371/JOURNAL.PONE.0075004

Piersanti, S., Rebora, M., Marri, G.C. and Salerno, G., 2024. Antennal olfactory responses in the black soldier fly *Hermetia illucens*. Journal of Insect Physiology 159: 104722. https://doi.org/10.1016/j.jinsphys.2024.104722

R Core Team, 2023. R: a language and environment for statistical computing (v 4.3.1). R Foundation for Statistical Computing, Vienna.

Reyes-Ramírez, A., Rocha-Ortega, M. and Córdoba-Aguilar, A., 2021. Dietary macronutrient balance and fungal infection as drivers of spermatophore quality in the mealworm beetle. Current Research in Insect Science 1: 100009. https://doi.org/10.1016/J.CRIS.2021.100009

Richardson, J. and Smiseth, P.T., 2019. Nutrition during sexual maturation and at the time of mating affects mating behaviour in both sexes of a burying beetle. Animal Behaviour 151: 77-85. https://doi.org/10.1016/J.ANBEHAV.2019.03.013

Ridley, M., 1989. The incidence of sperm displacement in insects: four conjectures, one corroboration. Biological Journal of the Linnean Society 38: 349-367. https://doi.org/10.1111/j.1095-8312.1989.tb01582.x

Savola, E., Vale, P.F. and Walling, C.A., 2022. Larval diet affects adult reproduction, but not survival, independent of the effect of injury and infection in *Drosophila melanogaster*. Journal of Insect Physiology 142: 104428. https://doi.org/10.1016/j.jinsphys.2022.104428

Shah, P.N., Hemerik, L., Dicke, M. and Van Loon, J.J.A., 2024. Dietary protein levels influence growth, adult emergence and susceptibility to bacterial infection in *Hermetia illucens* L. (Diptera: Stratiomyidae) larvae. Journal of Insects as Food and Feed 10: 2059-2070. https://doi.org/10.1163/23524588-00001206

Sheppard, D.C., Tomberlin, J.K., Joyce, J.A., Kiser, B.C. and Sumner, S.M., 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). Journal of Medical Entomology 39: 695-698. https://doi.org/10.1603/0022-2585-39.4.695

Simmons, L.W., Parker, G.A. and Hosken, D.J., 2020. Evolutionary insight from a humble fly: sperm competition and the yellow dungfly. Philosophical Transactions of the Royal Society London Series B: Biological Sciences 375: 20200062. https://doi.org/10.1098/rstb.2020.0062

Terzidou, A., Kouloussis, N., Papanikolaou, G. and Koveos, D., 2022. Acoustic characteristics of sound produced by males of *Bactrocera oleae* change in the presence of conspecifics. Scientific Reports 12: 13086. https://doi.org/10.1038/s41598-022-16888-8

Tettamanti, G., Van Campenhout, L. and Casartelli, M., 2022. A hungry need for knowledge on the black soldier fly digestive system. Journal of Insects as Food and Feed 8: 217-222. https://doi.org/10.3920/JIFF2022.X002

Therneau, T., 2023. A package for survival analysis in R (version 3.5-7). R Foundation for Statistical Computing, Vienna.

Tomberlin, J.K. and Sheppard, D.C., 2001. Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). The Florida Entomologist 84: 729. https://doi.org/10.2307/3496413

Tomberlin, J.K., Sheppard, D.C. and Joyce, J.A., 2002. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95: 379-386. https://doi.org/10.1603/0013-8746(2002)095[0379:SLHTOB]2.0.CO;2

van Huis, A., 2019. Welfare of farmed insects. Journal of Insects as Food and Feed 5: 159-162. https://doi.org/10.3920/JIFF2019.x004

Voulgari-Kokota, A., Van Loon, M.S. and Bovenkerk, B., 2023. Insects as mini-livestock: Considering insect welfare in feed production. NJAS: Impact in Agricultural and Life Sciences 95: 2191797. https://doi.org/10.1080/27685241.2023.2191797