



REVIEW ARTICLE

A decade of advances in black soldier fly research: from genetics to sustainability

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Received 14 January 2024 | Accepted 7 July 2024 | Published online 7 August 2024

Abstract

Black soldier fly (BSF), *Hermetia illucens*, is one of the most explored insect species mass-produced for feed, but also for food and technical purposes. Considering the rapid developments in both research and industrial production of this insect species in the last decade, this review intends to reflect on the most current scientific insights and define the future trends and needs for the most relevant associated research fields. The review reflects on the aspects of BSF production and reproduction, utilization of BSF biomass as components of animals' feeds and human food. It also provides reflection on genetics, microbiology and sustainability. The analysis identifies the need in future research associated with compositions of fungal and viral communities of insects and their environments and mapping the dynamics of BSF gut physiology and microbiota in varying conditions. High interest will be devoted to establishing genomic resources, to characterize genotypic diversity, and to harness its potential through selective breeding to improve BSF performance quantitatively and/or qualitatively. Further research will follow on the use of BSF for food and feed development, potentially for specific application cases, associated with animal gut microbiome improvement and antimicrobial properties of BSF biomass. The further in-depth exploration of the potential of BSF for waste biotransformation and the assessment of its circularity potential are also expected to be major focus points of research in the next decade.

Keywords

feed – genetics – *Hermetia illucens* – production – sustainability

1 Introduction

Black soldier fly (BSF) – *Hermetia illucens* is one of the most popular insect species used in mass production of insects for feed and petfood purposes. In recent years, there has been substantial growth in the field of sustainable insect production, focusing on improving efficiency and quality, reducing costs and environmental impacts. The number of research articles dealing with *H. illucens* production chains grew exponentially over the last decade. The studies concentrate on insect growth, their nutritional properties, and their dependencies on feed compositions, feeding trials for different animals with BSF larvae inclusion, genetic diversity, gut physiology and microbiota, insect processing techniques, application as food and feed, sustainability issues and circularity potential. The reviewed studies are an example of the areas in which the work is progressing, however there are other areas of work as well which have not been covered in this review. Among a few areas not included in the review, which require further research and critical synthesis of information via review following should be noted: frass management, insect welfare and biology including pathogens, variety of potential established and emerging methods for the analyses, standardization of experimental trials, diversity of BSF research in geographic perspective (the list is not complete).

During the last decade there has been considerable literature published on the mentioned issues. Obviously, they have different complexity and quality. It is becoming very challenging for experts and non-experts to analyze and draw conclusions from the constantly growing body of published studies (Auger *et al.*, 2023). There is a need in systematic, comprehensive analysis done by experts in the narrow specific fields (e.g. genetics or application of insects in feed or food) to determine the most prominent results generated during the decade of research and the gaps which should potentially be covered in the next decade of research. Therefore, the aim of the review is to provide expert highlight of scientific results in specific fields (authors are recognized experts in relevant fields) of *H. illucens* production, application, genetics, and sustainability. At the same time, this review also aims to define the remaining research gaps or future opportunities that might dictate the research in the upcoming decade in the defined areas of research. It should be noted that the results presented in this review are population specific and (biological) traits cannot always be generalized for all BSF strains.

2 Growth and cultivation of BSF

Fly housing and egg production

Egg production is essential when producing BSF larvae. These eggs can be collected from a wild population (Ewusie *et al.*, 2019), acquired from other BSF producers or produced as offspring from an inhouse colony. The BSF can be housed indoors or in a greenhouse (Sheppard *et al.*, 2002).

The first data on the development time of each life stage of the black soldier was already published two decades ago (Sheppard *et al.*, 2002; Tomberlin *et al.*, 2009). However, in the last decade new research has built upon this knowledge, leading to new insights. Malawey *et al.* (2021) observed that once the flies have eclosed from the pupa, peak fertility takes a few days, with male sperm count peaking after 48 h. The time for a fly to start laying eggs after eclosion is 5 days at 35 °C according to Chia *et al.* (2018a), while Bertinetti *et al.* (2019) observed oviposition 4 days after eclosion at 28 °C. Shumo *et al.* (2019a) tested oviposition at different temperatures and found 30 °C to be the optimum. Bertinetti *et al.* (2019) also concluded that feeding the flies improved fly longevity and total egg mass produced, reaching thrice the number of eggs when the flies had access to artificial milk, with on average 1,200 eggs per female. The benefit of providing nutrients to flies for improved egg production seems to be confirmed by Macavei *et al.* (2020) and Klüber *et al.* (2023), the former observed that sugar and water supplementation was better than supplying nothing or only water. The latter found that a glucose or protein rich micro-algae solution did not improve egg production over tap water, but a 5% honey solution did by 80% compared to tap water. Chia *et al.* (2018a) did not observe an unequivocal link between supplying a 10% sucrose solution and increased egg production despite improved adult longevity.

Another parameter affecting egg production is fly density, which has been investigated by Hoc *et al.* (2019). They found that 6,500 individuals per m³ of fly cage produced the highest total amount of eggs. Liu *et al.* (2022) tested up to a density of 3,700 individuals/m³ and observed highest total egg yield with the highest fly density. A female biased population (56% more females) also produces more eggs overall, but the output per female drops compared to a male biased population (Hoc *et al.*, 2019). The life history of a fly plays a role in its fertility and its number of offspring. Gobbi *et al.* (2013) observed differences in ovarian size in female flies of similar size, grown on different diets. Georgescu

et al. (2020) observed larger egg clusters (containing more eggs) in weight classes with heavier flies. Additionally, differences were observed by Jones and Tomberlin (2021) in mating behavior and oviposition rate depending on either male or female size. Finally, Barragan-Fonseca *et al.* (2019) showed that food macronutrient contents during the larval phase significantly affect egg yield of the BSF.

Furthermore, light quality, intensity and duration also have significant influences on the mating success and BSF fertilized egg production (Awal *et al.*, 2022). The BSF depends on direct sunlight for mating and therefore adult colonies used to be kept in greenhouses (Sheppard *et al.*, 2002). Sunlight still seems to outperform artificial light as demonstrated by Nakamura *et al.* (2016), where flies that received an additional 2 hours of daily sunlight had a higher fertilized egg rate than their counterparts that were exclusively exposed to artificial light. Especially in temperate regions artificial light sources are of interest due to seasonal and meteorological variation in light intensity and duration. Since Light emitting diode (LED) illumination has become popular, light sources can be designed to have a specific spectrum composition (Hitz *et al.*, 2019). But optimization to match the fly's demand is still needed. In 2016 Oonincx *et al.* (2016) determined that BSF ommatidia contain ultraviolet (UV)-, blue- and green-sensitive photoreceptor cells. They suggested the following LED ratio UV: blue: green = 1:1:3. This composition was tested by Macavei *et al.* (2020), they found that light with this specific composition performed better than fluorescent tube lamps or white LED's. Heussler *et al.* (2018) tested three different artificial lights (LED's, fluorescent lamps, and halogen lamps) and found no difference between the lights despite the absence of UV in the LED light. There also may be an effect of the genetic background of the flies as demonstrated by Liu *et al.* (2020). They tested 2 different BSF strains under four different artificial lights with clear differences in egg production between both strains.

Apart from light quality, light intensity is another key parameter. There are different ways on how to express light intensity, such as $\mu\text{mol}/\text{m}^2/\text{s}$, W/m^2 or lux. However, there is not one constant to convert one unit to another, as this conversion depends on the spectrum of the light source. As such, comparing results from different studies that use different light sources and units is difficult. Thus, Schneider (2020) observed a higher cumulative probability of mating (70%) at 431 W/m^2 compared to an irradiance of 0.92 W/m^2 (23%).

Hoc *et al.* (2019) found that egg yield increased logarithmically with increasing light duration per day, although 6 hours of light and 18 hours of dark did not differ significantly when compared one-on-one with 16 hours of light. This lets them conclude that 6 hours of light is the minimal recommended duration. In addition, Liu *et al.* (2022) found that increasing photoperiod from 8:16 to 16:8 (light:dark) increased neonate production, but that the number of neonates per watt decreased under artificial illumination.

Typically, oviposition and egg collection occur in a centralized ovitrap (oviposition or egg laying trap). Female flies are attracted to lay their eggs in an ovitrap, which is a device designed with numerous crevices to facilitate egg deposition (Sheppard *et al.*, 2002). The flies prefer an ovitrap made of wood or cardboard (Julita *et al.*, 2021). Bogdan *et al.* (2022) recommends crevices of 2 mm or smaller over wider crevices. It is even possible that the color of the ovitrap influences the attractiveness for flies, as seen by Romano *et al.* (2020), where flies were more prominent on white or green colored objects compared to those colored blue or pink. Moreover, smell is an effective way to lure female flies to the ovitrap, to achieve this the ovitrap is commonly placed over a wet decomposing organic substrate. According to Bogdan *et al.* (2022) a 50% dry matter content is optimal. Although various studies have tried different organic substrates as a lure (Lamin *et al.*, 2022; Nyakeri *et al.*, 2017; Sripontan *et al.*, 2017), no specific chemical compounds have been identified so far that trigger this process. However, there are indications that flies prefer to lay their eggs, which could be (partially) attributed to the presence of bacteria found in different life stages of the BSF (Zheng *et al.*, 2013). Moreover, Scieuzo *et al.* (2021) identified several odorant binding protein genes in adult BSF and candidate volatile organic compounds indicative of organic decomposition.

Optimal conditions for egg hatching were tested by Chia *et al.* (2018a), who observed the highest egg survival of 80% at 30 °C and 70% relative humidity with an eclosion time of 3.5 days with a wild caught Kenyan strain. A high relative humidity is preferred over a low one for egg eclosion as seen by Holmes *et al.* (2012).

Producing the next generation of flies

Larval growth follows a sigmoid curve with accelerating growth in the earlier instars and decelerating growth in the latest instar. Accordingly, the specific metabolic rate was reported to be high in instars 3, 4 and 5 and reduced in instars 6 and 7 (Gligorescu *et al.*, 2019). The last non-feeding instar (prepupa) can be self-harvested

(Sheppard *et al.*, 1994). Hoc *et al.* (2019) and Georgescu *et al.* (2020) observed that during self-harvest, initially predominantly male prepupae will escape. Later on, the ratio of male-female will shift in favor of a higher abundance of female prepupae.

The presence of a pupation substrate has a significant impact on facilitating prepupae to pupate, regardless of the type of substrate. In the absence of a pupation substrate, prepupae are slightly delayed from pupating (Dzepe *et al.*, 2020; Holmes *et al.*, 2013). Prepupae take the shortest time to pupate and have the highest rate of successful adult emergence in complete darkness (Ferdousi and Sultana, 2021).

Importance of nutritional composition of the feed

Being a generalist species, BSF larvae can feed on a wide range of diets, from food by-products and organic waste (Bava *et al.*, 2019; Chia *et al.*, 2018b; Diener *et al.*, 2009, 2011; Eggink *et al.*, 2022; Ewusie *et al.*, 2018; Gao *et al.*, 2019b; Jucker *et al.*, 2017; Lalander *et al.*, 2019; Meneguz *et al.*, 2018; Msangi *et al.*, 2022; Nguyen *et al.*, 2015; Oonincx *et al.*, 2015b; Scala *et al.*, 2020) to fish offal (St-Hilaire *et al.*, 2007), manure (Miranda *et al.*, 2019) and aquaculture sludge (Schmitt *et al.*, 2019), i.e. substrates with high variability in their dietary macronutrient contents and ratios. The biowaste and side streams that have been evaluated so far for BSF larvae rearing have been recently reviewed by several researchers (Gold *et al.*, 2018; Surendra *et al.*, 2020). Following the restrictions that apply to other farmed animals, the current European Union (EU) legislation mainly allows, with few exceptions such as milk, eggs, and blood products from non-ruminants, the use of feed-grade substrates of vegetal origins for BSF larvae rearing (European Council (EC), 2001), whereas similar restrictions apply also to the United States and Canada (Surendra *et al.*, 2020). This is not the case thought in other parts of the world, e.g. Asia, where BSF larvae can be grown on a wide spectrum of organic side-streams and wastes (Surendra *et al.*, 2020). Based on the published results, it has been clearly demonstrated that the nutritional composition of the feed greatly impacts the performance of BSF larvae. Indicatively, although BSF larvae can tolerate and develop on nutrient-poor substrates (Ribeiro *et al.*, 2022), it was early enough shown that a high dietary protein and fat content is positively correlated with growth rate and larval performance (Nguyen *et al.*, 2013; Oonincx *et al.*, 2015a; Tschirner and Simon, 2015). Hosseindoust *et al.* (2023) focused their research on the usage of tofu by-products (obtained from a tofu factory, formed as soybean curd residue), food waste

(collected from households, kitchens, and dining establishments at an environmental waste-collecting facility), and vegetables (cabbage, lettuce, carrot, onion, and garlic collected at an agricultural market) in terms of promoting BSF larvae growth and conversion efficiency. Their findings indicate that tofu by-products can be suitably employed for fostering larval growth and nutrient accumulation (Hosseindoust *et al.*, 2023). Several studies have investigated the nutritional requirements of BSF larvae (Barragán-Fonseca *et al.*, 2018; Barragan-Fonseca *et al.*, 2018; Beniers and Graham, 2019; Cammack and Tomberlin, 2017; Eggink *et al.*, 2023). The dietary content of the two main groups of macronutrients, i.e. the protein (P) and carbohydrate (C) content of the diet, as well as the crude protein to carbohydrate (P:C) ratio can significantly impact larval growth and performance (Barragán-Fonseca *et al.*, 2018; Barragan-Fonseca *et al.*, 2018, 2019, 2021; Beniers and Graham, 2019; Cammack and Tomberlin, 2017; Eggink *et al.*, 2023). For instance, Eggink *et al.* (2023) reported that feeding of BSF larvae on substrates with P:C between 1:2 and 1:3 resulted in the highest larval performance, in terms of survival, total biomass produced and feed conversion ratio, whereas larvae reared on protein- or carbohydrate-biased substrates in most of the cases low survival rates. Similarly, Barragán-Fonseca *et al.* (2018) reported good performance for larvae fed various vegetable waste-based diets with a P:C ratio of 1:2. Cammack and Tomberlin (2017) observed low growth rates and long development times in carbohydrate-biased diets; however, in their study larvae performed best when fed C:P balanced diets. Apart from the C:P ratio, the performance of BSF larvae is also affected by the sum of crude P and C content, with diets with P + C concentrations of 25 or 50% giving good results (Barragan-Fonseca *et al.*, 2019, 2021; Beniers and Graham, 2019). Regarding carbohydrates, their content between 10 and 60% has been shown suitable for supporting high larval performance (Barragan-Fonseca *et al.*, 2021). However, it is noteworthy to highlight the ability of BSF larvae to also grow on substrates free of digestible carbohydrates (e.g. manure, slaughterhouse waste, etc.) (Lalander *et al.*, 2019; Miranda *et al.*, 2019). Dietary protein strongly affects larval growth, protein contents between 10 and 15% supporting high larval growth. An excess of the protein content of the feed though can negatively affect larval survival and performance, probably due to the high energy consumption needed for the metabolism of the surplus protein (Barragan-Fonseca *et al.*, 2021; Tschirner and Simon, 2015), indicating that a nutritionally balanced diet may be more crucial to ensure high

larval performance than a high protein dietary content. High dietary fat content may also negatively affect BSF larvae performance, as it can result in development time increase or larval growth reduction, and this has been attributed to the difficulty of BSF larvae in processing fat (Nguyen *et al.*, 2013; Spranghers *et al.*, 2017). Further research is warranted though to properly define the nutritional requirements of BSF larvae and investigate not only the impact of the dietary macro- and micronutrient content, but also of the nutritional quality of the main macro- and micronutrients, e.g. amino acid and fatty acid profile, etc., as is the case for most industrially farmed animals. Apart from the effect of the nutritional value of the feed, its moisture content can also greatly impact larval growth, where larvae grow faster and bigger when fed diets at optimal moisture of around 70% (Cammack and Tomberlin, 2017; Cheng *et al.*, 2017). Finally, although still an overlooked topic with only few studies available, apart from the moisture content, the physical properties of the diet, e.g. particle size, water holding capacity, dry or wet bulk density, may also affect BSF larvae growth (Yakti *et al.*, 2023).

Dietary effects on BSF larval body composition

Apart from larval performance, the nutritional value of the feed may also alter insect body composition (Oonincx and Finke, 2021). For BSF, high variability has been reported in the composition of larvae fed on different organic substrates, with larval protein content ranging from 27.5 to 62.7% dry matter (DM) and fat content between 6.6 and 51.5% DM (Barragan-Fonseca *et al.*, 2017; Lu *et al.*, 2022). In general, BSF larvae have a high crude protein content regardless of the protein concentration of the diet, i.e. the rearing of BSF larvae on low quality and nutrient-poor diets in most cases results in protein-rich larvae (Spranghers *et al.*, 2017; Tschirner and Simon, 2015). It should be kept in mind though that chitin (3.9 to 7.2% DM) (Lu *et al.*, 2022) accounts for part of the nitrogen content of BSF larvae and this should be taken into account when calculating the BSF larvae protein content. Indicatively, larvae fed on kitchen waste and spent grains with protein content of 20 and 12.2% had a protein content of 33 and 41.3% DM, respectively (Shumo *et al.*, 2019b). According to Fuso *et al.* (2021), significantly lower protein contents were detected in BSF larvae grown on fruit by-products (exotic fruit, pineapple, kiwi, apple, melon), while higher contents were observed when autumnal leftovers (pomace, legume, corn) were employed, BSF larvae protein content being mainly correlated to fiber and protein content in the diet. Among amino acids,

lysine, valine and leucine were most affected by the diet (Fuso *et al.*, 2021). Essential amino acids satisfy the Food and Agricultural Organization (FAO) requirements for human nutrition, with an essential amino acid index (EAAI) (1.44-1.94) (Fuso *et al.*, 2021; Huang *et al.*, 2019; Miron *et al.*, 2023) comparable or higher than the EAAI of other edible insects or protein sources, e.g. soybean or casein (Yi *et al.*, 2013). It should be kept in mind though that chitin (3.9 to 7.2% DM) (Lu *et al.*, 2022) accounts for part of the nitrogen content of BSF larvae and this should be taken into account when calculating the BSF larvae protein content (Fuso *et al.*, 2021). Larval crude fat is strongly affected by the feed composition and varies over a wide range (6.6-39.2% DM) depending on the dietary composition (Barragan-Fonseca *et al.*, 2017). Based on the results of several studies, BSF larvae have the ability to accumulate high amounts of total fat in their body irrespective of the low-fat diet content (Barbi *et al.*, 2020; Ewald *et al.*, 2020; Meneguz *et al.*, 2018; Spranghers *et al.*, 2017). The regulation of BSF larvae composition through the feed offers a valuable tool for the manipulation of their composition and the production of larvae with varying nutrient content for specific feed applications (Oonincx and Finke, 2021). For instance, Ferrari *et al.* (2022) fed BSF larvae with seaweed and other selenium-rich substrates and produced pre-pupae fortified with selenium, an essential trace element for farmed animals. Similarly, Oonincx *et al.* (2020) fed BSF larvae with diets enriched with flaxseed oil, a rich in omega-3 fatty acids (FA) ingredient, and reported increased omega-3 polyunsaturated fatty acids (PUFA) concentrations, particularly that of alpha-linolenic acid, and decreased omega-6/omega-3 ratios to levels close to the ones considered optimal for human health. Along the same lines, a number of studies has investigated the feed effect on the fatty acid (FA) composition of BSF larvae, and particularly on their enrichment with omega-3 FA through their feeding on omega-3-rich sources (Barroso *et al.*, 2017; El-Dakar *et al.*, 2020; Erbland *et al.*, 2020; Ewald *et al.*, 2020; Georgescu *et al.*, 2022; Lawal *et al.*, 2021; Leong and Kutty, 2020; Liland *et al.*, 2017; Meneguz *et al.*, 2018), highlighting the potential for manipulation of the BSF larval composition through the diet.

Bioaccumulation of contaminants

Apart from exerting a significant effect on insect composition, feedstock is also a crucial factor affecting the chemical safety of farmed insects. The use of substrates contaminated with heavy metals, mycotoxins, pesticides, hormones, dioxins, etc., could potentially lead

to the bioaccumulation of these contaminants to the insect body (Meyer *et al.*, 2021). In the case of BSF larvae, although only limited studies are available on their bioaccumulative potential, all evidence gathered so far suggest that heavy metals, such as cadmium, lead, zinc or arsenic, can bioaccumulate in the larval body when larvae are reared on feedstocks naturally or artificially contaminated with those heavy metals (Biancarosa *et al.*, 2018; Diener *et al.*, 2015; Proc *et al.*, 2020; Purschke *et al.*, 2017; van der Fels-Klerx *et al.*, 2016, 2020). For instance, Van der Fels-Klerx *et al.* (2020) reported bioaccumulation factors up to 20 for cadmium when BSF larvae were fed diets containing plastic or paperboard carton packaging material. In contrast, mycotoxins and pesticides do not seem to bioaccumulate in the larval body of BSF (Bosch *et al.*, 2017; Camenzuli *et al.*, 2018; Leni *et al.*, 2019; Purschke *et al.*, 2017; Gold *et al.*, 2023). A critical review of the bioaccumulation potential of various chemical compounds in BSF larvae was recently provided by Lievens *et al.* (2021). As the bioaccumulation of chemical contaminants in the tissue of BSF larvae is directly related with the safety of BSF larvae as nutrient source for animal feed, e.g. see Heuel *et al.* (2023), further research is required to properly assess the risks posed by the different bioaccumulation scenarios.

3 Genetics

Contrasting a steep publication increase across various BSF research fields in the last decade, dedicated studies on the genetics of BSF emerged only very recently. Availability and exploitation of genetic and genomic resources in this insect farmed for food and feed are thus still limited (Eriksson and Picard, 2021), contrasting routine implementations in conventional livestock.

After the release of the BSF complete mitochondrial genome (Qi *et al.*, 2017), the global scale phylogeography of BSF based on sequences of the cytochrome oxidase subunit I (COI, 'barcode gene') by Ståhls *et al.* (2020) represented a pioneering milestone. Concurrent studies providing complementary samples (Khamis *et al.*, 2020), partly focusing on selected biogeographic regions such as Southeast Asia (Park *et al.*, 2017), India (Ebenezar *et al.*, 2021) or South America (Pazmiño *et al.*, 2023) complemented fascinating evolutionary patterns, that were conclusively compiled recently by Guilliet *et al.* (2022). Altogether, these investigations document almost 60 haplotypes characterized by a deep phylogenetic split of up to 4.9% COI divergence that sepa-

rates two major clades. While one clade comprises all South American origins and exhibits high and markedly structured diversity, shallower topology of the other clade captures all North American origins. Both mitochondrial clades include Central American BSF to a different extent plus non-American samples of variable geographic origin, whereas few haplotypes occur widespread outside the Americas though some supposedly non-native regions show surprisingly high local diversity. Inferring a putative cradle of the BSF within the Americas yet remained difficult because the barely diverse North American clade appears phylogenetically ancestral, whereas the highly structured South American haplotype topology renders merely recent radiation there unlikely. Such patterns may point to a cryptic species complex within the BSF, with paramount implications (see below) for the sector. The comprehensive global population genetic study based on less conserved nuclear markers by Kaya *et al.* (2021) profoundly recorded the evolutionary history of the BSF. This seminal work refined the BSF genetic hot spots are located in central-eastern South America, and that from there ancient northwards range expansions across the Americas followed that are characterized by distinct genetic cluster formation along geographic gradients. Further, by overcoming constraints of single maternally inherited markers (Guilliet *et al.*, 2022; Ståhls *et al.*, 2020), microsatellites elucidated historic and contemporary demographic trajectories to non-native regions outside the Americas (Kaya *et al.*, 2021) that shaped the BSF's cosmopolitan distribution. Only a few colonizations, e.g. West Africa and the Mediterranean, were traced to single sources. Instead, most naturalizations across large geographic scales, such as entire Australasia, south-eastern Africa and Central Europe were initiated via decisive admixture events between distinct clusters from different indigenous and adventive regions. All reconstructed non-native dispersal routes appear human-mediated and not older than one or few centuries. Yet they gave rise to unique global population genetic structure capturing substantial variation across and differentiation between 16 distinct clusters. Conversely, only a limited number of closely related BSF strains that show signatures of domestication and go back to a south-eastern North American origin is used by the majority of BSF farms virtually worldwide (Kaya *et al.*, 2021). Evidence of occasionally massive introgression emanating from mass reared BSF into local wild populations may indicate the need for monitoring (Guilliet *et al.*, 2022; Kaya *et al.*, 2021). Several of these mentioned patterns have recently been corroborated by

Generalovic *et al.* (2023) using genomics approaches. Importantly, this study also highlighted geographic patterns of mito-nuclear discordance for American BSF that could point at an ancient hybridization event between two formerly isolated lineages after the formation of the Isthmus of Panama. Future studies may resolve to which extent nuclear genetic introgression from South American invaders into a putative North American sister-lineage, or an extinct ancestor thereof, might have taken place, and how this may have shaped the genetic makeup of presently widely farmed domesticated populations.

The first full genome sequence was published by Zhan *et al.* (2020). This paper identified BSF gene orthology falling basal to the suborder of Brachycera within the order of Diptera, and further highlighted a number of rapidly evolved pathways in BSF. Substantially expanded gene families involved amino acid metabolism, detoxification, chemoreception, immune system and regulatory modules, jointly reflecting key cascades for environmental interactions and adaptation. Specifically, immune signaling pathways, including genes encoding antimicrobial peptides (AMPs), exhibit substantial expansions compared to other Dipterans, and are considered to facilitate responses to diverse challenges in pathogen-rich environments. Similar patterns were observed for detoxification genes, notably the cytochrome P450 family, and chemoreception and pheromone signaling inferred to endorse oviposition site perception, feed avoidance, and the BSF's complex mating behavior. Shortly after, an even higher resolved chromosome-level genome assembly followed (Generalovic *et al.*, 2021). Comparison of the two genomes identified some insertions and deletions tentatively suggestive of chromosomal rearrangements that might be congruent with cryptic taxa mirrored also at the nuclear genetic level. In addition to six autosomes Generalovic *et al.* (2021) identified sex chromosomes indicating a XY sex-determining system in the BSF, which was later specified to depend on an *intersex* homolog largely conserved within Brachycera (Chen *et al.*, 2023). Presumably, similar to mechanisms in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), *intersex* interacts with *doublesex female* to produce female-specific phenotypes, but further work is needed to characterize effective transducers and downstream effectors (Chen *et al.*, 2023). Relative contributions of drift (such as founder-effects) and effective selection to genomic signatures of domestication are notoriously difficult to disentangle, but as exemplified by Generalovic *et al.* (2021, 2023) inspecting structures of runs of homozygosity and pre-

dicting functions of candidate genes highlighted by potential selective sweeps will ensure further insights in the light of increasing genomic resources.

In this sense, transcriptomics can consolidate candidate genes causally involved in functional mechanisms. Yet, in the absence of meaningful hypotheses, e.g. supported by genome sequences or robust empirical data, defining target life stages and tissues can be challenging. Moreover, comparison to appropriate reference genes for quantifying differential gene expression is imperative (Gao *et al.*, 2019a). An excellent example of whole-transcriptome (RNA-seq) analyses for BSF differential gene expression over two generations in two different selection regimes was provided by Hull *et al.* (2023). They illustrate temporal dynamics in differential gene expression according to selective pressure and characterize the functional pathways of candidate genes responding to selection for larval weight. Genes associated with this trait responded over time, involving positive selection on metabolic pathways, such as protein, fat and carbohydrate metabolism and negative selection on defense pathways (P450). This suggests higher energy assimilation for increased growth and defense mechanisms may be antagonistic phenotypes, but not necessarily mutually exclusive targets for combined selection. Interestingly, while initial intra-population plasticity appeared to be mirrored to some extent in expression patterns, variation thereof across individuals decreased over time, suggesting that both unintended adaptation and directional selection can drive allelic homogenization (Hull *et al.*, 2023).

Specific pathways have already been explored through targeted gene expression profiling. By investigating antennal transcriptomes, Xu *et al.* (2020) identified chemosensory genes of different classes and highlight a frequent lack of ortholog Dipteran amino acid sequences plus extended phylogenetically recent gene duplications resulting in many species-specific clades that likely evolved during ecological niche adaptation. Interesting sex-specific expression patterns were described for several genes (Xu *et al.*, 2020), suggesting roles in female oviposition, differentiated pheromone detection in either sex, and male-male/female interactions during lekking.

Some initiatives addressed transcriptomics related to lipid metabolism pathways, thus providing a reasonable understanding of the molecular regulatory basis of a trait as complex as fat accumulation. Dynamics of life stage dependent expression profiles for numerous candidate genes and their functional characteristics were provided (Zhu *et al.*, 2019) and later corroborated and

extended for selected genes and developmental stages (Giannetto *et al.*, 2020). Recently, the relevance of tissue for monitoring specific genes involved in BSF lipid metabolism pathways has been stressed, as well as far-reaching consequences of pinpointed ribonucleic acid (RNA) interference in fatty acid synthesis on multiple biological processes (Peng *et al.*, 2023).

The major antimicrobial peptide (AMP) pathway and its role in nutritional immunology has also been investigated by several studies. These key components of innate immune defense synthesized within BSF larval fat bodies are remarkably diverse and subject to diet-borne alteration of expression profiles supposed to provide protection against specifically encountered spectra of pathogenic bacteria, as first described by Vogel *et al.* (2018). A comprehensive characterization of putatively active peptides across different AMP classes, including defensins, cecropins, attacins and lysozymes, and their predicted functional roles followed (Moretta *et al.*, 2020), as well as more specific applications on differential expression of selected AMPs, e.g. for inferring relative influences of diet and developmental stage for immune stimulation (Candian *et al.*, 2023).

Profiling microRNAs could offer another approach in the context of possible modulations of BSF mass rearing and product properties via manipulating regulatory pathways (DeRaedt *et al.*, 2022), supposed our knowledge of individual functions and specificities will improve. To this end, fundamental research on BSF gene editing has also been explored (DeRaedt *et al.*, 2022; Zhan *et al.*, 2020), yet so far targeted phenotypes remain principal academic examples defying applied relevance. Irrespective of more tangible progress there, enhancing BSF genomic resources based on overtly existing (natural) diversity and (combined) dedicated phenotyping efforts is advised for adapting classical breeding concepts, such as basic population genetic monitoring, marker-assisted selective breeding and genomic prediction.

For instance, monitoring genetic makeups of newly established founder populations to counteract initial bottlenecks or subsequent management-induced drift is key, given that any artificial regime exerts also unintended selective pressure with consequences on segregating allelic diversity up to fitness-relevant inbreeding rates and population collapse at worst (Rhode *et al.*, 2020). While deploying basic genetic markers to mitigate adverse effects during establishing wild-sourced BSF naïve to controlled environments is arguably recommended (Rhode *et al.*, 2020), other studies notably found no indication for inbreeding depression not

even in populations with documented severe bottlenecks (Generalovic *et al.*, 2021, 2023; Kaya *et al.*, 2021). Conversely, outbreeding depression could pose a veritable challenge to sustained breeding. The founder population of Rhode *et al.* (2020) comprised differentiated gene pools by chance. Apart from the documented successful hybridization between genetically distant populations (Ståhls *et al.*, 2020), significant deviations from genetic equilibrium across nearly all hybrid strains between North American and Asian clusters was intriguing (Kaya *et al.*, 2021). This raises suspects of constant incompatibility-derived trade-offs, possibly due to structural genomic variation (Generalovic *et al.*, 2021) or other forces that disrupt co-adapted gene complexes in certain backcrosses (Kaya *et al.*, 2021). Yet, regardless of putative genomic causes, the previously speculated reinforcement by positive assortative mating of more closely related individuals in mass rearing of BSF (Kaya *et al.*, 2021) was indeed convincingly proven by Hoffmann *et al.* (2021), despite their coinciding evidence for multiple paternity seemingly contributing to most sibling cohorts. Maintaining adequate diversity in BSF populations under consideration of actual mating systems and appropriately controlling fates of key population genetic measures is no complicated but still widely ignored task, with eventually huge implications on trait performance and fitness (Hoffmann *et al.*, 2021; Rhode *et al.*, 2020). However, although natural global colonization routes of the BSF feature interspecific admixture as a key driver for successful diversified ecological adaptation (Kaya *et al.*, 2021), routinely implemented outcrossing like in other livestock may not be generally advised for this insect, particularly in combination with prevalently farmed North American domesticated strains. Moreover, while captive populations in semi-artificial contexts may benefit from dosed introgression in case of superiority of locally adapted wild populations, occasional outcrossing to restore fitness in entirely closed mass-rearing settings might conflict with genetic improvement for desired traits. Selective inbreeding was indeed shown to result in increased performance for fitness-relevant strains such as egg production and adult eclosion, while not affecting larval growth and adult sex ratios (Cai *et al.*, 2022). Yet, lower survival until the pupal stage (Cai *et al.*, 2022) (suggests such effects may be temporary only, and without accompanying genetic data it remains unclear if comparatively improved performance upon recurrent inbreeding was merely influenced by eradicating outbreeding burdens in a hybrid strain (Kaya *et al.*, 2021).

Emerging complex selection approaches revealed reasonable heritability suggesting that considerable performance gain can be achieved for commercially interesting traits like larval weight and crude protein or crude fat contents within relatively short periods (Facchini *et al.*, 2022).

More studies of this kind would be valuable, including more transparent trait definitions, e.g. larval weights versus effects on developmental time, as well as genetic data to track correlating changes and evaluate quantitative trait loci and more complex genetic architectures. This can be accomplished using genomic resources (Generalovic *et al.*, 2021, 2023), such as resequencing-based SNP-calling in the lack of meaningful SNP-panels, via transcriptome analyses, or a combination of both. An appealing case-setting for selection-informed gene expression profiling has been summarized above (Hull *et al.*, 2023). Facchini *et al.* (2022) yet correctly point out that some commercially relevant phenotypes, such as more favorable feed conversion rates, should be considered composite traits, hence requiring multi-trait selection approaches that account for putative positive or negative correlations.

Crucially, any future breeding program requires being tailored to specific environments. Alleged routine management practices should not be underestimated. Unintended selective forces or unnoticed parameter fluctuation, vitally including the adult part of BSF rearing cycles, may jeopardize breeding progress, falsify candidate genes due to hitchhiking, or mask trait heritability through excessive plasticity responses. From a practical perspective, the readily controlled factor larval diet receives most attention. Besides unsurprisingly overarching diet effects on larval growth performance, feed conversion and body composition traits, Sandrock *et al.* (2022) report pronounced genotype-by-diet ($G \times D$) interactions for virtually all investigated traits based on a fully crossed design of rearing four genetically distinct BSF strains on three nutritionally different diets. Comparable $G \times D$ interactions were impressively demonstrated by Greenwood *et al.* (2021). Both studies (Greenwood *et al.*, 2021; Sandrock *et al.*, 2022) corroborate phenotyping naturally evolved BSF genetic diversity can be a solid alternative for outlining breeding programs compared to domesticated strains, which may suffer from compromised adaptive responses after decades of intense (intended or unintended) selection (Generalovic *et al.*, 2021, 2023; Kaya *et al.*, 2021). However, the previously implied link between selection for larval weight and upregulation of genes involved in glycerol synthesis (associated with insect cold stress) (Hull *et al.*,

2023) could hold a mechanistic reason for this distinct representative from temperate North American dominating BSF farms worldwide.

4 Insights in the gut microbiota and physiology

Two other important parameters, aside from BSF genetics, are key to understanding and optimizing BSF larval performance on different diets: the gut physiology, including digestive processes/functions (e.g. water flows, enzymes and activities in different regions, etc.), and gut microbiota. While only six years have passed since one of our co-authors summarized the (then) existing knowledge on the dynamics and functionality of the latter (De Smet *et al.*, 2018), the speed of new research being generated in those years has been astonishing. As predicted, these new studies on the microbiota produced both interesting insights and new questions. Knowledge on the digestive capabilities of BSF larvae is even more in its infancy. In fact, apart from the description of two gut serine proteases (Kim *et al.*, 2011), the BSF digestive system has been neglected until 2019, when Bonelli and collaborators reported the first characterization of the larval midgut of this insect (Bonelli *et al.*, 2019). Therefore, the most recent insights into both aspects will be discussed in some detail here.

BSF gut physiology and microbiota

The midgut is the central and largest region of the insect alimentary canal, which is involved in nutrient digestion and absorption (Caccia *et al.*, 2019). In BSF larvae, this organ represents the core unit of the bioconversion process and is largely responsible for the extraordinary dietary plasticity of the larvae. Accordingly, the midgut is characterized by a high morphofunctional specialization and regionalization since it is formed by three districts, each with peculiar features, pH, and functions (Bonelli *et al.*, 2019). Interestingly, every midgut district is equipped with a specific microbial community, which derives from a progressive selection of bacteria along the antero-posterior axis of the gut, that contributes to digestive activity (Bruno *et al.*, 2019b). Similar to other holometabolous insects (Tettamanti and Casartelli, 2019), BSF larval midgut undergoes a significant rearrangement during metamorphosis and is completely replaced by a new one in the fly (Bruno *et al.*, 2019a). Interestingly, the fly gut is functional and endowed with digestive capabilities (Bruno *et al.*, 2019a), thus subverting the common belief that adult BSF does not feed (Bruno *et al.*, 2019a) and open-

ing valuable opportunities, as discussed in the first section, on the modulation of adult longevity and oviposition through the nourishment provided (Bertinetti *et al.*, 2019; Bruno *et al.*, 2019a; Nakamura *et al.*, 2016).

While the morphological and functional complexity of the midgut plays an important role in the capability of BSF larvae to grow on a wide variety of organic waste, three additional factors deserve attention as well. The first is the functional plasticity of this organ, since the larvae can modulate their digestive activity and nutrient absorption, through post-ingestion mechanisms, to adapt to nutritionally poor substrates and match their nutritional requirements (Bonelli *et al.*, 2020). Secondly, BSF larvae have well developed mouthparts that support them in the ingestion of semisolid substrates with different texture and moisture content (Bruno *et al.*, 2020; Lievens *et al.*, 2023). The last factor is the associated microbiota in the (mid)gut that can provide metabolic products by decomposing macronutrients.

In fact, aiding in digestion is just one way by which the associated microbiota is known to affect insect health and fitness (Ridley *et al.*, 2012; Singh *et al.*, 2019) and research over the last decade did show that BSF larvae are no exception (Mannaa *et al.*, 2024). The absence of a microbiota was found to trigger extensive changes in transcriptional profiles of BSF larvae during development, indicating its vital role in normal host functional activity through microbiota-dependent co-expression of multiple BSF genes across key pathways (Auger *et al.*, 2023). Other examples of processes in BSF that are affected by the presence of microbes in general, or of specific isolates, are oviposition in the adult stage (Zheng *et al.*, 2013), immunity in the larvae (Auger *et al.*, 2023), and waste reduction by the larvae (Mazza *et al.*, 2020). Hence, it would be best from a production point of view to ensure a stable microbiota consisting of such beneficial microbes. In line with this, Klammersteiner *et al.* (2020) found that the type of diet could play a lesser role in guts of BSF larvae than previously assumed and that, instead a stable autochthonous collection of bacteria (*Actinomyces* spp., *Dysgonomonas* spp., and *Enterococcus* spp. as main members of this community) provides the tools for degrading of a broad range of substrates (Engel and Moran, 2013). However, the past decade has generated many studies that used metagenomics to map the microbial community either in the whole BSF larvae or specifically in their gut and its dynamics in response to a wide range of parameters. These studies revealed a mixed message. On one hand a recent meta-analysis of eleven studies using 16S rRNA gene sequencing did reveal the presence of several core

bacterial genera in this community: *Enterococcus*, *Klebsiella*, *Morganella*, *Providencia*, and *Scrofmicrobium*. On the other hand, the overall structure of the community varies clearly depending on a range of parameters such as diet (Bruno *et al.*, 2019b; Tanga *et al.*, 2021), rearing temperature (Raimondi *et al.*, 2020), developmental stage (Querejeta *et al.*, 2023), gut region (Bruno *et al.*, 2019b), genetics (Greenwood *et al.*, 2021) and rearing facility (Gorrens *et al.*, 2022).

This plasticity due to changing environmental conditions can also be a major risk as it has been revealed that, for example, nutrient restrictions can result in stress-associated dysbiosis of the microbiota (Marasco *et al.*, 2022). Auger *et al.* (2023) recently offered interesting insights from a microbial ecology perspective and pose that these are observations of an adaptive dysbiosis, where dietary changes trigger a new balancing in the BSF larvae microbiota between less-adapted pioneering communities inherited from the parents and more adapted microbes ingested from the diet. Nevertheless, such a disruption of the normal gut microbiome network entails significant risks to health and fitness, as it reduces the resilience of the microbiome to new stresses (Sommer *et al.*, 2017). For example, Clark *et al.* (2015) could link dysbiosis of the intestinal microbiota to intestinal barrier dysfunctioning and mortality in *Drosophila*. This stresses once more to consider the need to establish a stable microbiota with beneficial microorganisms during the industrial rearing process at the right developmental stage and dependent on the utilized substrate. This does require a clear understanding of which are key members of the microbiota and what their role is, especially the latter is often uncharted terrain. To address this, several groups have been generating, identifying, and characterizing microbial isolate collections from the BSF larvae (gut) over the past decade (Callegari *et al.*, 2020; Cifuentes *et al.*, 2022; Gorrens *et al.*, 2021; Klüber *et al.*, 2022; Tegtmeier *et al.*, 2021). These studies largely retrieved the same core members as identified by the metagenomic studies. Callegari *et al.* (2020) determined the hydrolytic profile of their collection, and one interesting observation was the widespread ability (16.1% of collection, including *Providencia*) to degrade uric acid, the main nitrogenous waste compound excreted by Malpighian tubules into the insect hindgut (Liu *et al.*, 2022). Nevertheless, this functional characterization of associated microbes will be a key research domain in the decade to come.

5 Application of BSF for feed

Considering the interesting content in terms of protein and lipids, a large number of *in vivo* studies on monogastric animals such as pigs, poultry, and fish has been carried out in the last decade. A critical review of the principal results obtained in terms of growth performance, nutrient digestibility, product quality and gut health related to the above-mentioned animal species, is presented.

In weaning pigs, both full-fat black soldier fly (BSF) larvae meal and defatted BSF larvae has been tested, and generally, no negative effects were reported on blood profile, nutrient digestibility, small intestinal morphology, or histological features, as highlighted in a study where pigs fed the control diet with toasted soybeans were compared to animals fed 8% full-fat BSF prepupae (Ether Extract, 41%) or 5.4% defatted BSF prepupae (Ether Extract, 8%) (Spranghers *et al.*, 2018).

In hybrid grower pigs (crossbreeds of purebred Large White and Landrace) (Chia *et al.*, 2019) reported no difference in growth performance, red blood cell indices, and white blood cell when 100% of fishmeal (10% of inclusion) was replaced with BSF larvae meal. However, in finishing pigs (crossbreeds of purebred Large White and Landrace) (Chia *et al.*, 2021) carcass weight feeding animals with increasing level of BSF larvae meal (9 to 14%) replacing 50 to 100% of fish meal, increased.

In terms of meat quality, (Altmann *et al.*, 2019) showed that instrumental tenderness, cooking loss, and 2-thiobarbituric acid reactive substances value, were not affected in the longissimus muscle of pigs fed partly defatted BSF larvae meal in substitution of 50 or 75% of soybean meal. Herrera *et al.* (2022) have investigated the effect of replacing soybean meal with BSF larvae meal in the guinea pig diets, and found no significant differences in the protein content and amino acid profile of meat nor in the n-6:n-3 and polyunsaturated/saturated ratios, however the results showed an augmentation in the desirable fats (mono- and polyunsaturated fatty acids) in the guinea pigs fed with BSF larvae meal.

In terms of gut microbiota, in weaned pigs a diet containing BSF larvae meal (10%) showed a positive influence on cecal microbiota when compared to the control diet. This was observed by an increase in short-chain fatty acids-producing bacteria (Biasato *et al.*, 2020). Additionally, in growing pigs, the consumption of dietary BSF larvae meal resulted in a more enriched small intestinal microbiome compared to dietary Soybean meal (SBM), and this change did not cause any systemic inflammatory response (Kar *et al.*, 2021). The

colonic microbiota of finishing pigs benefited from a supplementation of 4% BSF larvae meal leading to an increased abundance of *Lactobacillus* and butyrate-producing bacteria. Consequently, there was an increase of short-chain fatty acids concentrations in colonic digesta with potential beneficial impact on animal health (Yu *et al.*, 2019).

As far as poultry feed is concerned, as reported in nutritional trials where soybean meal and oil have been replaced by BSF larvae derived products, the use of these alternative ingredients has generally shown positive results in terms of livestock performance, without negatively impacting meat and egg quality characteristics. No changes or positive effect, such as improved feed conversion has been recorded with the inclusion of BSF larvae meal in the diet of the broiler chickens up to 10% of their total diet (Dabbou *et al.*, 2018). However, higher inclusion levels seem to have a negative impact on performances, possibly due to the presence of high levels of chitin in the BSF larvae (Altmann *et al.*, 2020). Regarding the effects of BSF larvae meal on poultry meat quality, most studies agree that its dietary inclusion does result in any change in carcass characteristics, physicochemical parameters of the meat, or its sensory quality (Heuel *et al.*, 2022b; Pieterse *et al.*, 2019).

In trials with laying hens, it has been observed that a 7.5% inclusion of defatted BSF meal (6% of fat content) leads to an increase in feed intake and feed conversion, without affecting daily egg production and egg weight in birds aged between 19 and 27 weeks. Moreover, the inclusion of BSF larvae meal has shown benefits such as greater eggshell thickness and increased yolk color intensity (Mwaniki *et al.*, 2018). Similar interesting results were also reported by Secci *et al.* (2018), in a study involving chicken fed diets containing 17% BSF larvae meal inclusion. The eggs produced showed a higher proportion of yolk and a more intense red color: Moreover, the yolks contained 11% less cholesterol than those in the control group. Even complete replacement of soybean cake by BSF meal revealed promising results (Heuel *et al.*, 2021b, 2022a), including findings for limited transfer of short-chained saturated fatty acids to egg yolk (Heuel *et al.*, 2021a).

Together with poultry, the aquaculture sector is likely the one where the largest number of studies using insects as alternative ingredients has been performed. These studies investigated ingredients and diets digestibility, animal performances and health, as well as product quality in both fresh and marine species (Hua, 2021; Liland *et al.*, 2021; Weththasinghe *et al.*, 2022). The nutrients' digestibility, and in particular the crude pro-

tein digestibility, is impacted by the level of processing (Gasco *et al.*, 2023). Indeed, Basto *et al.* (2020) performed investigations in European sea bass (*Dicentrarchus labrax*) using different insect meals and reported a crude protein digestibility 89 and of 91% for a full fat and a defatted BSF larvae meal, respectively, with results similar to the fish meal based reference diet that recorded a 93%. Also, Gasco *et al.* (2022) reported values of about 85% when assessing defatted BSF larvae meal in rainbow trout (*Onchorynchus mykiss*). In both trials the digestibility of essential amino was usually equal or even higher than the ones for other processed animal proteins, such as feather meals, blood meal and meat meal (Basto *et al.*, 2020; Gasco *et al.*, 2022). Similarly, in Atlantic salmon (*Salmo salar*), (Fisher *et al.*, 2020) found a higher crude protein digestibility for BSF larvae meal than for corn protein concentrate. Results on fish performances when fed diets containing BSF larvae meal are controversial, probably due to the different composition of the BSF meal tested or the inclusion BSF meal utilized. In Atlantic salmon, diets added with full or defatted meals reported no negative impact in fish fed up with 60% of meal/oil insect product (Belghit *et al.*, 2018, 2019; Weththasinghe *et al.*, 2021). Similarly, in sea bass no adverse effects were reported when including BSF larvae meals up to a level of 25% (Abdel-Tawwab *et al.*, 2020; Magalhães *et al.*, 2017; Mastoraki *et al.*, 2020). Moreover, the inclusion of 15% of BSF larvae meal as a substitute for plant protein in diets that entirely eliminate fish meal has demonstrated positive effects. Indeed, this inclusion has resulted in improved growth performance of sea bass, as well as enhanced feed efficiency and nutrient digestibility (Pérez-Pascual *et al.*, 2020). When it comes to feeding rainbow trout, the inclusion of BSF larvae meals at levels up to 32% has shown either an increase in performance parameters or no significant impact (Biasato *et al.*, 2022; Caimi *et al.*, 2021; Kumar *et al.*, 2021; Melenchón *et al.*, 2022; Stadlander *et al.*, 2017). However, not all results are positive. For instance, salmon fed 30% of BSF meal in substitution of 30% of herring and soybean meals showed a reduced weight gain, specific growth rate and a higher feed conversion ratio than fish fed the conventional protein-based diet using herring meal and soybean meal (Fisher *et al.*, 2020). Reduced performances and feed efficiency are also reported for seabass at 18% of BSF larvae meal inclusion (Reyes *et al.*, 2020). Differences are presumably due to the BSF larvae meal origin and/or the process applied to produce it, or the excessive presence of chitin which is not digested by fish (Gasco *et al.*, 2023). Moreover, as compared to fishmeal, BSF larvae

meal can present deficiencies in lysine and tryptophan, as well as some limitation in threonine and sulfur amino acids (Oonincx and Finke, 2021), particular care has to be paid to accurately match fish requirements when formulating.

Regarding product quality and the influence of BSF larvae meal on fish fillets, Dumas *et al.* (2018) have reported a negative linear relationship between body protein and BSF larvae meal inclusion while a positive relationship between body lipid and BSFLM inclusion, while other studies have not found significant differences (Belghit *et al.*, 2018; Borgogno *et al.*, 2017; Dumas *et al.*, 2018; Gasco *et al.*, 2019; Melenchón *et al.*, 2022). An important consideration is the impact of including insect meal in fish feeds on sensory properties (Borgogno *et al.*, 2017). This aspect is crucial, along with psychological factors, in determining consumer acceptance of fish that have been fed with insect meals (Baldi *et al.*, 2022; Mancuso *et al.*, 2016; Rumbos *et al.*, 2021). Recognizing insects as a source of protein to produce more sustainable food products holds significant importance in this context.

BSF larvae meal can also play an important role in animal health due to the presence of bioactive compounds (Gasco *et al.*, 2021). Indeed, a positive microbiota modulation (Biasato *et al.*, 2022; Leeper *et al.*, 2022) have showed enhanced antioxidant activity (Moutinho *et al.*, 2021; Stenberg *et al.*, 2019), overall immune system improvement (Abdel-Latif *et al.*, 2021; Weththasinghe *et al.*, 2022) and enhanced gut health (Kumar *et al.*, 2021; Melenchón *et al.*, 2022) in several studies carried out in a different fish species.

6 Application of BSF for food

BSF larvae is quite known for its application in the feed sector, however, there are also some cases (even though not very common) of its application for food purposes. Even historically populations in a few regions of the world were consuming BSF larvae (Wang and Shelomi, 2017). For example, the larvae were grown on fermented tapioca and consumed in a raw form with the beverage from the fermented tapioca “tapai” (Chung *et al.*, 2002; Wang and Shelomi, 2017). But even recently there have been some developments associated with the potential to use BSF larvae for food purposes. Thus, in 2018 Enorm Biofactory A/S from Denmark applied for the Novel Food authorization with BSF larvae meal (dried grounded whole BSF larvae), with planned inclusion of 10% to the bakery and snack products (Enorm Biofac-

tory A/S, 2018). Up to the publication of this article, the submission is still being processed. Furthermore, there are a few experimental trials oriented towards the application of BSF larvae flour for bakery products (Amoah *et al.*, 2023; Mshayisa *et al.*, 2022), especially for bread (González *et al.*, 2019; Montevicchi *et al.*, 2021) and tortilla chips (Widya Saraswati *et al.*, 2021). Such trials relate to the suitable physico-chemical and colloidal properties of BSF proteins (Queiroz *et al.*, 2021). While addition of BSF larvae meal could increase the protein and fat content, it also worsens the textural properties of the final product if more than 15% of ingredients are added. Similar effect was observed for the extrudates (Jorge Iñaki *et al.*, 2022), used as potential matrix to produce meat substitutes (Alam *et al.*, 2019) or using whole BSF larvae in emulsified sausages (Bessa *et al.*, 2019).

It is foreseen that use of lipids extracted from BSF larvae for food purposes should increase in the future, as they are associated with favorable properties (Loho and Lo, 2023). It is suggested that BSF larvae lipids can be suitable ingredients to substitute some fats in margarine and baked goods, following the processes of deodorizing and balancing the melting points (Delicato *et al.*, 2020; Smetana *et al.*, 2020). Lipid refining should eliminate the strong fishy and earthy aroma (Delicato *et al.*, 2020; Wang and Shelomi, 2017), making them suitable for the butter or margarine replacement in baked goods (Delicato *et al.*, 2020; Smetana *et al.*, 2020). There are also trials to use complete content of BSF larvae for the creation of milk substitute, however, are not confirmed in the recent study (Tello *et al.*, 2021).

7 Sustainability aspects of BSF chains

Although BSF larvae has proven to be a beneficial alternative protein source for reducing the greenhouse gas (GHG) emissions related with food production and consumption, there are still some knowledge gaps that need to be addressed before adopting a large-scale industrial production (Boakye-Yiadom *et al.*, 2022). Given the complexity of tasks involved in BSF value chains (e.g. processing, rearing, valorizing, breeding, operations, product processing), further research is crucial to identify their effects on mass production (Ravi *et al.*, 2020). An LCA done on production of dried BSF larvae showed a high energy attribution at the pre-pupa drying stage (39.3%) when compared with the other processing stages in the system boundary (prepupa production, bioconversion of biowaste, egg production, and production of crushed biowaste) which had the biggest

share on Global warming potential, terrestrial eutrophication, and eco-toxicity. Therefore, alternative single raw materials usage as feed and another drying method for prepupa will help minimize these impacts (Nugroho *et al.*, 2023). Another study observed that microbial metabolism of fresh feed substrate (30% bran and flour, and 70% water) without larvae was solely responsible for 34% of the total CO₂ emissions during BSF larvae rearing and suggested that each component involved in the process needs to be untwined (Parodi *et al.*, 2020). This shows that when the components contributing to the overall CO₂ emissions involved in the BSFL rearing process are detangled, new research pathways are established which could further help in limiting the total CO₂ emissions in future.

BSF rearing is accompanied by direct emissions. Various studies have quantified emissions of methane, ammonia, carbon dioxide, and nitrous oxide, although not all gases are constantly measured across all studies. (Chen *et al.*, 2019; Ermolaev *et al.*, 2019; Guo *et al.*, 2021; Mertenat *et al.*, 2019; Pang *et al.*, 2020a,b; Parodi *et al.*, 2020, 2021). These emissions are highly relevant as some (methane and nitrous oxide) contribute to global warming as greenhouse gases and others (ammonia) influence local ecosystems and public health. Moisture content (Chen *et al.*, 2019), pH (Pang *et al.*, 2020a), carbon-protein ratio (Pang *et al.*, 2020b) and the presence or absence of manure (Chen *et al.*, 2019; Pang *et al.*, 2020b; Parodi *et al.*, 2021) are all shown to have an effect.

BSF production on different diets can have a high impact and low performance (in comparison to soybean meal and fishmeal), regardless of the diets, unless production environment and/or heating source from non-renewable to renewable is changed (Beyers *et al.*, 2023). Insects produced on high quality diets for food and feed purposes have high environmental impacts, which can be lowered if insects are grown on food waste (Smetana *et al.*, 2021). During the last decade there have been quite a few studies performed on LCA of BSF application for waste treatment (Beyers *et al.*, 2023; Boakye-Yiadom *et al.*, 2022; Ferronato *et al.*, 2023; Guo *et al.*, 2021; Lalander *et al.*, 2019; Mertenat *et al.*, 2019; Mondello *et al.*, 2017; Pahmeyer *et al.*, 2022; Salomone *et al.*, 2017). They often confirm the beneficial potential of using waste as feed for BSF. For example, one LCA study in Indonesia (developing country) shows that waste treatment by BSF has 47 times lower CO₂ emissions than open windrow composting, helping in managing the increasing amount of produced waste. Methane and nitrous oxide samples were extracted and analyzed by

gas chromatography (Mertenat *et al.*, 2019). Considering codesigning of food system for a circular economy, localization of BSFL gives several solutions (Jagtap *et al.*, 2021). A study that developed a geographical model identifying the hotspots for the production of BSFL as feed and waste bioconversion processing revealed that locally produced feeds can contribute positively to the circular economy as according to a study, connectivity between supply needs of feedstocks and the related production and manufacturing needs to be localized to achieve a sustainable resource use and demand (Jagtap *et al.*, 2021; Martindale *et al.*, 2020). BSF frass with abundant plant nutrients and high-potential for income generation, also needs investigation for its full-potential (Smetana *et al.*, 2021). A study reviewing the potential of BSF larvae frass suggested a need for future research on the frass post-treatment processes impacts, bio stimulants and plant growth-promoting rhizobacteria and fungi and substance formations and others (Lopes *et al.*, 2022). A study also observed that BSF larvae frass from a formulated diet of fruit and vegetable components (39% fruits, 36% vegetables, 15% bread and 10% spent brewer's grain) with balanced amount of protein, lipid and carbohydrates and reduced fiber had high macro and micronutrient value like the commercially available organic fertilizers (Arabzadeh *et al.*, 2022).

There is scope for future technological advancements in real-time gas monitoring during BSF larvae production since direct GHG emissions might represent a considerable amount in overall life cycle emissions and factors involved in changing amounts such as insect diet quality and other breeding conditions (Boakye-Yiadom *et al.*, 2022). Moreover, the direct GHG emissions might be associated with the substrate changes. For example, a study found that increasing the pH of the food waste (FW) used as substrate is beneficial in reducing environmental footprint by recycling C and N from FW to BSF larvae biomass (Pang *et al.*, 2020a). Such an example demonstrates a trend for the research needed to define the niche of BFS production according to the function of the production system: for the waste treatment, for food or feed production or mixed options.

A range of life cycle assessments (LCAs) were recently completed for different production technologies of BSF larvae (Ites *et al.*, 2020), feed and food products (Smetana *et al.*, 2016, 2019, 2020), and application of different LCA approaches (Boakye-Yiadom *et al.*, 2022; Siegrist *et al.*, 2023; Smetana, 2023; Smetana *et al.*, 2019; Spykman *et al.*, 2021). An interesting approach was applied to the LCA by employing a modular modelling approach of BSF larvae production chains (Spyk-

man *et al.*, 2021). It provided a possibility to generate multiple scenarios, adjusting one or few modules, interconnected via developed coefficients. The study opened new opportunities for the quick assessment of environmental impacts and interconnection with economic efficiency. It was further used as a basis for multi-objective optimization of BSF larvae production and identification optimal sustainable insect production chains for different regions (Mouhrim *et al.*, 2022) but also for whole Europe (Smetana *et al.*, 2023). Due to a wide range of uncertainties in LCA studies using different functional units, processes, techniques, system boundaries, allocation and impact assessment methods, there is a need for product category rule (PCR) guidelines for insect products with the same functionality (Boakye-Yiadom *et al.*, 2022; Siegrist *et al.*, 2023). Moreover, specific methods for circularity potential estimation and quantification of insect chains are envisioned to be developed in the near future (Smetana, 2023). Such methods will be beneficial to determine the potential of insects for the recovery and return of nutrients in the food system.

8 Outlook for the next decade of research

It is clear that research during this last decade has unveiled key new findings in a range of domains and is, at a rapid pace, elevating our understanding of this insect species while also triggering new research questions. First, it can be expected that future research will continue to target the nutritional requirements of BSF larvae, providing a systematic overview of connections between complex compositions of feeds and nutritional profile of insects. Moreover, the research on impact of insect nutrients included in animal feed will continue to define the synergetic and antagonistic connections between insect composition and livestock wellbeing. Insect processing such as fractionation (separation of fat, chitin, proteins, etc.) will also be a focus of the research defining the potential for the enhancement of target properties useful for feed and food industries.

The few existing studies on BSF genetics used closely related genetically uniform strains (Kaya *et al.*, 2021). As a result, present insights into phenotypic variation and associated estimates for genetic parameters and correlations tend to be specific to these populations. Many technical aspects and research goals demand being tackled to achieve BSF breeding progress comparable to conventional livestock sectors (Jensen *et al.*, 2017). Different genetic markers and genomic approaches per-

mit addressing questions of variable complexity, ranging from basic population genetic monitoring to elucidating genetic architectures of traits for optimizing desired phenotypes (Eriksson and Picard, 2021). Accumulating genomic resources from diverse populations will elevate marker density and, in conjunction with functional genomics studies, facilitate the identification of candidate genes responding to selection, ultimately improving sustainability and efficiency of BSF farming alike. Research on symbiotic interactions of *H. illucens* and microorganisms both in the insect but also in environment will continue in the next decade, concentrating on the possibilities for the feed pretreatment, insect-microbiome co-digestion of unfavorable feeds and wastes and utilization of microorganisms for the biotransformation of insect biomass to reach the required properties.

For example, the main digestive features of BSF larvae and the composition of their gut microorganisms have been characterized and revealed for both plasticity depending on many (a)biotic parameters, like the diet. This convinced the BSF community of the importance of knowledge on these aspects to improve insect performance and productivity, as reflected by the exponential increase of publications on this topic (Tettamanti *et al.*, 2022). As far as BSF larvae substrate digestibility is concerned, a very recent study proposes asymptotic Estimated Digestibility (ED) as a new indicator corresponding to the maximal fraction of initial macronutrients that are not in the frass at the end of the feeding period (Guillaume *et al.*, 2023). Asymptotic ED corresponds to the maximal proportion of each macronutrient that BSFL and associated microbial community can potentially digest. Therefore, crude macronutrient content multiplied by the asymptotic ED of that macronutrient should be considered for each ingredient, rather than the sole crude macronutrient content, when formulating a diet for BSFL based on several ingredients. These authors propose further studies focused on determining asymptotic ED of macronutrients in different types of ingredients that could be used as BSF larvae feed (such as agricultural side-streams, food waste or animal manure) and evaluate the effect of rearing conditions on asymptotic ED. However, some major gaps persist: (1) the unpredictable or variable exploitation of different organic wastes and byproducts by the larvae. This point could be, at least partially, addressed thanks to the development of ad hoc *in vitro* gut digestion models that are able to simulate the complexity of the BSF larvae digestive system and predict the outcomes of *in vivo* feeding experiments (Gold *et al.*, 2020).

Recognizing that doing a feeding experiment will likely be still much easier to determine performance on variable wastes than a complex *in vitro* digestion in the lab; (2) the role of the BSF larvae digestive system in reducing foodborne pathogens, with the consideration of the accumulation and concentration pathways in the body of BSF larvae and treated substrate; (3) the relationship between nutrient digestion/absorption, and the accumulation of long-term storage molecules in the larva, systemic immunity, reproduction, etc.; (4) the composition of the fungal and viral community is, respectively, less and hardly studied to data, while they also determine both the functionality and plasticity of the whole microorganisms; and (5) the mechanisms by which specific members of the microorganisms are able to persist throughout the life cycle and on different diets. In short, the research in the last decade has focused on mapping the BSF gut physiology and microorganisms in varying conditions. Fundamental research in the next decade will hopefully transition our field from mapping to understanding the underlying mechanisms that dictate these interactions, so we can start using these insights to positively impact BSF performance.

The leading trend of research on BSF gut and related applications for the next decade could deal with the five following aspects. (1) A better knowledge on BSF gut physiology, microorganisms, and nutrition, as well as the possibility to trace causal cascades through ribonucleic acid interference (RNAi) and Clustered regularly interspaced palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) tools in this insect (Kou *et al.*, 2023; Zhan *et al.*, 2020), could establish BSF as a reference model among Diptera for digestion-related studies. (2) Future research should further target the mapping of the genome-wide diversity present in the global BSF population (Kaya *et al.*, 2021) combined with genomic selection within dedicated breeding schemes could play a key role in promoting superior and/or purpose-tailored BSF strains (Eriksson and Picard, 2021). (3) A fine characterization of BSF gut physiology could not only favor the use of larvae for the reduction and valorization of tricky, e.g. fiber-rich, substrates (underexploited so far), but also promote the isolation of new enzyme activities and bioactive compounds, likely associated to the gut microbial community, that are relevant from a biotechnological or medical perspective. (4) The exploration of the relation between the microorganisms and the immunity could help understand recent observations that link immune activity to the absence of micro-organisms (Auger *et al.*, 2023) and help develop strategies to boost larval immunity dur-

ing processing. (5) The understanding of the functional roles of specific microbes could help develop microbiological tools for feed pretreatment, insect-microbiome co-digestion of unfavorable feeds and wastes or the utilization of microorganisms for the biotransformation of insect biomass to reach the required properties.

Application of BSF for the food is currently under research and development. Despite defined suitable nutritional and physico-chemical properties, there are only a few scarce cases of *H. illucens* applications for food development. Next decade of research will tackle the lack of the studies associated with the use of lipids, proteins, or complete biomass in different foods. Moreover, specific methods for circularity potential of insect chains are envisioned to be developed in the near future (Smetana, 2023).

Acknowledgements

The research is partially supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Program for Ecological Farming and Other Forms of Sustainable Agriculture in the scope of the ERA Net CORE Organic project “Poultrysect” grant agreement 2819OE152. The authors would like to acknowledge the financial support received for this project from transnational funding bodies, partners of the H2020 ERA-NETs SUSFOOD2 and CORE Organic Cofund, under Joint SUSFOOD2/CORE Organic Call 2019, as part of the Poultrysect project (ID: 48). This paper is also supported by the EU-PRIMA program project ADVAGROMED (Prima 2021 – Sect. 2). The project is funded by the General Secretariat for Research and Innovation of the Ministry of Development and Investments of Greece under the PRIMA Programme. PRIMA is an Art.185 initiative supported and co-funded under Horizon 2020, the European Union’s Programme for Research and Innovation. And partially funded by the Federal Ministry of Education and Research (BMBF) (grant agreement 02WPM1651). This paper is also supported by the EU-PRIMA program project CIPROMED Grant Agreement No: 2231. Call 2022 Section I Agri-food IA]. CS was supported through the “Innovation Space NewFoodSystems” (Project *reKultI4Food*) funded by the German Federal Ministry of Education and Research (BMBF), grant no. 031B1206A. The paper is also supported by Fondazione Cariplo (RICH project, grant number 2020-0900) and Italian Ministry of Univer-

sity and Research PRIN 2020 (NICE-PET project, Prot. 2020ENH3NZ). De Smet, J. holds a postdoctoral fellowship (12V5222N) from the Flanders Research Foundation (FWO).

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