Quantitative analysis of mimetic traits in butterflies using deep learning: An attempt to imitate predator responses.

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Batesian and Müllerian mimicry in butterflies has long been studied, and its elaborateness is remarkable. On the other hand, in recent years, attention has also been paid to the mechanism maintaining "imperfect mimicry", thus increasing the need to "precisely evaluate the accuracy of mimicry." To achieve this, quantitative comparisons have conventionally been made through behavioral experiments, where predators are presented with prey and their responses are recorded. Alternatively, image analysis focusing on specific traits (e.g., ground color or spot color of the wing) has been employed. Because the former involves substantial costs for preparing predator species, while the latter may not accurately reflect the predator's response, there is a need for the development of an image analysis technique that imitates the predator's perception. In recent years, artificial intelligence has demonstrated amazing performance in various aspects. In particular, deep learning using convolutional neural networks (CNN) is a technology that plays a central role in the field of image analysis. CNN learns the vast features required for image classification by itself, enabling advanced similarity evaluation without human intervention. Furthermore, by refining the task to be optimized and input images used for training, it might be possible to achieve image analysis that imitates the predators' response. Here we introduce an attempt to evaluate the accuracy of mimicry from predators' perspectives with the help of CNN, using mimetic butterflies in Japan, such as *Papilio polytes* and *Hypolimnas misippus*. Specifically, we have attempted methods for (1) refining the output of CNN based on the results of predation experiments, (2) focusing on the time required by CNN for species, and (3) inputting images of prey along with their "taste" into the CNN to train it to avoid less palatable prey. In this presentation, we will discuss the validity and generality of these approaches.

Potential of Black soldier fly business in Japan, based on the Taiwan model

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In recent years, social problems such as environmental pollution and protein crisis have demanded effective food waste recycling. The saprophagous insect black soldier fly (BSF), *Hermetia illucens*, produces high-quality protein from organic waste, and hopes for its use in creating an environmentally recycling society in the future are growing. BSF recycling technology has already been implemented in Southeast Asia, in Taiwan and Vietnam, and start-up progress is also seen around the world.

Japan is lagging behind in launching start-up companies that operate on a large scale. The reasons are the high cost of energy and labor, and limited sales channels for products, which impact both production cost and market development. Therefore, we studied the feasibility of a BSF business in Japan by studying the business models of two Taiwanese private companies (A and B) already in business.

The production scale and products of A and B companies were compiled (including some speculation by the authors). Japanese waste disposal fees and product sales prices were obtained by interviews with several related companies. The results were converted into parameters per volume of residuals treated. Production costs and unit product prices were calculated for a Japanese business model.

Waste disposal volume of Taiwanese companies ranged from 90 to 150 t (expand first time)/month, but the larval feed produced varied widely from 0.5 to 9 t/month, depending on the density of larvae placed in the waste. Profit structures including production, were classified into three segments - industrial waste disposal fees, larval feed production, and plant fertilizer production. The percentages of profit for each segment were: for Company A: 30, 40 and 10; for Company B: 40, 40 and 20. Assuming a waste disposal volume of 100 t/month in Japan, it was estimated that approximately 2 t/month of larval feed and about 70 t/month of plant fertilizer would be collected. Labor costs were estimated to account for 80% of the total production cost, with almost 45% of it required for adult rearing (egg collection). While labor costs were lower in Taiwan than in Japan, the amount of waste disposal was 170-210 USD/t, which was similar to that in Japan. Thus, the Taiwanese model was more likely to establish a profitable structure.

This study revealed that BSF businesses in Taiwan differ in the number of larvae required for the different types of residues, which results in differences among product collections. It is important to carefully consider the treatment of waste residue because it greatly affects the overall stability and efficiency of the business, such as ease of treatment, larval uniformity, and odor control. In order for social implementation of the BSF technology in Japan, it was concluded that automation without manual labor, more efficient egg collection, and securing sales channels through higher value-added products will be essential.

(2,954 characters)

High-quality genome assembly of the zoophytophagous stink bug, *Nesidiocoris tenuis*, offers clues to their food habit adaptation.

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Nesidiocoris tenuis is an important natural enemy of tomato insect-pests such as whiteflies, thrips, and the tomato leaf miner. It is both carnivorous and herbivorous, which enables it to maintain an effective population density even when pest prevalence is low by relying on insectary plants until a pest outbreak occurs. However, when *N. tenuis* population becomes too dense, the bugs sometimes feed on the tomato crops themselves and damage them. This dual nature appears to originate from the predator's food preferences and chemosensory capabilities. To better understand these features and apply them to breeding techniques for this species, we have constructed a high-quality genome sequence assembly and provide comprehensive gene annotation.

For assembling a highly quality contiguous genome sequence, preparing a strain with a low heterozygosity and extracting high molecular weight DNA are essential. Therefore, we established an inbred strain from single mating pair of the Japanese commercial strain of *N.tenuis* and inbred the resulting population for ten generations. Genomic DNA was extracted using a gravity-flow column, and longer HMW DNA strands were isolated through centrifugation-based size selection. We sequenced these DNA strands using a long-read sequencer (the Oxford Nanopore Technology MinION), assembled the resultant sequence reads, and scaffolded them based on chromosome conformation captured by Hi-C. This constructed genome was structurally annotated based on protein homology predictions from closely related species and transcriptome analysis. Finally, we examined food habit adaptations by comparing our constructed genome with those of zoophytophagous, phytophagous, and hematophagous hemipterans.

We obtained a highly continuous (scaffold N50 of 16.4 Mb) and high quality (BUSCO completeness of 94.6%) genome sequence assembly, comprising of 20 scaffolds. Of these, 17 longest scaffolds cover 99.5% of the whole genome sequence reads and align with previously reported karyotype of this species. In addition, we unexpectedly obtained as byproducts the complete genome of symbiotic microorganisms, confirming that *N. tenuis* harbors both *Spiroplasma* (Molicutes) and *Rickettsia* (Alphaproteobacteria). Our phylogenetic analysis of chemosensory receptor genes showed that orthologous olfactory receptor genes are commonly shared among closely related species irrespective of their food habits. In contrast, gustatory receptor (GR) genes appear to be more species-specific, suggesting that each species tends to develop unique GRs that align with its specific diet. These results suggest that the gustatory gene set plays a pivotal role in adaptation of food habits and can be a promising target for selective breeding. We believe that our chromosome-level genome analyses of *N.tenuis* paves the way for new breeding technologies in insect natural enemies using genomics and genome editing technologies.

(2,934 characters)

Vertical Movement of Black Soldier Fly Larvae and the Design of Mass Production Bioreactors

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Our research is focused on the mass cultivation of black soldier flies (*Hermetia illucens*), in the context of their role in food waste disposal and their potential as a sustainable alternative protein source. The larvae of black soldier flies have gained particular attention for their ability to convert food waste into protein resources. However, the current rearing methods rely on manual labor, limiting both the quantity of food waste processed and the scalability of production. Our study is aimed at improving black soldier fly rearing by designing and constructing a bioreactor that accommodates large-scale breeding, optimizes larval growth, and facilitates efficient harvest of the final products within the food waste processing cycle.

Traditional methods employ stacked trays for rearing larvae. For developing a large-scale bioreactor, the first aim is to monitor the vertical movement of larvae for determining the optimal height of the bioreactor. For this purpose, we have designed a unique rectangular chamber with specific features that are suited for observing and analyzing the vertical behavior of black soldier fly larvae.

Our chamber is equipped with an air pump for air circulation, a Raspberry Pi system for recording temperature and humidity, and an air sensor for detecting air composition. The system also includes a video setup to capture images for analyzing larval movement and an image analysis system (using YOLOv8) to automatically detect larval behavior for the identification and analysis of various larval density regions. This whole system will enable us to study not only the impact of various feed compositions on larval growth but also monitor air quality within the chamber to evaluate its potential influence on larval survival rates and weight. Thus, our holistic approach is designed to facilitate both the identification of optimal feed compositions and the assessment of overall larval productivity efficiency.

Preliminary results show several patterns of larval movement across the chamber and suggest the potential for rearing black soldier fly larvae in an elevated chamber. Planning is underway to evaluate the capability of the larvae detection equipment to observe larval movement. With these preliminary results on hand, our next phase of the research will center on scaling up the chamber to bioreactor dimensions for mimicking practical applications. We anticipate these experiments to involve testing of shapes such as cubes or cylinders. The design of the large-scale bioreactor will be intricately linked to the larval monitoring technologies developed in this study and promises a substantial reduction in manual labor costs.

(2,684 characters)

The search for attractants controlling aggregation behavior in black soldier fly larvae

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Black soldier fly larvae (BSFL) possess the remarkable capability to convert organic waste, including kitchen garbage, into insect proteins. This capability has garnered global attention, particularly as societies aim to transition towards recycling-oriented practices. To harness BSFL for protein production, there is a growing trend of rearing them in mass production facilities, commonly referred to as insect factories. However, the behavioral aspects of BSFL are poorly understood, which is impeding the quest for achieving efficient mass production.

Aggregation behavior is commonly observed in the larvae of numerous dipteran insects as it presents various advantages such as accelerated growth due to elevated temperatures and societal digestion. Our study is focused on identifying chemical cues that trigger the aggregation behavior of BSFL and understanding the mechanisms controlling this behavior.

Initially, we conducted aseptic breeding experiments with BSFL. Here, the eggs underwent meticulous sterilization in sodium hypochlorite solution and were maintained in microtubes until hatching to obtain germfree larvae. Next, we conducted experiments to determine the larvae's preference between pre-colonized artificial food, on which they are typically reared, and fresh artificial food. For this purpose, we used two distinct sets of larvae: those reared on regular artificial food under the normal condition (non-aseptic) and those reared under the aseptic conditions (described above). For assessing BSFL food preference, we set up these ‘choice’ experiments within a circular field measuring 15 cm in diameter. Ten larvae were placed at the center of the field, and we recorded their attraction to the two types of food positioned equidistant from each other. To identify the attractants, we performed chemical analyses using gas chromatography–mass spectrometry (GC–MS). We will present the results we have obtained so far.

Identification of the attractant substance(s) is only the first step toward understanding the mechanism behind the frequently observed aggregation behavior of BSFL. A better understanding of the mechanisms controlling this behavior, which offers numerous benefits to larvae, may help us devise methods to improve the efficiency of BSFL mass production process.

(2,466 characters)

Reduction of ammonia emission in black soldier fly larvae based food waste conversion system using *Thiobacillus thioparus* inoculation.

Fang Jitao, Masami Shimoda (The University of Tokyo)

The escalating global challenge of managing food waste has sparked interest in innovative and sustainable solutions. Black Soldier Fly (*Hermetia illucens*) larvae have emerged as promising agents for organic waste conversion to valuable resources such as protein-rich biomass and nutrient-rich frass. However, the processing of food waste by these larvae often results in the release of ammonia, a pungent and environmentally detrimental gas.

Thiobacillus thioparus is a species of sulfur-oxidizing bacteria renowned for its distinctive metabolic pathways, especially its ability to utilize ammonia as a nitrogen source. Thus, *T. thioparus* has the potential to assimilate ammonia and transform it into nitrate, a less volatile and more environmentally friendly chemical.

Our experimental design involved the co-cultivation of Black Soldier Fly larvae and *T. thioparus* bacteria in food waste compost. To measure the generation of ammonia, we utilized the GX-6000 Portable Multi Gas Monitor manufactured by Riken Keiki Co., Ltd., Japan. Each day, we suspended the instrument 2 centimeters above each sample for 5 minutes to collect ammonia and compared the highest values of ammonia emissions recorded within that 5-minute interval for the different samples. The results indicate that, relative to samples containing only Black Soldier Fly larvae, the samples co-cultivated with *T. thioparus* bacteria exhibited a significant reduction in the release of ammonia. We also performed weekly extractions of 2g of food waste from the different samples three times and conducted relative quantitative analysis of specific DNA from *T. thioparus* using qPCR. This information allowed us to assess the relative growth of *T. thioparus* in the different samples. Finally, we compared the growth of Black Soldier Fly larvae through weight measurements three times a week. Our results in these experiments indicate that co-cultivation had no negative impact on the growth of either *T. thioparus* bacteria or Black Soldier Fly larvae.

We conclude that *T. thioparus* inoculation has the potential to be an effective solution for mitigating ammonia emissions in the Black Soldier Fly's food waste processing system. Our next step is to develop a novel approach for reducing ammonia emissions that aligns well with the sustainable waste management goal of minimizing environmental pollution.

(2,366 characters)

Growth of black soldier fly larvae is unaffected by suppression of gut microbiome.

Rina Fukunaga, Takeshi Aizawa, Masami Shimoda (The University of Tokyo)

The Black Soldier Fly (BSF), *Hermetia illucens*, plays a crucial role in an innovative insect-based method for recycling organic waste. BSF larva's strong digestive capacity is thought to derive from its gut microbiome (GM). To explore the potential symbiotic relationship between BSF and its GM, we compared the growth of germ-free larvae with those containing natural microbial content. Previous studies on germ-free larval rearing did not include comparisons of dry weight or growth rates. As a foundational study to precisely determine the growth of germ-free larvae, the following experiment was conducted.

【Exp.1 Artificial diet feeding】 Germ-free larvae were generated by sterilizing eggs with 1% sodium hypochlorite solution and subsequently feeding them an autoclaved artificial diet. Additional germ-free larvae were prepared using antibiotics. For the control 'with-germs' treatment, food was inoculated with frass obtained from typical BSF rearing debris. Surprisingly, germ-free larvae, including those treated with antibiotics, exhibited growth rates comparable to larvae with natural microbial content. The maximum weight of germ-free larvae reached 0.43g, similar to that of larvae with natural microbes. These findings suggest that BSF larvae do not depend on the GM for normal growth, casting doubt on the GM's role in their robust digestive capacity.

【Exp.2 Household waste feeding】 In this experiment, typical household waste was introduced. We prepared the sterilized household waste by homogenizing the components of the waste with a mixer and autoclaving them. Results showed suboptimal growth compared with larvae with natural microbes. This led us to investigate how the size of the mixer-processed diet grains and external bacterial activity might influence the growth. These results prompt a closer examination of the interplay among diet characteristics, external bacterial inputs, and the growth performance of BSF larvae.

This research indicates that BSF larvae are not dependent on the GM for their normal growth and question the role of the GM in BSF's strong digestive capacity. Further research is needed to understand the complex relationships between BSF, its microbiome, and dietary factors. Such studies will be critical to improve organic waste recycling methods using BSF.

(2309 characters)

Does larval compensatory growth reaction to restricted growth experience differ from that to smaller body sizes at birth?

Sugihiko Hoshizaki (University of Tokyo)

Juvenile animals may face the challenge of inadequate nutrition, which may adversely affect subsequent body growth and possibly leads to decreases in fertility or survival. Compensatory growth (CG) often occurs to animals that are re-provided with enough nutrition after a period of restricted body growth; i.e., they can compensate for a reduced growth by increasing the growth rate during the recovery period (diet-induced CG). The diet-induced CG has been widely found in invertebrates and even vertebrates. While previous studies have reported accelerated body-growth during the recovery period, a few studies have examined the degree of body-size compensation during the recovery growth period. Furthermore, previous studies have found similar compensatory patterns of body-growth associated with factors other than the poor nutrition experience. In particular, in several insects, larvae that have relatively smaller body sizes at their birth subsequently gain more body mass during larval development (size-induced CG). Then, the size-induced CG may be a confounding factor in the test of diet-induced CG. However, few studies have distinguished the diet- and size-induced CGs. As a null hypothesis, if the two CGs share physiological mechanisms in response to the body size at the beginning of recovery growth phase, the allometric relationship between body sizes at the beginning and the end of a developmental stage is identical irrespective of the restricted growth experience.

In the present study, I tested this hypothesis using larvae of the scarab beetle *Trypoxylus dichotomus* as a study system. The egg size, i.e., neonate body size, in *T. dichotomus* is negatively associated to their mother's age. In the subsequent larval development, the size-induced CG for body size occurs for every larval instar. The diet-induced CG has not been examined in previous studies. In the laboratory condition, I separated neonates of *T. dichotomus* into two groups. Larvae of one group (control group) were fed with the humus (standard diet) during the experiment. Those of the other (treatment group) were fed with poor quality diets during instar 1 (poor growth phase), and subsequently fed with the standard diet in instar 2 (recovery growth phase). The larvae were reared individually, and their body growth was followed longitudinally. The allometric relationship of body size at the instar 2 end to that at the instar 2 beginning was compared between the treatment and control group. Preliminary analyses suggested that the treated larvae gained more body-mass than the control larvae during instar 2. Based on results, I would like to discuss the developmental effect of experiencing a transiently restricted body-growth on subsequent larval development.

Subsidiary effects of spent coffee grounds in black soldier fly production

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The black soldier fly (BSF; Helmetia illucens) feeds on a wide variety of food wastes, including kitchen scraps, and on livestock manure. BSF larvae have excellent nutritional value with potential for use as a component of livestock and fishery feeds.

Large quantities of spent coffee grounds as waste from industrial beverage manufacturers are difficult to recycle. Although the growth of BSF reared on coffee grounds alone is poor, growth on coffee grounds mixed with other food wastes is good.

In this study, we examined the use of spent coffee grounds to improve the rearing environment of BSF when mixed with breadcrumbs, bean curd, and vegetable scraps, which are unsuitable alone for rearing BSF.

Larvae reared on breadcrumbs alone developed slowly. In contrast, larvae reared on breadcrumbs mixed 3 to 1 by weight with coffee grounds developed normally, and final instar larvae were 65% larger than those fed on breadcrumbs alone. In this diet, the coffee grounds created air gaps, which helped to soften the diet's viscosity, enabling the larvae to move freely in the diet and facilitating development.

The survival rate of final instar larvae reared on bean-curd mixed 3 to 1 by weight with coffee grounds increased from 30% on bean curd alone to 95%, and average weight increased by 183%. In this diet, larvae did not move around under drier environment, and therefore fewer individuals escaped from the rearing cage.

Although larval development did not improve when coffee grounds were mixed into vegetable scraps, the number of final instar larvae was increased, suggesting a positive effect of coffee grounds on larval survival. This increase of survival rate disappeared as the variety of vegetables blended in the diet increased.

Food waste obtained from a single food plant can be unsuitable for rearing BSF larvae owing to narrow nutritional and physical properties. Our results show that spent coffee grounds can improve the environment of the diet and promote larval growth. Spent coffee grounds therefore offer promise as a dietary component in the production of BSF.

(2087 characters)

Advanced insect nets: Red-colored nets effectively control micro pest

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In recent years, micro insect pests such as thrips (Frankliniella occidentalis and F. intonsa) and whiteflies (Bemisia tabaci and Trialeurodes vaporariorum) have become common in tomato cultivation in Japan, causing damage to production yields and quality loss. F. occidentalis and F. intonsa are also known as a vector of Tomato Spotted Wilt Virus (TSWV) (Zawirska, 1976; Inoue et al., 2004), and B. tabaci is a vector of Tomato Yellow Leaf Curl Virus (TYLCY), respectively. In addition, these micro pests have been reported to have reduced susceptibility to insecticides (Fukuda et al., 1991; Nauen et al., 2002), and insecticide control is reaching its limits (Tokumaru and Hayashida, 2010).

Insect nets is one of the physical control methods. Although the control effects of mesh size of the net on the insect invasion have been reported, there are few reports about the effects of the net color. Oya et al. (2011) reported that the density of T. tabaci on cabbage covered with the red-colored nets was very low. However, there are lots of unclear points about the behavioral response of the micro pests other than T. tabaci against the red-colored nets. In this study, therefore, we examined the behavioral response of F. occidentalis, F. intonsa, and B. tabaci against the red-colored nets, and the control effectiveness of red-colored nets against the micro insect pests was also studied in tomato cultivation fields.

1. Behavioral responses of Frankliniella occidentalis, F. intonsa, and Bemisia tabaci against the red-colored nets

To evaluate the control effects of the red-colored nets against F. occidentalis, F. intonsa, and B. tabaci, we investigated the invasion rates of the pests into the 0.8 mm mesh nets on kidney bean fully covered with six types of colored nets under laboratory condition. As a result, in F. occidentalis, black-white and red-black nets significantly reduced thrips invasion to about 1/3 compared with the white nets, while in F. intonsa, red-white nets reduced thrips invasion to about 1/3 compared with the white nets, but no significant differences were found between the nets. In Bemisia tabaci, the red-black net significantly reduced whiteflies invasion to about 1/5 compared with the white nets.

2. The effectiveness of red-colored nets in controlling the micro pests in tomato fields

The effectiveness of red-colored nets in controlling micro pests was investigated in tomato greenhouses in Kyoto Prefecture, Japan. The results showed that the number of F. occidentalis and F. intonsa in greenhouses with red-white and red-red nets was about one-third and one-half of those in plots with white nets, respectively. Trialeurodes vaporariorum infestations in greenhouses with red-white and red-black nets was about one-tenth and one-twelfth of those with white nets, respectively.

These results indicate that red-colored nets are effective in controlling thrips and whiteflies.

Violet light complements biological control tools in enhancing Nesidiocoris tenuis predation potential on whiteflies in protected systems; an integrative approach to whitefly control.

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Pest control in protected systems have evolved around chemical pesticides for the obvious reason, to eliminate pests quickly and completely at a given time. However, there are consequences in using chemical pesticides, i.e. pest resistance, negative effects on beneficial insects, health and environmental impacts and the list goes on. Hence, the use of biological control or integration of biological control tools to minimize chemical pesticide use. Biological control tools are essential tools that augments and enhances biological control agents. Biological control tools include insectary/banker plants, artificial/factitious diets, pheromones/repellents, selective biopesticides, protective nets, capture/attractant devices that utilizes light, color and/or odor stimuli are but few to name that have been extensively studied to augment and enhance biological control agents. Amongst these biological control tools, attractants utilizing light and color takes the focus in this discourse. There are a variety of light wavelengths and intensities studied so far that manipulates various species of insects. In the case of natural enemies, violet light (405nm) has shown positive phototaxis on natural enemies such Orius sauteri and Nesidiocoris tenuis. The use of N. tenuis as a natural enemy of various greenhouse pests in protected systems is widely reported around the globe, mainly in the Mediterranean basin, Asia, and the North and Central America. Furthermore, N. tenuis is a polyphagous insect, and there are reports supporting N. tenuis release with banker plants to augment its predation potential on pests. However, N. tenuis seems to prefer banker plants more than crops as the banker plants provide conducive environments for it to breed and reproduce. Since, violet light has a positive phototaxis effect on N. tenuis, violet lights could be a useful tool in attracting N. tenuis on banker plants to the target cash crops. To verify this conjecture, we performed field experiments under greenhouse conditions to test the effect of violet lights in attracting N. tenuis from banker plants (sesame, cleome, verbena) to tomato plants, aided with selective pesticides and their collective effect on silverleaf whitefly (Bemisia tabaci) densities, the target greenhouse pest in this study. Before field studies, we performed toxicity assays in in-vitro conditions to identify selective pesticides compatible with N. tenuis. In-vitro results revealed that neonicotinoids and spinosyns were toxic to both N. tenuis and B. tabaci. On the other hand, Avermectins & milbemycins, pyridine azomethine derivatives and diamides were less to moderately toxic (compatible) to N. tenuis but highly toxic to B. tabaci. Separately, in the field studies, first, we confirmed that the movement of N. tenuis from banker plants to tomato plants was accelerated in the presence of violet lights. The rapid movement of N. tenuis onto tomato plants aided with occasional spraying of selective pesticides significantly reduced B. tabaci egg and nymph densities compared to control treatment. Furthermore, these densities were also relatively comparable to those in the conventional pest control treatment. Here, we will discuss the integration of violet lights with N. tenuis and banker plants in suppressing cosmopolitan pests such as B. tabaci and the collective role of violet lights, natural enemies, and banker plants in advancing integrated pest management in protected systems.

Enhancing Amino Acid Productivity and Profile in Black Soldier Fly Larvae through NAT Transporter Suppression in the Excretion System.

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Larvae of the black soldier fly (*Hermetia illucens*, BSFL) can efficiently recover protein from biodegradable wastes, making it a promising alternative to fishmeal—an essential protein source for domestic animals. However, the composition of essential amino acid (EAA) in BSFL differs from that of fishmeal and requires adjustment to meet the nutritional requirements of various animals. Given that EAAs cannot be synthesized, we aimed to modify the profile of EAAs in BSFL by inhibiting the excretory system.

To identify nutrient amino acid transporters (NATs) responsible for EAA excretion, the total RNA was isolated from different tissues including whole gut, midgut, Malpighian tubules from 5th instar larvae, and the larva with the gut removed and the heads of male and female adults for RNA sequencing (RNA-seq) analyses. Totally, 5 NATs (*HiNATt*, *HiNATg*, *HiNATh*, *HiBLOT*, and *HiSERT*) were identified and named them on the tissue specificity or functions. The *HiNATt* showed high expression in Malpighian tubules of BSFL, suggesting its role as the excretory NAT.

To confirm the function of *HiNATt*, its dsRNA was injected into 7-day-old BSFL to silence the *HiNATt* ($BSFL^{HiNATt-}$) expression, with *EGFP* dsRNA injection as a control ($BSFL^{egfp-}$) . The effects of RNAi were evaluated at 14 days post-injection. Although the survival rate of $BSFL^{HiNATt-}$ and $BSFL^{egfp-}$ was comparable, $BSFL^{HiNATt-}$ displayed a 56.2% decrease in body weight. Nevertheless, the total EAA content in $BSFL^{HiNATt-}$ was 77.3% higher than in $BSFL^{egfp-}$.Remarkably, some valuable EAAs were strongly increased such as histidine (156.8%) and valine (98.1%). These results suggest that inhibiting *HiNATt* could alter the composition of EAAs in BSFL.

The accessibility of BSFL products is influenced by the EAA content, yet fine-tuning the specific EAA content in BSFL remains a challenging task. To achieving a fishmeal-free aquaculture and animal husbandry, breeding BSF strains with improved accessibility for different purposes is imperative. This approach opens a new avenue for producing of BSF larvae with improved nutritional value and customized amino acid compositions via biotechniques such as genome editing. The customized BSF larvae could greatly encourage the aquaculture sector, a major consumer of fishmeal, to accept BSF-based products as an alternative protein source.

Recent progress in optical pest control using light and color.

Masami Shimoda (The University of Tokyo)

Optical Pest Control (OPC) using light and color has been attracting attention as one of the physical pest control techniques in Integrated Pest Management, It is a pest control method that artificially manipulates the light environment in horticultural facilities and outdoor cultivation to suppress the entry of pests and reduce their proliferation. While chemical pesticides aim to eliminate pests in agricultural fields, OPC, in combination with various other control measures, aims to suppress the density of pests below economically acceptable levels so that damage to crops does not become severe. OPC can lead to a reduction in the amount and frequency of chemical pesticide use, and can be expected to reduce the development of insecticide resistance, which is the primary cause of the increasing difficulty in controlling pests worldwide.The basic principle of OPC is the use of insect's behavioral responses to lights and colors. Insects have compound eyes that allow them to recognize the shape, color, and movement of objects. Since compound eyes are composed of several hundred to several thousand individual eyes, they have low resolution and cannot see objects in detail. However, with the individual eyes being arranged in a sphere, they have acquired a much wider field of vision (than humans). The most important feature of many insects' vision is their high sensitivity to near-ultraviolet light, shorter than 400 nm wavelength, which helps them to find flowers (nectar) and mates. Some insects can sense polarized light, and some have a higher temporal resolution in vision. Processing visual information with simpler brain structures (fewer neurons) makes them better suited for quick movements in flight, that help them to catch moving prey.Research to date has provided detailed information on how insects behave under specific light conditions. The response of insects to light is highly variable, depending on the insect species, developmental stage, and the light environment. Newly developed OPC technologies in the last decade include: (1) red light irradiation method to suppress pest density by camouflaging the green color of crops; (2) blue light irradiation method to suppress mating behavior of nocturnal moths by disrupting their biological clock; (3) red color netting to prevent micro-pests from entering cultivation facilities; (4) blue light irradiation method to kill a variety of pests by causing physiological disorders; (5) purple light irradiation to attract natural enemies of pests to crops; (6) light sources or sticky sheet traps with visual contrast as insect trapping devices. This presentation will provide an overview of these pest control technologies that are becoming basic tools for establishing environmentally-friendly pest control systems.