

Book of Abstracts

of the 1st EAAP Workshop on Insect Genetic Improvement, Implementation, Impact



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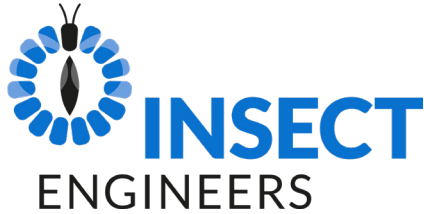
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Scientific programme

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Chair: Petersen / Obšteter

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A Preliminary Draft Pangenome of Black Soldier Fly (*Hermetia illucens*): Structural and Genetic Insights from Diverse Populations.

H. Rosche-Flores¹, C. Picard¹

¹ *Indiana University Indianapolis, Biology, 723 W Michigan Street, 46202 Indianapolis, United States*

The black soldier fly (BSF, *Hermetia illucens*) is increasingly used in sustainable insect agriculture, yet its genetic diversity across wild and commercial populations remains underexplored. We constructed a preliminary pangenome from four assemblies that includes a reference genome generated from a captive strain (GCF_905115235.1), another captive strain, and two global wild-caught genomes using a variety of sequencing technologies. The pangenome graph revealed extensive conservation, with over 1 Gb of rank-0 (core) segments shared across assemblies, alongside isolated regions of high variability. These variable regions, representing accessory sequences, included loci with significant structural complexity and population-specific contributions. This preliminary pangenome complements earlier findings of selective pressures in BSF populations. Furthermore, regions of high variability in the pangenome align with loci previously associated with traits such as heavy metal tolerance, metabolic shifts, and cuticle formation. Additional research is necessary to determine whether these observed patterns indicate analogous mechanisms of adaptation or selection with additional sequenced specimens representing the diversity of the species.

Diverse Genomic signatures of *Hermetia illucens*: Tools for comprehensive analysis of diversity"

B. Gradus¹, S. Klompus¹, S. Espinoza²

¹ *NRGene, BSF, Ilan ramon 2, 7403635 Nes Ziona, Israel,* ² *NRGene Canada, BSF, 110 Research Dr., SK S7N 3R3 Saskatoon, Canada*

Insect farming, and specifically the bioconversion of organic material into animal feed, is increasingly making use of the Black Soldier Fly (BSF). However, in comparison to other farm animals, the genetic resources of farmed insects remain poorly characterized. NRGene is a leading molecular genomics company, we utilize our pipelines to develop modern molecular breeding tools for the BSF industry. As part of the Israeli BSF consortium, we have generated the first two BSF high-quality PHASED (separate reconstruction of the sequences corresponding to the two copies of each chromosome) chromosome-scale genome assembly (male and female). These genomes were then used for downstream analysis for population genetic characterization and diversity analysis. To achieve this, we leveraged our proprietary DeNovoMAGIC™ pipeline, which combines Pacific Bioscience long reads with Illumina short reads sequencing to serve as input for the assembly (BUSCO benchmark resulted in a score of 96.04% for BSF_IL_Male and 96.13% for BSF_IL_Female). While investigating the assembled genomes, we noticed that the coverage of the contigs of chromosome 7 (the suspected X chromosome) is 50% compared to the rest of the chromosomes. This finding supports the XY dependent sex determination in BSF. Further comparison of the two phased genomes with the un-phased (collapsed) iHerIII genome, identified unmapped scaffolds unique to the BSF IL Male assembly, suggesting that these scaffolds may belong to the previously uncharacterized male Y chromosome. The male BSF_IL_Male assembly served as a reference genome in diversity analysis and population genetics comparison. Whole genome sequences of more than 16 populations (5 male and 5 females per population) were produced for this purpose. The analysis results illustrate the high diversity that exists between populations from around the world. From this diversity we generated 2 genetic SNP panels: a high density panel composed of 62K SNPs and a minimal panel of 520 SNPs used as the first high throughput genotyping platform for BSF. Both panels are harboring this diversity and include male-female specific identification SNPs.

Building a reference imputation panel and optimizing imputation strategies for low-pass sequencing of Black Soldier Fly

P. Muchina³, J. Kinyua¹, F. Khamis², C. Tanga², G. Gebreyesus³, G. Sahana³, Z. Cai³

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The black soldier fly (BSF) *Hermetia illucens* plays a critical role in waste management, sustainable agriculture, and animal feed production. However, their genetic resources remain poorly characterized. Accurate genotyping of BSF is crucial for addressing fundamental questions in conservation management, evolutionary biology, and ecology. These insights are vital for optimizing selective breeding programs. While genotyping for several agriculture species is frequently performed using arrays, they are unavailable for BSF. Sequencing-based methods such as whole-genome sequencing (WGS) are an ideal alternative, and although the cost has significantly decreased over the decades, sequencing many samples at high coverage is often cost-prohibitive. Low-coverage whole-genome sequencing (lc-WGS) combined with genotype imputation is a promising solution for lower-cost datasets. Several imputation methods have been proposed and successfully applied in genomic studies in other species. However, there is limited information on the performance of these methods in BSF. Our study combined sequenced high-coverage WGS data with publicly available WGS datasets to build a custom BSF reference panel for imputing a set of lc-WGS data. We evaluated the performance of two reference-based imputation methods, i.e., GLIMPSE2 and QUILT v1.0.5, and one reference-free method, i.e., STITCH v1.7.2, across varying low sequencing depth (0.5x, 1x, and 3x). Reference-based methods performed well, with an imputation accuracy of over 0.8. QUILT v1.0.5 performed slightly better than GLIMPSE2 in lower coverages (0.5x and 1x). STITCH had a lower performance, highlighting the importance of the reference panel, though it could be an option in the absence of a reference panel. This study establishes a valuable resource for imputing lc-WGS data to full genome variants. This resource is anticipated to enhance population genomic studies and support the development of more informed and effective selective breeding programs in BSF.

Session 1

Theatre 4

If one is not enough - Using whole-genome sequencing data from pooled workers to explore the admixture pattern of honey bee populations

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Assessing the admixture level of native honey bees, such as *Apis mellifera mellifera* in Ireland (M-lineage), is crucial before implementing conservation actions in beekeeping. Currently, especially pooled drone genomes are utilized to determine the admixture levels of honey bee colonies through an ancestry informative marker panel, which includes microsatellites or single nucleotide polymorphisms (SNPs). However, due to the hemizyosity of drones, they only capture a fraction of the genetic diversity, since multiple paternal origins contribute to the formation of honey bee colonies. To address this limitation, we used whole-genome sequencing data from 30 pooled workers to assess the admixture levels of honey bee colonies. We included 38 *Apis mellifera mellifera* and 10 *Apis mellifera carnica* (C-lineage) colonies genotyped for 773,705 genome-wide SNPs in the admixture analyses using three commonly applied methods: F4-ratio, FST and Admixture. Furthermore, we computed Spearman rank correlations to determine the concordance between the different methods, by re-ordering the colonies according to their admixture level. Using FST the C-lineage admixture proportion ranged between 27% and 73%. In contrast the F4-ratio and Admixture methods showed lower C-lineage introgression patterns, ranging between 3% and 72% and from 5% to 59%, respectively. Despite the different admixture patterns, we observed high rank correlation between all three methods. Admixture and FST had the highest Spearman rank correlation with 98%, while F4-ratio and FST had the lowest at 97%. Our results demonstrate that sequence data from pooled honey bees can be successfully used to accurately determine the admixture level of honey bee colonies. The high concordance between the three applied admixture methods indicates that they performed equally well in detecting the most hybridized colonies. Therefore, it is more important to define appropriate reference populations (M- and C-lineage) prior to admixture computations and apply adequate admixture thresholds to enhance the conservation of local native honey bee colonies.

The bug and the bridgehead: global colonisation patterns of the black soldier fly inferred from metapopulation genetic structure

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During the past decade, the black soldier fly (BSF) has become a promising insect to improve agricultural sustainability by promoting circular economies. Based on a comprehensive global sample, deciphering hierarchical genetic structure among 150 subpopulations revealed that the recent advent of BSF farming had huge impact on the genetic profiles of strains widely used for commercial production and academic research, being indicative of a single, demographically unique domestication event followed by a quick and wide spread across global production settings. However, prior to the worldwide triumph of a single strain there must have been comparatively dynamic colonisations during the last decades or centuries, likely unintended yet mediated by human commensalism. The origin of a major admixture event between evolutionarily distant lineages from the native ranges in the Americas that established a so-called bridgehead population outside the native ranges will be explored. Similarly, the various demographic trajectories of this bridgehead population as well as subsequent secondary or tertiary admixture events with independent single source founders that led to successful colonisations on all different continents will be reconstructed. The genetic profiles shaped at different times (ancient, historic and contemporary) and geographic scales offer vast opportunities as well as challenges for the BSF breeding sector. While manifold unique genetic resources wait for more detailed genetic characterisation as well as phenotypic exploration, virtually all native and naturalised wild populations are subject to ongoing interference with a genetically uniform domesticated BSF. This will likely change the current global population genetic architecture profoundly and irreversibly, with largely unpredictable long-term consequences at both sides of the farm fence.

Session 1

Theatre 6

Geography and Climate Shape Genetic Diversity in Black Soldier Fly Populations Across Native and Introduced Ranges

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We investigated the ecological drivers of differentiation in the black soldier fly (BSF) using 541 samples from 32 populations across the native range via reanalysis of published microsatellite data. Bayesian clustering identified $K=3$ as the optimal number of clusters, yet also revealed substructure within the clusters. Kriging maps across sub-continent point to major breaks around the Isthmus of Panama and the Andes Range. North and Central American populations were monophyletic. Ordination approaches confirmed three major groups, with spatial structure in higher-order axes, suggesting potential temperate adaptations further North, as well as bottlenecks, island adaptations, or recolonization events in Puerto Rico. Gradient Forest analysis highlighted key climatic and geographic predictors, with cumulative importance values of 0.54 compared to 0.38 for Moran's Eigenvector Maps. A combination of environmental and spatial variables were strong predictors of native population structure. The Southern/Central-South ancestry interface was driven by precipitation variables, while the Northern/Central-South interface was shaped by temperature and seasonality. Applying the model in Africa, where BSF introduced from multiple sources established wild populations, predicted ecological dominance of one ancestry in more humid regions. This prediction aligns with precipitation variables being significant in the native range and also possible resistance to hybridization in West Africa due to ecological adaptation, suggesting success of historic translocations might be non-random. Our results emphasize the combined influence of geography, climate, and history and provide a basis for predicting responses to environmental change across native and introduced ranges. Further research investigating signatures of ecological adaptation at the genome level might be necessary to shed light on specific morphological, physiologic, or behavioral adaptations and their relevance for breeding. Unlike honey bees or silkworms, BSF offers early-stage breeding potential.

AI and Molecular Taxonomy: A Comprehensive Approach to Aquatic Macroinvertebrate Identification and Genetic Diversity in the Balkans

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Accurate and efficient species identification is pivotal for advancing genetic diversity research, population structure analysis and ecosystem monitoring. Through this research we developed an automatic identifier for aquatic macroinvertebrates (MI) by combining artificial intelligence (AI), molecular taxonomy, and morphological approaches. Using Convolutional Neural Networks (CNNs), we classified over 90 taxa, including Ephemeroptera, Plecoptera and Trichoptera (EPT), with 98.7% accuracy and 100% precision for major taxa groups. For chironomids, we achieved 92.3% accuracy. Gradient-weighted Class Activation Mapping (Grad-CAM) highlighted the importance of specific morphological features, such as the head for EPT and the mentum and mandibles for chironomids. Molecular validation of MI taxa was achieved through DNA barcoding, culminating in the deposition of over 100 gene sequences in the BOLD Gene Bank (https://v3.boldsystems.org/index.php/MAS_Management_UserConsole). These sequences, the first-ever from Serbia and the Balkans, provide foundational data for future research on population genetics, inbreeding management and evolutionary studies. Our findings underscore the necessity of integrating traditional morphological, molecular and AI-based tools for comprehensive species identification and biodiversity applications. By uniting these methodologies, this research enhances taxonomic workflows and establishes a framework for future studies on Balkans taxa. The alpha version of the standalone application is available on GitHub (Imagelytics). This research was supported by the Science Fund of the Republic of Serbia (#7751676) and COST Action CA22140 (Insect-IMP), which assisted in disseminating the results of the presented study.

Population genomics from a food matrix: estimating csd variability using next generation sequencing on *Apis mellifera* DNA present in honey

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The complementary sex determiner (*csd*) gene is a crucial genetic factor in the sex determination of *Apis mellifera* L. Females only develop if fertilized eggs have functional heterozygous genotypes at this gene. Males, being haploids, are hemizygous. Two identical *csd* alleles result in non-viable males. Given the recent decline in honey bee populations, it is imperative to monitor allele variability at this gene to prevent negative effects of high inbreeding levels. In this study, we evaluated the use of next generation sequencing in conjunction with environmental honey DNA as a source of honey bee genomic information to obtain extensive sequencing data for analysing variability at the hypervariable region (HVR) of the *csd* gene. DNA was extracted from several honey samples directly collected from honeycombs of various colonies. A bioinformatic pipeline was utilised to analyse approx. 1.5 million reads, identifying a total of 160 different *csd* alleles, 55% of which were novel. The average number of alleles per sample corresponded with the expected number of patrines per colony, based on queen mating behaviour. The diversity of alleles at the *csd* gene could also offer valuable information for reconstructing the honey bee population genetic history.

Evidences of recent genome domestication in *Tenebrio molitor* using population and quantitative genomics

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Tenebrio molitor, commonly known as the yellow mealworm, is now reared globally in controlled farming environments for its nutritional value. Mealworm mass selection began in academia in the early twentieth century in Europe, but it was also likely practiced by farmers, who may have selected individuals with faster growth rates, larger mass and shorter development duration. This study aims to identify modern genomic signatures of domestication by combining population genomics with genome-wide association studies (GWAS) of key agronomic traits. Whole-genome sequencing was performed on 18 pools of 40 individuals, representing wild and farmed populations, to measure population genomic metrics, such as FST, Tajima's D and nucleotide diversity. Moreover, the GWAS was performed using 281,549 markers in 3,660 farmed individuals across 26 traits. We identified multiple regions with high genomic differentiation and QTLs associated with growth traits. Two genomic loci showed both significant differentiation structure and associations with growth QTL, suggesting they were likely under artificial selection. These findings support the hypothesis that recent genome domestication has occurred in yellow mealworms. These identified regions and QTLs could serve as targets for further genetic research or breeding programs, contributing to a better understanding of *T. molitor* domestication through its genome modifications.

Exploring the fitness traits of cross-breeding in *Tenebrio molitor*: Insights from laboratory and large-scale trials

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The rapid expansion of the insect-based food and feed industry has driven researchers and commercial producers to explore novel strategies, including the genetic improvement of fitness traits through insect breeding. Among these, the yellow mealworm, *Tenebrio molitor* L., has emerged as a key species due to its extensive utilization as food and feed. Recent studies have demonstrated that strain-specific factors significantly influence the growth, development, and reproductive performance of *T. molitor*. However, the impact of cross-breeding between distinct strains on adult reproductive performance and offspring fitness remains largely unexplored. This study aimed to assess the mating compatibility and performance outcomes of inter-strain crosses compared to intra-strain matings in *T. molitor*. In the initial laboratory trials, cross-mating experiments were conducted between two strains of *T. molitor* (originating from Greece and Italy), evaluating two intra-strain and two inter-strain combinations. Subsequently, pilot-scale trials were performed using four pure strains (Inagro, Greek, Italian, and USA), encompassing four intra-strain and six inter-strain mating combinations. Inter-strain mating was facilitated by either pairing adult beetles in oviposition boxes at a 1:1 male-to-female ratio (laboratory trials) or mixing pupae of equivalent age from different strains (pilot-scale trials). Sexually mature beetles were allowed to mate and oviposit under undisturbed conditions, with egg production, hatching rates, and larval performance to be systematically recorded at regular intervals. First-instar larvae from specific intervals were further evaluated to assess growth and developmental traits. Results from the laboratory trials indicated no significant differences between intra-strain and inter-strain crosses in terms of egg production, hatching rate, or larval growth performance. Conversely, the pilot-scale trials revealed significant differences in hatching rates between mating combinations, although egg production remained unaffected.

Can we use crossbreeding in black soldier fly to improve production?

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As our world faces a growing demand for sustainably produced food and feed, the production of farmed insects has been put forward as a viable solution. The black soldier fly (*Hermetia illucens*, BSF) has proven to be a promising novel source of protein, both as feed and as food. For this species, breeding practices that are common in traditional livestock breeding are currently understudied. One such practice is crossbreeding. Therefore, we explore in this research the possibilities of crossbreeding using four different BSF strains. We created four reciprocal crosses (F1) to investigate parental differences in the offspring, followed by breeding four second-generation populations (F2). All populations were reared at pilot scale on chicken feed in triplicate. Each generation was evaluated for growth, feed conversion, bioconversion efficiency, mating behaviour, and egg production. Although the final larval mass of the F2 generation did not significantly differ from the F0s, the larvae developed faster, resulting in a higher percentage of prepupae at the end of the rearing phase. Furthermore, egg production was 19% higher in the F2 generation compared to the average of F0. Taking both production and reproduction traits into account, we calculated an increase of 16.5% in total larval mass production compared to F0. Although outbreeding depression is frequently mentioned for BSF, our findings demonstrate the potential for crossbreeding in future BSF breeding programs and highlight the need for further research on crossbreeding in BSF.

Genomic phylostratigraphy reveals a wealth of *Hermetia illucens* species-specific genes of unknown function

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Genomic phylostratigraphy is a powerful bio-statistical method used for determination of the evolutionary age of protein-coding genes of a given organism. Proteins encoded by the focal genome are classified into phylostrata, each of them being populated with genes whose protein families emerged at a certain time-point in the tree of life. With this method, gene age may be inferred by considering an orthologous group of genes to represent descendent lineages of the deepest speciation node, or as a result of divergence between the two most distant homologs. Thus, the age of a gene is defined as the most recent common ancestor of the species found in that phylostratum. It has been shown that if a new biological function emerged at a given time-point in evolution, the genes related to that function should be enriched in the corresponding phylostratum. We applied the phylostratigraphic approach on the proteome of *Hermetia illucens*. The analysis resulted in 14,081 proteins assembled in the *H. illucens* proteome scattered across the 31 phylostrata, representing distinct evolutionary steps between the emergence of the first cellular organisms and our focal species. From all proteins included in *H. illucens* proteome, 835 proteins (5.93%) were assigned to the youngest phylostratum containing exclusively the proteins specific to *H. illucens*. Surprisingly, none of the 835 proteins in the last phylostratum could be functionally annotated through the EggNOG database. Since the black soldier fly is one of the most important commercially reared insect species today, such a result is highly indicative of the insufficient knowledge of its genetics, and specifically recently emerged gene functions in this evolutionary line. Our results offer a highly valuable and exploitable genetic resource for further research in the topic of *H. illucens* gene functions. By understanding the recently emerged gene functions, new biotechnological applications and breeding strategies can be developed to address the known challenges in today's breeding processes and to elevate the breeding yield and quality of the black soldier fly.

Using artificial selection tools to increase neonates' survival rate in Black Soldier Fly (*Hermetia illucens*) after suspension

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The Black Soldier Fly (*Hermetia illucens*) Larvae is a pivotal species in sustainable waste management and alternative protein production. Optimizing neonate survival in the supply chain remains a critical challenge under industrial-scale and decoupled breeding from rearing conditions. We investigated the use of artificial selection tools to enhance the survival rate of Black Soldier Fly neonates within the innovative PasuseM™ system, a FreezeM suspension technology designed to preserve and manage the insect's viability to enable stock management and enhance shelf life. This study aimed to increase the survival rate for an extended time periods than PasuseM™ is offering so far and by that to improve the shelf life of the product. By selectively breeding for traits associated with resilience under suboptimal environmental conditions, we implemented a targeted breeding program over multiple generations. We suspended the neonates for long periods in PasuseM™ units as a selection pressure, in which the surviving neonates were grown to adulthood and their offsprings were collected and used for the next generation in the breeding program. Results demonstrated a significant increase in neonates' survival within the PasuseM™ unit, attributed to enhanced physiological adaptations conferred through artificial selection. These findings underscore the potential of genetic optimization to improve the operational efficiency of industrial insect-rearing systems, paving the way for scalable and sustainable deployment of Black Soldier Fly technology in waste recycling and protein production industries.

Correlated Response to Selection for Increased Body Weight on Fecundity in *Hermetia illucens*

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Genetic improvement through artificial selection holds potential for improving production of the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae). A long-term artificial selection for increased larval body weight is in place for the black soldier fly. To investigate the impact of body weight selection on egg production, four tests were conducted, assessing the phenotypic relationship between pupal body weight, egg-clutch weight, number of eggs and egg size. To measure fecundity, egg clutches were collected from individual females. The performance of the selected body weight line (BW) selected for 14, 21 and 32 generations were compared with the base population (BP) line for measurements on egg clutches. The pupal body weight of individual females showed a strong positive correlation (0.73) with egg-clutch weight and a moderate positive correlation (0.47) with the number of eggs. The egg-clutch weight showed a strong positive correlation (0.79) with the number of eggs and a moderate positive correlation (0.51) with the length of egg. The BW line showed significantly higher performance over the BP line over all variables measured with about 18-49% increase in egg-clutch weight per female compared to the BP line and 24-30% increase in number of eggs per clutch. The linear mixed model showed that the selection had significantly increased egg-clutch weight over the generations of selection in the BW line. Assessment of phenotypic relationships showed no evidence of reproductive trade-offs with higher body weight in female black soldier flies in this study. This research provides the first empirical evidence of increased black soldier fly egg production in response to artificial selection for increased larval body weight.

A Novel PCR-Based Method for Early Sex Identification in Black Soldier Fly (*Hermetia illucens*) LarvaeI. Nevo-Yassaf¹, A. Sloutskin¹, I. Alyagor¹¹ FreezeM, Nachshonim 1, 7319000 Nachshonim, Israel

Sex identification in the Black Soldier Fly (*Hermetia illucens*) has traditionally only been achievable at the adult stage through labor-intensive manual procedures. In this study, we report the development of a novel, high-throughput Polymerase Chain Reaction (PCR)-based method for molecular sex identification at the larval stage. Our method utilizes a newly identified sex-specific genetic marker that displays distinct differences between male and female larvae. By amplifying this marker through PCR, we can accurately distinguish between the sexes at an early developmental stage. Early sex identification has significant implications for optimizing breeding strategies and rearing efficiency, as male and female larvae exhibit different growth rates, nutrient profiles, and reproductive capacities. This non-lethal method involves the collection of small larval biopsies, allowing tested individuals to continue normal development into adulthood without compromising viability or reproductive performance. As a result, this approach enables early and sustainable selection for breeding programs. The ability to accurately determine larval sex supports more efficient resource allocation, selective rearing, and enhanced productivity in insect-based protein production systems. Our PCR-based approach is scalable, enabling high-throughput sex identification that facilitates selective breeding for desired traits and the establishment of genetically superior lines, ultimately improving production efficiency and profitability. This work represents a significant advancement in Black Soldier Fly research and provides a powerful tool for optimizing the management and utilization of this key insect species in biotechnology and sustainable agriculture.

High larval density and exposure to entomopathogenic fungi adversely affect key life history traits and lead to increased immune investment in the black soldier fly.L. O. Opare¹, A. B. Jensen², A. Lecocq², S. Holm¹, T. Esperk¹¹ University of Tartu, Institute of Ecology and Earth Sciences, Juhan Liivi 2, 50409 Tartu, Estonia, ² University of Copenhagen, Department of Plant and Environmental Sciences, Thorvaldsensvej 40, 1871 Copenhagen, Denmark

Larval density, or crowding, is an important biotic factor often overlooked in studies of its impact on organisms. High conspecific density can create a heightened risk of infection and a suboptimal environment for many insect species. While larval density is expected to correlate with immune response, significant immune investment can have notable fitness costs for larvae. The effects of entomopathogenic fungi on economically important insect species reared at different larval densities have not been extensively studied. This study investigated the effects of larval density and exposure to entomopathogenic fungi (EPF) on the life history traits of economically important black soldier flies (BSF). We reared BSF larvae at 1 and 5 larvae/cm² densities and exposed them to two entomopathogenic fungi, *Beauveria bassiana* strains. Our results showed that larvae reared at higher densities exhibited significantly higher phenoloxidase (PO) activity than those reared at lower densities. Additionally, EPF-exposed larvae showed higher PO activity than the control treatments, indicating that larvae in high-density environments can respond to additional stress from EPF exposure. Increased development time and reduced prepupal and pupal mass were also observed at high larval densities. In conclusion, larval density, EPF exposure, and their interactions significantly affect BSF larval life history traits and immune response. Although higher immunity at elevated densities may provide better protection against pathogens, it comes at the cost of longer development times and reduced body size.

Proteomic Analysis of Invertebrate Iridescent virus-6 infected DS2 CellsI. A. Ince¹, S. Boeren²¹ CNRS, CBS, 29 rue des navacelles, 34090 Montpellier, France, ² Wageningen UR, Biochemistry, Dreijenlaan 3,, 6703HA Wageningen, Netherlands

The pathogenesis of a model dsDNA virus performed by using a unique virus-host system. As a DNA virus model, we selected a dsDNA virus with known genome and virion proteome called Invertebrate iridescent virus 6 (IIV-6). As a host organism we have selected fruit fly (*Drosophila melanogaster*) that are popular model organism for investigation innate immunity mechanisms. Fruit fly has fully annotated genome and possible to make/get mutants of the fly with an intended gene mutation. The main reason of the selection of this model system is to explore novel metabolic pathways responsible from dsDNA virus infection in non-natural host species as so far there is no identified DNA virus infection in *Drosophila* naturally. The current in silico interaction models can allow us to select a group of interacting proteins generated by GePS to work on understanding fundamental mechanism behind this phenomenon. In future I may provide good model to study virus evolution and development adaptation to new host which ultimately let the establishment of permissive infection and possible emerging infection from non-native host (a new host). So far, in *Drosophila* we learn that RNAi targets virus infection and JAK-STAT metabolic pathway is involved in antiviral defence. In *Drosophila*, the current knowledge on innate immunity responses against DNA virus infections are still very limited. We identified the viral and host proteins in a quantitative manner which may take crucial role in infection process. The Label free quantitative (LFQ) proteomic approach was applied to reach this goal. Quantitatively determined host proteins analyzed in silico to define the protein- protein interaction . Therefore, it will now be possible to perform functional analysis of the crucial genes for viral infection processes in *Drosophila* S2 cells. The proposed virus-host system provided initial understanding on molecular mechanisms of innate immunity and antiviral responses of host. Recently, it is realized that the viruses can shift to new organisms and eventually they gain new properties to emerge diseases in new organisms. Due to this phenomenon, it is prerequisite to work virus biology on non-natural host models.

Session 2

Theatre 6

Silkworms as an Alternative Insect Protein Source: Nutritional Value and Environmental SustainabilityS. Mylonas¹, M. Georgiadou², M. Stamouli¹, K. Mountzouris², A. Tsagkarakis¹¹ Agricultural University of Athens, Laboratory of Sericulture & Apiculture, Department of Crop Science, 75 Iera odos str., 11855 Athens, Greece, ² Agricultural University of Athens, Laboratory of Nutritional Physiology & Feeding, Department of Animal Science, 75 Iera odos str., 11855 Athens, Greece

The increasing global demand for sustainable protein sources has intensified research into insect-based proteins. This study investigates the potential of silkworms, *Bombyx mori* L. (Lepidoptera: Bombycidae), as an alternative insect protein source by analyzing the crude protein, crude fat, and other nutritional factors of the produced larvae and pupae. Feeding experiments were conducted, and the nutritional content of silkworms was compared with that of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) (Black Soldier Fly – BSF), a well-established insect protein source. Results indicate that silkworms have a high nutritional value, showcasing significant levels of crude protein and fat, comparable to or exceeding those of BSF. Despite their nutritional benefits, concerns arise regarding the environmental impact of silkworm cultivation, primarily due to their dependence on plant-based feed that requires substantial water resources. This study highlights the need to balance the nutritional advantages of silkworms with the environmental costs associated with their production, emphasizing the importance of assessing sustainability in the development of alternative protein sources.

Comparative larval growth of *Alphitobius diaperinus* populations on various substrates

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The lesser mealworm, *Alphitobius diaperinus* (Panzer), is a promising species for mass production due to its potential as a nutritious source for feed and food. Nonetheless, there is still plenty of room for improvement regarding its production. For instance, traditional livestock animal production typically involves the utilization of strains or breeds with favorable production characteristics for intensive farming. However, in the case of *A. diaperinus* the effect of the genetic material has not yet been thoroughly evaluated. To this end, the larval growth of two wild populations from Turkey and USA, one laboratory and one wild population from Greece, and two populations from commercial rearing facilities in the Netherlands was comparatively evaluated in laboratory trials. All populations were evaluated in flour of 100% wheat bran, rice hulls or sunflower seeds. The results indicated considerable differences among the populations with regard to their larval development, as well as to their feed conversion efficiency parameters. The study aims to highlight the significance of selecting *A. diaperinus* populations with specific traits to improve the overall efficiency of the farming systems. The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 11297).

Do strains affect insect farming efficiency? The effect of *Tenebrio molitor* strains on adult fecundity and larval growth

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Optimizing mass-rearing practices for *Tenebrio molitor* is crucial for the insect industry, yet the impact of strain variability is often overlooked. This study evaluated the growth, development, and reproductive performance of seven *T. molitor* strains from Greece, Italy, Turkey, Germany, the USA, and Spain. In the first laboratory bioassay, the egg production of adults of the seven strains was investigated. More specifically, 5 males and 5 females were placed in plastic vials, together with white flour as oviposition substrate, and left undisturbed to mate and oviposit. Laid eggs were collected and counted every 4 d. Adult survival and larval hatchability were also recorded. In a second bioassay, the larval development of the seven strains was recorded. After a 4-week period in which 1st-instar larvae were allowed to feed undisturbed, larval weight and survival were evaluated every two weeks until the emergence of the first pupa. The feed conversion ratio and the total development time were also determined. Results revealed significant differences across strains, with larval weights ranging from 87-154 mg and development times spanning 67-156 days. Egg production and adult survival also varied considerably, highlighting the substantial impact strain selection can have on biomass production. These findings emphasize the importance of strain choice for improving efficiency in commercial mealworm farming.

Pervasive plasticity: Genotype-by-environment interactions for larval performance and body composition traits in the black soldier fly

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Black soldier fly larvae (BSFL) serve as vital source of dietary protein for livestock and aquaculture as well as an innovative solution for organic waste management. However, the plasticity in converting various dietary substrates for which BSFL became a prime insect candidate in circular agriculture may still entail limitations that are highly relevant for economic viability in a challenging framework of regulations and market. The impact of BSF genetics remains poorly investigated due to a lack of effective monitoring tools. In this study, four genetically distinct BSF strains were characterised using microsatellites, and tested across three different feed substrates. A range of growth and compositional traits were measured, revealing that both feed substrate and genetics—and their interaction—significantly influenced nearly all examined phenotypes, including mortality rates, growth patterns, average larval weight, and key body composition metrics like dry matter and crude protein content, as well as amino acid profiles. Comparably complex patterns including strong genetic effects were observed for feed conversion ratio, nitrogen efficiency, fiber degradation, and emissions. This emphasizes the important yet underestimated role of BSF genetics in interpreting phenotypic variation across studies that so far largely miss to report genotypes. The findings further suggest that, depending on targeted objectives in BSF production, efficiency and sustainability may be substantially increased by thoroughly considering BSF genetics and interactive effects with diet in a particular context, ranging from overall biomass to fine scale nutritional profiles, or performance variation in dynamic contexts. A stronger focus on screening a variety of differentiated populations for genetic effects and breeding-relevant GxE effects appears timely. Such basic population-level genotype-phenotype information will be very valuable for designing dedicated breeding programs selecting for specifically superior or generalist strains.

Modelling the Impact of Non-Native Honey Bee Importation on Native *Apis mellifera mellifera* Populations

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The human mediated introduction of queens from the C mitochondrial lineage, particularly *A. m. ligustica* and *A. m. carnica*, has led to increased hybridization and introgression events, threatening the unique adaptations of the local bees in Ireland. Using the SIMplyBee stochastic simulator, we developed a model to simulate hybridization events between native *A. m. mellifera* and non-native C-lineage honey bee populations over a 20-year period. We modelled the impacts of varying import percentages, gene-by-environment interaction simulated as genetic correlation between the environments, spatial introgression patterns with a single point of entry of the imports, and the effects of stopping importation practices. Our results revealed a direct correlation between import percentages and introgression rates, with higher imports leading to greater introgression levels in the native Irish population. This resulted in a trade-off where increased honey yields were accompanied by declining fitness levels of the local population. Simulated genotype-by-environment (GxE) interactions demonstrated that negative genetic correlations between native and non-native fitness traits led to greater fitness declines. The spread of introgression across the simulated landscape showed that even colonies far from importation zones were significantly affected over time. Importantly, stopping importations did not rapidly reverse introgression, as the rate of decay was slow, underscoring the long-term risks posed by importation practices and the need of measures to prevent the disappearance of the local subspecies.

How genetic integrity in honey bees, addressed in a regional law, can be monitored using mitochondrial DNA

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The conservation of the genetic integrity of *Apis mellifera* subspecies has become a key objective, particularly in Europe where several national and regional initiatives have been introduced to protect native honey bee populations. In 2019, the Emilia-Romagna region in northern Italy became the first Italian authority to pass a law specifically aimed at protecting the native *Apis mellifera ligustica* subspecies. This law banned the breeding and introduction of other subspecies into the region. In this study, we investigated the diffusion and distribution of mtDNA haplotypes in the honey bee population of the Emilia-Romagna region, by examining 1,143 honey bees sampled in the years 2020, 2021 and 2022 just after the introduction of this law. We analyzed a highly informative portion of mitochondrial DNA (mtDNA), commonly used to assess the genetic diversity of honey bee populations and we were able to provide a snapshot of the distribution of mtDNA haplotypes in the region. A total of 14 different mtDNA haplotypes were identified, the two most prevalent were C1 (characteristic of *A. m. ligustica*) and C2 (found in *A. m. carnica*), present in 86.5% and 11.0% of the bees analyzed, respectively. A small percentage (1.3% and 1.1%) of the bees carried haplotypes belonging to the A and M lineages (A1a, A1e, A4, A26, A65, A2w, A6a, M3, M3a, M4, and M79). These results suggest that the regional law has been effective in reinforcing the conservation of the *A. m. ligustica* genetic pool. Ongoing genetic monitoring will be crucial to evaluate the long-term impact of this law. Based on these findings, we recommend implementing even stricter measures to prevent further erosion of the genetic integrity of the native *A. m. ligustica*.

A Glow in the Waste: Advancing Food Safety with Transgenic Fluorescent Black Soldier Fly Larvae

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Food waste has emerged as a critical public health challenge, threatening the sustainability of food systems globally (United Nations, 2023). With millions of tonnes of food waste generated annually, innovative and sustainable management solutions are urgently needed. Bioremediation of organic waste through black soldier fly larvae (BSFL) offers a promising approach, as BSFL can transform organic matter into valuable products, including animal feed, organic fertiliser, and biofuel (Gabler, 2014; Liu et al., 2008; Siddiqui et al., 2022). Despite these benefits, the European Union prohibits the re-entry of BSFL raised on food waste into the agrifood chain, due to concerns over animal proteins and potential contaminants in the substrate (Commission Regulation (EU) No 142/2011). To address these safety concerns and improve traceability, we propose the development of a fluorescent transgenic line of BSFL, allowing for efficient monitoring throughout the bioremediation process and preventing inadvertent re-entry into the food chain. Our methodology employs transposase-mediated plasmid DNA integration to generate fluorescent green and yellow BSFL. Specifically, we cloned the *Hermetia illucens* actin promoter into a pGEM-T Easy vector for insertion into enhanced green and yellow fluorescent protein plasmids, which will be microinjected into BSF eggs. A CMV-hyPBBase plasmid will serve as a helper in the integration process. Functional studies will follow to assess the efficacy and safety of the transgenic BSFL. This transgenic approach will significantly enhance traceability within bioremediation, supporting further advancements in sustainable waste transformation practices while ensuring the integrity of the agrifood chain.

Optimizing COI Barcoding for Molecular Detection of Hidden Insect Infestations in Rice Grains: Evaluating Primer Efficiency and Detection Limits

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Hidden insect infestations in rice grains pose significant challenges to food security, economic stability, and public health worldwide. Two genetically close species, *Sitophilus oryzae* and *Sitophilus zeamais*, known as rice weevils, complete much of their lifecycle inside grains, evading detection through concealed entry holes. Traditional detection methods, such as CO2 measurement, ninhydrin staining, grain flotation, X-ray imaging, and acoustic monitoring (ISO 6639-4:1987), lack the specificity and sensitivity for precise identification. This study developed a specific and rapid multiplex real-time PCR (qPCR) assay. Using DNA barcoding targeting the cytochrome oxidase I (COI) gene, species-specific primers and probes were designed and evaluated. The primers achieved efficiencies of 107% for *S. oryzae* and 116% for *S. zeamais*. The assay's limit of detection was 0.00005 ng/μL for *S. zeamais* DNA and 0.0025 ng/μL for *S. oryzae* DNA, demonstrating its ability to detect very low DNA concentrations. This qPCR approach provides an effective tool for stakeholders to detect, monitor, and manage hidden insect infestations in rice grains, improving strategies for pest control and food quality assurance.

Session 3

Theatre 1

Ancient DNA, museomics and insect domestication

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Palaeogenomics has deeply revolutionized our understanding of animal domestication, revealing our early interaction with traditional livestock, and their ancient dispersal routes. Thanks to breakthroughs in ancient DNA, the origin of modern domestic horses was for example traced back to the lower Don-Volga region, in the Central Pontic Caspian steppe. Around 4,200 years ago, they spread out from their homeland, ultimately colonising the world and replacing almost all local lineages that pre-existed in Eurasia. Compared to their wild relatives, the ancestors of modern domestic horses carried mutations at genes experimentally associated with enhanced docility and mobility. Mirroring such a fascinating domestication history, we here present ancient and modern genomes from the most important commercial and laboratory strains of *Tenebrio molitor*. We find that domestic strains form a monophyletic clade, at the exclusion of most ancient (wild) specimens, suggesting a single origin for all domestic strains, including the so-called German laboratory lines. Captive specimens, dating back to ~1991 (National History Museum of Madrid), are basal to this “domestic” clade, attesting what today represents the earliest genomic evidence of domestic *T. molitor*. Finally, we pinpoint genes and genomic regions that underwent positive selection along the phylogenetic branch leading to all domestic stocks, as this internal branch represents their ancestral population at early domestication stages, to fully understand the molecular essence of this fascinating evolutionary process.

How Genomics Revolutionized Crop and Animal Genetic BreedingG. Ronen¹¹ NRGene, Ilan Ramon St 2, 7404905 Ness Ziona, Israel

The growing global demand for food and feed requires continuous improvements in farm productivity. Modern farms are significantly more productive than 70 years ago, largely due to advancements in plant and animal breeding. Genetic breeding involves mating individuals and selecting superior progeny based on their performance. Differences in genetic codes among progeny, however, make identifying and linking these variations to performance a complex challenge. Genomics, the study of genomes—the complete DNA blueprint of a species—has revolutionized this process. A decade ago, the genomes of most crops and farm animals were unavailable. NRGene's innovative algorithms, such as DenovoMAGIC™, enabled the assembly of complex genomes for key species like wheat, corn, cattle, and shrimp. PanMAGIC™ further advanced genomics by comparing multiple genomes within a species, creating large databases of genetic variations. These tools have accelerated the development of DNA kits for efficiently detecting genetic differences, mapping traits, and producing elite varieties in significantly less time. NRGene's cutting-edge genomic tools are being intensively employed to study the Black Soldier Fly (BSF) genome and map key traits, with the aim of improving its productivity and establishing it as the next big farm animal.

Session 3

Theatre 4

Population and Functional Genomics of Black Soldier Fly Mass RearingC. Rhode¹¹ Stellenbosch University, Department of Genetics, Private bag X1, 7602 Matieland, South Africa

Black Soldier Fly (BSF) farming has been proposed as an alternative livestock production system that may meet the challenges for future food security, with low resource requirements, higher feed conversion ratios and similar nutritional value as conventional animal protein. BSF has the added advantage that its larvae act as efficient bioremedial agents, converting organic waste into useable biomass creating a sustainable, circular agricultural economy. Despite the industrial scale of BSF mass rearing, globally, little is known about the drivers of genetic and phenotypic variation under these production conditions. Using classical genetic and multi-omic approaches the interplay between organismal biology, population genetics, and functional genomics were investigated to elucidate micro-evolutionary forces that impact phenotypic development of BSF larvae under commercial mass rearing. Genetic drift seems to be the major evolutionary force shaping genomic diversity in such populations, even in the presence of direct artificial selection for production traits. Drift is also the major cause for rapid and significant generational genetic differentiation. Additionally, few loci under selection were significantly associated with production traits, further illustrating the influence of stochastic evolutionary processes during mass rearing. The effects of selection on gene expression were also weak and differential transcriptomic profiles highlighted functional trade-offs between growth metabolism and immune function. Metagenomic analysis found significant associations between bacterial taxa and protein-fat ratios in BSF larvae, and that both feed-substrate, host genetics and the interaction between feed- and host genetics played a significant role in the composition of larval gut microbiomes. The findings highlight the multidimensional and complex nature of BSF production and its impact on genetic diversity and phenotypic development, with applications for future genetic management and improvement strategies for enhanced production.

Aquaculture Breeding development and what we can learn from insect breedingP. Haffray¹*¹ SYSAAF, Station LPGP/INRAE, Aquaculture genetic unit, Campus de Beaulieu, Université de Rennes, 35042 Rennes, France*

Insects farming and aquacultures share key elements conditioning investment in genetic improvement among which a recent historical domestication, a relative small size of the individuals, a high diversity of species and modes of production, very diverse final utilizations and products, very diverse kind of actors from individual farmers to integrated farms. From the application of genetics in the last decades in fish, shellfish and shrimp aquacultures, some biological, economical and organizational driving factors or constraints will be questioned and illustrated from aquaculture practices based on the development and use of reproductive biotechnologies, genomic tools, phenotyping technologies, sanitary practices and selection methods in a perspective of more sustainable and less environmentally impacting practices.

Session 4

Poster 1

Understanding the reproduction of Hermetic illucens under various conditionsG. Çalışkan Koç¹, Ö. Süfer²*¹ Uşak University, Food Processing Department, Food Technology Program, Eşme Vocational School, 64600 Uşak, Turkey, ² Osmaniye Korkut Ata University, Food Engineering Department, Engineering Faculty, 80000 Osmaniye, Turkey*

ABSTRACT The aim of this review is to examine in detail the reproductive behavior of the Black Soldier Fly (BSF) under different conditions. The Black Soldier Fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae), which inhabits tropical, subtropical, and warm temperate regions, has emerged as a promising option for industries such as food, feed, fertilizer, and biofuel. This fly, which can be easily reared on various waste materials, is considered an alternative protein source due to its richness in protein and fat. It is easy to rear, and compared to many other insect species, BSF grows rapidly. However, the most critical processes in mass-rearing BSF involve obtaining large amounts of organic matter, ensuring consistent larval production, and producing eggs efficiently in both quantity and quality to maintain a stable progeny. BSF reproduction consists of two stages: mating and egg-laying. In tropical regions, BSF mates continuously under natural conditions, while in warm temperate regions, it mates a few times per year. Two days after mating, females become ready to lay eggs, triggered by the release of volatile organic compounds from decaying organic matter. Eggs are deposited in dry crevices near a moist food source. Studies in the literature have examined BSF reproduction in natural habitats, using semi-artificial (greenhouse regulated by sunlight) and artificial breeding methods (i.e., rooms regulated by artificial light). Both mating and egg-laying are influenced by environmental conditions such as light, humidity, and temperature. Seasonal changes, particularly the intensity of sunlight, affect the number of mating events. Additionally, the sex ratio and population density impact BSF reproduction. In conclusion, environmental and biological factors influence BSF reproduction and must be optimized. It can be suggested that stimulating BSF with temperatures above 26°C and optimal light intensity could enhance reproductive and egg-laying efficiency.

Impact of genetic variation and substrate choice on the performance of the black soldier fly (*Hermetia illucens*)

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The black soldier fly (*Hermetia illucens*) holds great potential for sustainable protein production and organic waste recycling in agriculture. Successful BSF farming, however, depends on understanding the genotype-environment (G×E) interactions and the genetic diversity across different strains. In this study, we evaluated the larval performance of nine genetically distinct BSF strains, sourced from facilities in Europe (4 strains), Africa (2 strains), and Oceania (3 strains), using three different substrates over three non-consecutive generations. Results indicate substantial variation in larval yield, growth rates, feed conversion ratios (FCR), waste reduction, and protein content among strains. Notably, European and African strains, particularly Europe 2 and Africa 2, displayed superior bioconversion efficiency, waste reduction, and adaptability to varied nutrient conditions compared to Oceanian strains. For example, on high-fiber substrates, the Europe 4 strain showed a 13% bioconversion efficiency (BE), significantly outperforming Oceania strains by up to 22%. On chicken start mash, Europe 2 achieved the highest BE at 22%, significantly outperforming strains such as Oceania 2, which achieved a BE of 18%. Feed conversion ratios (FCR) also varied; for example, Africa 1 exhibited a superior FCR of 1.63 on wheat bran, compared to 1.94 for Oceania 1, indicating a 19% efficiency increase. These findings underscore the importance of selecting genetically suited strains for BSF farming, as strain-specific traits can significantly enhance productivity based on environmental and substrate conditions. Additionally, our findings confirm the robustness of experimental results through two validation experiments conducted over non-consecutive generations, with intervals of four and five generations between experiments. This consistency highlights the reliability of these performance traits, highlighting the potential of targeted breeding programs to match the right BSF strains with the right substrates for sustainable agricultural applications.

Session 4

Poster 3

The Potential of Edible Insects as Sustainable Protein Sources in Animal Nutrition: Opportunities and Challenges

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As a consequence of the increase in the world population, there is a rapid increase in the consumption of animal products. It is predicted that world animal product production will increase over the next 25 years. Meeting the increasing demand will be possible by increasing and diversifying the resources allocated for animal nutrition. The intensive use of traditional protein sources (e.g. soy and fish meals) in animal nutrition causes serious environmental problems. The increases in animal production and environmental problems associated with traditional protein sources have led researchers to search for alternative protein sources, such as insect proteins. Protein-rich insects are considered an alternative to reduce the cost of protein additives. Insects are also very rich in essential amino acids, fats, vitamins, and minerals. In terms of protein quality, they are similar to soybean meal and fish meal. However, there are pros and cons to using insect protein as animal feed. Pros include sustainability, as insects require less land, water, and feed compared to traditional livestock, making them a more sustainable option. They also offer high nutritional value and can be more cost-effective. Cons include potential consumer resistance due to cultural and psychological barriers, regulatory hurdles that vary by region, and challenges in scaling up production to meet large-scale demand. In conclusion, the use of insects as a sustainable protein-rich feed ingredient in diets is technically feasible and offers new perspectives in animal nutrition. This review will discuss the potential of insects as animal feed and the factors limiting their use.

Turning food waste and agri-food byproducts into animal feed ingredients and organic fertilizer using insects – Towards pilot implementation in Greece

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Our food system is dysfunctional: 10% of human population is undernourished. Yet, food-grade materials are fed to livestock and annually 1 billion tons of food are wasted. To break this pattern, certain insect species can be reared on food waste and agrifood byproducts and processed into animal feed, while their frass can be used as organic fertilizer. In Greece, this circular technology can: Divert food waste from landfills, reducing GHG emissionsSupport agrifood businesses via byproduct valorizationReduce dependence of aqua- and livestock farming on imported, price-unstable feed ingredients with environmental burdenReduce dependence on mineral fertilizers, manufactured energy-intensively from imported non-renewablesDespite these environmental and socioeconomic benefits, the technology is not yet applied at a commercially relevant scale in Greece. Lack of pilot implementation limits techno-economic and regulatory knowhow, access to finance and general awareness. To fill this gap, we will: Adapt the technology to Greece, with focus on:Bioclimatic facility design for energy efficiencyOptimal insect diets based on low-value, local agrifood byproducts to maximize resource-efficiencyLocal and/or selectively bred insect strains for high performance under the local climatic and dietary conditionsSet up a pilot facility in Crete/Greece to:Assess techno-economic and environmental performanceCharacterize product quality and safety in collaboration with local end-usersAcquire license for commercial activity, to unveil a national regulatory pathwayMake a case for “insect business” in Greece via:Showcasing the facility to create awarenessBusiness plans to encourage investmentGuidelines and training to insect businessesPromotion of insect products in the domestic marketsWith this stepwise strategy, we will create a hub of technical, regulatory and business knowhow, necessary for a thriving Greek insect sector on par with the rest of Europe.

Session 4

Poster 5

The Role of Consumer Knowledge and Attitudes in the Adoption of Insect-Fed Poultry

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The rapid growth of the world's population has led to a surge in demand for meat and meat products, posing significant challenges to modern agricultural and food systems. This increased demand has driven up the need for feed ingredients such as soybean meal and fishmeal, the production of which often has a negative impact on the environment. Insects, such as the Black soldier fly (BSF), offer a promising sustainable alternative to these traditional protein sources. The use of insect larvae in animal feed can significantly reduce the environmental footprint of livestock systems and increase European self-sufficiency. However, public acceptance of insect-fed chicken is a crucial factor for the successful implementation of such solutions. To investigate the factors influencing consumer acceptance of insects in animal feed, a survey was conducted among 773 Slovenian consumers. The results show that consumers are most receptive to the use of insects in chicken feed (74%), with the safety of the resulting chicken meat being a primary concern. In addition to safety, familiarity with insects as a food source and previous consumption experience are important factors in acceptance. Over 90% of respondents were aware of insect consumption and 24% had personal experience of eating insects. Younger, more educated people were more likely to accept chicken meat from insect-fed chickens. While 75% of respondents were aware of the use of insects in certain EU foods, only 33% were aware of their possible use in animal feed. More than half (65%) believed that insects in animal feed posed no risk to human health, while only 14% believed that Slovenian citizens would readily accept this practice. Educating Slovenian consumers about the sustainability benefits of insects as a protein source is crucial to overcoming food neophobia and increasing acceptance of insects. Future research should explore the individual factors influencing acceptance and develop strategies to improve public knowledge and awareness of the use of insects in animal feed.

Filling the gap - Genetics of Selection in Honeybees

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For a long time, the genetic improvement of honey bees has been subject to significant interest among beekeepers, breeders and scientists. However, most available literature addresses the practical aspects of how to organize breeding, what traits to select for, how to phenotype colonies, and how to organize mating control and queen rearing. On the other hand, selection, in the narrow sense and as an element of the breeding cycle, has never been treated thoroughly and with critical attention. To fulfil that “aperture”, we set down a booklet entitled Genetics of Selection in Honeybees, where we elaborate on the complexity of defining the Breeding Goal, discuss the quantifying gene effects on traits, heritability, repeatability and correlation in the chapter Quantitative Genetics, further disentangle and visualizing the Breeding Value Estimation and finally, the chapter Selection where we address the design of a breeding program, annual genetic change and inbreeding, generation interval. In this book we aim at a readership not trained in quantitative genetics, not fond of equations. The book only contains a few essential equations but dozens of figures and tables, and highlighted text boxes. In that way, we aim to motivate the readership and, hopefully, to assess the material for further improvements critically. We consider it as work in progress and welcome your comments in the future to improve the accessibility of the text. The booklet is freely available on the Wageningen University E-depot at the following unique web address: <https://edepot.wur.nl/675067>.

Genome-wide association study in *Tenebrio molitor* reveals reproduction, development and growth quantitative trait loci

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Tenebrio molitor, also known as the yellow mealworm, is farmed as a new alternative and sustainable source of protein and is valued for its high nutritional content and ability to thrive under various conditions. Identifying genomic regions, genes and markers associated with agronomic traits will be a first step for genomic selection. Our study aims to understand the genetic basis of complex traits involved in growth, development and reproduction through genome-wide association studies (GWAS). We performed single and multi-locus GWAS methods on 3660 individuals genotyped by using Axiom Chip and phenotyped for 26 quantitative traits involved in growth, development and reproduction. The analysis of around 281,549 markers allowed the identification of several significant QTLs and their associated genes. The genomic regions identified and their associated molecular markers represent a key step towards breeding programs for the improvement of yellow mealworm production.

Utilization of the edible insects in food industryO. Sufer¹, G. Çalışkan Koç²¹ *Osmaniye Korkut Ata University, OKÜ Karacaoglan Campus, 80000 Osmaniye, Turkey,* ² *Uşak University, Eşme MYO, 64600 Uşak, Turkey*

The aim of this study is to investigate the factors affecting the chemical composition of edible insects and their potential uses in food industry. Edible insects have garnered increasing interest in food technology due to their environmental friendliness, economic and sustainable nature, and nutritional qualities (rich in proteins, healthy fats, vitamins, and minerals). The most commonly consumed insects belong to the orders Coleoptera, Lepidoptera, Hymenoptera, Orthoptera, Hemiptera, Isoptera, Odonata, and Diptera. Edible insects can be classified into two groups based on their life cycle patterns: hemimetabolous and holometabolous insects. Hemimetabolous insects (Orthoptera, Hemiptera, Isoptera, Odonata, Diptera, etc.) undergo incomplete metamorphosis, also known as paurometabolism, passing through three stages: egg, nymph, and adult. Holometabolous insects (Coleoptera, Lepidoptera, Hymenoptera, etc.), on the other hand, experience a four-stage development process, including egg, larva, pupa, and imago (or adult). The life cycle stage of insects affects their nutritional composition. In general, high fat content is observed in the pupal stage of edible insects, while high protein content is found in the larval stage. Therefore, the developmental stage of insects chosen may vary depending on the valuable component intended for extraction. Additionally, the nutritional composition of insects varies depending on the species and diet. The edible insect sector is rapidly growing, producing new products for various applications, including powders, liquids, and oils. In many Asian countries, edible insects are consumed by roasting, frying, or boiling them whole. Studies have shown that, in countries such as Europe and the United States, consumers tend to prefer using valuable components extracted from insects rather than consuming insects directly. Moreover, insect flours/powders can be used in fortified dry foods, protein supplements, high-protein cereals, meat substitutes, chitosan, protein-rich beverages, sports supplements, and various snacks such as burgers, energy bars, and compound feeds.

Session 4

Poster 9

Can mate-choice enhance or inhibit genetic rescue in commercially reared parasitoid biocontrol agents?L. Corral¹, A. Karley², R. Boulton¹¹ *University of Stirling, Biological and Environmental Sciences, 3A120 Cottrell building, FK9 4NF Stirling, United Kingdom,* ² *James Hutton Institute, Ecological Sciences, The James Hutton Institute Invergowrie, DD2 5DA Dundee, United Kingdom*

Millions of parasitoid wasps are reared commercially each year for biological control of agricultural pests. However, these wasps are typically bred on hosts that are made susceptible to parasitism for logistical efficiency, which prevents them from co-evolving with their field hosts. When pests evolve resistance to parasitism, commercially reared parasitoids often fail to keep up, reducing the effectiveness of inundative biological control. To address this, biocontrol companies use “genetic rescue,” introducing wild-caught parasitoids into rearing populations to boost genetic variants that can overcome host resistance. Mate choice could influence the success of this strategy, enhancing genetic rescue if males from wild-type hosts are preferred as mates but hindering it if they are not. We investigated mate preferences in two commercially reared aphid parasitoid species, *Aphidius ervi* and *A. colemani*. Female wasps were tested in arenas containing two males separated by mesh barriers. Males emerged either from aphid lines that were parasitoid resistant (*Sitobion avenae* for *A. ervi* and *Rhopalosiphum padi* for *A. colemani*) or parasitoid susceptible. Female wasps, reared from either resistant or susceptible hosts, were observed for 30 minutes, with their proximity to each male being recorded at 5-minute intervals. In both species, females spent more time near males from resistant hosts, regardless of the host type they themselves emerged from. However, this preference varied among individuals: some females showed a strong bias, while others exhibited no preference. These results suggest mate choice could be used to enhance genetic rescue, increasing the ability of commercially reared parasitoids to overcome host resistance but further research is needed to confirm how widespread these preferences are and whether they translate to differences in mating success.

How many samples are sufficient for testing the reliability of mating stations in honey bees (*A. mellifera*)?

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Controlled mating in honey bees is often organized in isolated areas (mating stations), which are assumed to be free of drones from unknown origins. The reliability of mating stations is tested by confirming the paternity of worker brood by comparing the genetic composition of queens in drone-producing colonies (DPQs) or their drones with that of mated queens used at the same mating station. In the BeeConSel project, the concept was to sample DPQ queens, mated queens and 20-30 samples of worker brood per colony. However, such extensive exploration of genetic composition (microsatellites, SNPs or sequencing) is laborious and costly. To cut costs, the reliability of mating stations is proposed to be tested based on a few samples per brood/colony. We aimed to see how few samples per brood would give a similarly accurate assessment of the reliability of the mating station as the whole 20 – 30 samples of brood per mating nuc. We used the data obtained during the BeeConSel project in which all samples were genotyped (n=483, 20 colonies), and reads of 5 microsatellites (A0007, A0013, Ap043, Ap055, and B0124) were used to detect the presence of 81 alleles. The calculated reliability was done on a set used as a baseline. We proceeded by 100 times random sampling 1-5 worker bees from brood per colony from the existing dataset, effectively performing an undersampling of the dataset. The estimated reliability approached the baseline by increasing the number of worker bees sampled per colony from 1 to 5. If only one worker bee is sampled per colony, the assessment can be over/underestimated by 20%, reducing to 7% when five samples are considered. The standard errors range from ± 0.09 when one worker bee is used to ± 0.04 when using five samples of worker bees per colony. The results indicate that using 4 – 5 samples is necessary for testing the reliability of the mating stations and still cutting the material and labour costs.

Session 4

Theatre 2

Optimizing pedigree reconstruction and patriline determination in honeybees

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Tracking inheritance patterns in breeding programmes is essential for managing genetic diversity, avoiding inbreeding, and ensuring accurate pedigree records that enable genetic improvement. With the rise of systematic breeding programmes, this is becoming important also for the Western honeybee, *Apis mellifera*. However, due to their complex reproductive biology and difficulties in controlling mating, honeybee breeders face challenges in acquiring accurate pedigree information. Genomic information offers another source of information to infer relationships and can be used to reconstruct or verify a pedigree. The aim of this paper was to evaluate pedigree reconstruction and patriline determination in honeybees using both real and simulated data. Specifically, we assessed the accuracy of different pedigree reconstruction methods, performed genotype phasing using the reconstructed pedigree, and developed custom R functions to assign the parental origin of haplotype, and determine the number of patrilines per queen. The results highlight that the choice of software and SNP array size significantly impacts the accuracy of paternal assignments and pedigree reconstruction. Our work provides valuable insight into method and dataset sensitivity, and underscore the difficulties of accurately mimicking real data with simulation. This study will significantly contribute to the evaluation of mating control in honeybees, but also other species, and to improved population management by guiding honeybee breeders and researchers in choosing the best method for their dataset but also understanding the limitations.

Mating behaviour checking in the black soldier fly (*Hermetia illucens* L.) using SNP data: parentage assignment for Pedigree reconstruction

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Recently, we developed the first family pedigree breeding scheme in the black soldier fly (BSF). This scheme has been used to estimate the first genetic parameters (heritability, genetic correlations, common environmental effects), with the successful development of an experimental line selected for body weight over several generations. According to this scheme, clutches are isolated and collected per female, with the assumption that one clutch is mated by a single male and a single female. However, some monitoring data on reproductive behavior has revealed that one female may mate with several males, which may in turn mate with several females. The aim of present study was to test the monogamy hypothesis in our pedigree selection scheme through a parentage assignment design. The experimental set-up consisted in breeding 21 males and 19 females (tagged with different colors) within a cage fitted with cameras to record all matings. From the 26 recorded matings, 32% of females and 33% of males behaved polygamously. In order to quantify the paternal contribution to offspring, polygamous females and males and a random sample of around 30 larvae/offspring were genotyped using the 96 KASPAR SNP genotyping chip developed on BSF. Using the probability method implemented in the *r* APIS package, we obtained 55% of the assignment rate corresponding to recorded mating patterns, and of which some did not automatically translate in progeny. Unassigned offspring either had poor quality genotypes and/or one of their parents was not genotyped. In conclusion, the monogamy hypothesis has not been confirmed and, based on the assignment results, there would be a 10-20% error to be assumed in our pedigree selection model developed for the genetic improvement of traits of interest in the BSF.

Session 4

Theatre 4

Mating behaviour and mating control (in farmed insects), what can we learn from biological control?

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Insect rearing for food and feed has gained momentum as an emerging sector aimed at addressing global food security and enhancing sustainability. Understanding insect mating behaviours is crucial for optimizing breeding in controlled environments, as these behaviours impact both yield and genetic diversity. Insights from the biological control industry, where insect breeding is well-established, can offer valuable frameworks for controlled mating in insect farming. In biological control, selective breeding and environmental manipulation are essential for producing robust insect populations that meet production demands. Selective breeding prioritizes insects with favourable traits, such as high fecundity, rapid growth, and resilience to environmental stressors. Adjusting environmental factors like lighting, temperature, and humidity helps synchronize mating behaviours and cycles with ideal breeding conditions. Additionally, understanding population dynamics is key for managing insect rearing systems, influencing how insects find mates, compete, and reproduce. Leveraging these aspects, with learned lessons from insect biological control breeding practices, insect farming can achieve enhanced productivity, reduce inbreeding risks, and develop scalable, sustainable solutions that support global food security. This presentation will explore how insect rearing for food and feed can benefit from established knowledge in biological control.

Can cryptic sex enhance the performance of an asexual aphid parasitoid?R. Boulton¹¹ *University of Stirling, Biological and Environmental Sciences, Stirling, FK9 4LA Stirling, United Kingdom*

Sexual reproduction has considerable genetic benefits, for instance it increases adaptive potential and helps to purge deleterious mutations. These benefits come at a cost however, because sexually reproducing females must produce male offspring their reproductive success is expected to be half that of an equivalent asexually reproducing female. Parasitoid wasps commonly commercially reared for biocontrol exhibit both sexual and asexual reproductive strategies, but do asexuals or sexuals make better biocontrol agents? While asexual biocontrol agents should in theory, be more efficient at controlling their host pests, in a changing world, where hosts can rapidly evolve resistance and environmental conditions can rapidly change, the adaptive potential that sex provides may become increasingly important. In this study I test whether rare facultative sex in a mostly asexual aphid parasitoid, *Lysiphlebus fabarum* represents the 'best of both worlds'. I predicted that facultative sex would result in higher recruitment of female offspring and increased fitness due to genetic diversity when compared to obligate sexual or asexual conspecifics. While facultative sex does result in complete production of daughters and genetically diverse offspring, this does not translate to improved fitness and higher parasitism. In fact, facultative sex was associated with high rates of reproductive failure compared to obligate sexual and asexual reproduction, possibly due to disrupted ploidy. The next step is to determine the extent to which these costs impact the parasitism rate over multiple generations and in environmental conditions that are relevant to biocontrol.

Session 4

Theatre 6

Unlocking the Potential of *Zophobas morio*: Nutritional and Reproductive InsightsP. Soulioti¹, C. Adamaki-Sotiraki¹, C. I. Rumbos², C. G. Athanassiou¹¹ *University of Thessaly, Department of Agriculture, Crop Production and Rural Environment, Fytokou Str., 38446 Volos, Greece,* ² *University of Patras, Department of Agriculture, G. Seferis Str., 30200 Messolonghi, Greece*

In recent years, mini livestock has gained considerable attention as an alternative protein source for food and feed applications. Although the insect species *Zophobas morio* (F.) (Coleoptera: Tenebrionidae) has not yet received approval as food or feed it demonstrates notable potential for such purposes. However, research on the biology and optimization of rearing practices for this species remains limited. Thus, this study aimed to optimize adult reproductive performance and larval growth through a series of bioassays. In a first bioassay the impact of three female-to-male sex ratios, i.e., 5:5, 6:4 and 8:2, on reproductive output and adult longevity, with weekly observations of adult survival, egg production and hatching rates over 91 days was recorded. While, in a second bioassay the impact of dietary protein content on larval growth, using wheat bran-based substrates with increasing levels of dry instant yeast to achieve protein contents of 15.2%, 20%, 22.5%, 25%, 17.5%, and 30% was evaluated. Larval weight and survival were recorded biweekly. Finally, in a third bioassay, larvae were reared on isoproteinic (20% protein) wheat bran substrates supplemented with different protein sources, i.e., dry instant yeast or soybean, egg white, pea, and brown rice protein powders. The results of this study revealed that adult sex ratio can significantly affect the reproductive output of *Z. morio*, with the highest number of eggs per female produced by the balanced sex ratios. Larval performance was also significantly affected by dietary protein content and source. Optimal larval growth was achieved with a 20% protein diet supplemented with soybean protein powder, while larvae fed a diet supplemented with pea protein powder did not perform well. These findings contribute to the understanding of *Z. morio* reproductive output and nutritional requirements providing valuable insights for optimizing its rearing practices.

Is age just a number? Investigating the effects of male and female age on the reproduction of *Tenebrio molitor* L.

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Tenebrio molitor (TM) is one of the major insect species reared in Europe for food and feed purposes. So far, most of the attention has been given to the larval stage, while adult breeding strategies is a topic that has not been investigated thoroughly. This study investigates the effect of adult age on their fecundity and fertility. A total number of 960 pupae were collected, sexed and divided in 4 age classes (1-4 weeks old [WO]). Sixteen treatments were created, each composed of 5 males (M) and 5 females (F) belonging to a specific age (1WOM:1WOF; 1WOM:2WOF; 1WOM:3WOF; 1WOM:4WOF; 2WOM:1WOF; 2WOM:2WOF; 2WOM:3WOF; 2WOM:4WOF; 3WOM:1WOF; 3WOM:2WOF; 3WOM:3WOF; 3WOM:4WOF; 4WOM:1WOF; 4WOM:2WOF; 4WOM:3WOF; 4WOM:4WOF). Adults were placed in plastic vials containing white flour which served as oviposition substrate, while agar was provided 3 times per week as moisture source. Per each treatment, 6 replicates were performed. Adult survival, egg production, and hatchability were recorded every 7 days for a 4-week period. Based on the statistical analysis conducted using SPSS software ($P < 0.05$), it was observed that pairs involving younger females produced significantly higher number of offsprings compared to pairs with older females. This outcome was consistent regardless of the age of the males.

Impact of Adult Body Size on Reproductive Performance in *Hermetia illucens* (Diptera:Stratiomyidae)

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The black soldier fly (BSF, *Hermetia illucens*) efficiently converts organic waste into protein, oil, and fertilizers, leading to its global industrialization (Van Huis, 2013). However, little information is known about adult BSF biology. The objective of research was to investigate how adult body size influences the reproductive performance in BSF. Insects were reared under controlled conditions. Thorax length (TL) was measured as an indicator of adult body size in newly emerged flies. Mating cages were set up with large males and females (L), small males and females (S), small males and large females (SL), and large males and small females (LS), each with a 1:1 sex ratio. With 60 flies per cage and three replicates, the complete experiment included a total of 720 flies. Females were randomly selected daily, dissected, and assessed for mating status and ovarian development. Infertile egg percentages were also determined. Analysis showed no significant difference in TL within S and L populations ($FS = 0.363$, $p > 0.001$; $FL = 0.16$, $p > 0.001$), but TL differed significantly within both SL and LS populations ($FSL = 63.5$, $p < 0.001$; $FLS = 73.4$, $p < 0.001$). Small females exhibited a delay in ovarian development of approximately 24 hours. This delay may be attributed to their lower fat reserves, which are essential for proper ovarian development. The lowest number of mated females was observed in the LS population (72%), while the SL population had the highest (96%) and showed the greatest egg production. In BSF the small males appeared to have a mating advantage, probably because of the better maneuverability of a small body compared with a large one. Egg fertility showed no significant difference, with infertile eggs ranging from 4% to 8%, indicating that body size had no notable impact on fertility across groups. The ratio of body size between males and females is a major factor regulating reproductive performance. The number of successful matings were greatest when the sex size ratio was 1.12 (TL female:TL male). Below 0.81 ratio threshold resulted in the lowest number of mated females ($p < 0.001$). These findings could be practically applied in the optimizing of the industrial rearing of BSF and the practice of breeding process.

Crossroads of preference: oviposition and developmental success in black soldier fly rearing

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The anticipated rise in global food production, including that of meat, cereals, and fish, is expected to significantly increase the generation of organic waste. Using insects presents a viable solution for degrading and valorizing the waste, with larvae serving as a protein-rich alternative to conventional livestock feed. However, maximizing the targeted deposition of fertilized eggs is a key challenge in the industrial breeding of the black soldier fly. In this study, we focused on identifying chemical signals produced by microbes that enhance oviposition behavior in black soldier flies (BSF) and evaluated the use of these attractant substrates in larval feeding trials. Among a variety of organic substrates, we aimed to identify the most attractive one for BSF oviposition. We presented the substrates to adult flies in Petri dishes with perforated lids to release odorants and attached egg traps for oviposition. Results showed that adult BSF females preferred chicken manure for oviposition. In a follow-up experiment, we aimed to evaluate whether the chosen substrate was also suitable for different developmental stages of BSF. The same substrates used in oviposition experiments were fed to the larvae to determine their effects on development. The results showed that the prioritized substrate from the first experiment did not support optimal development at any stage – chicken manure slowed the transition from the larval to pupal stage, led to the highest larval mortality, produced smaller adult flies, and resulted in no oviposition and thus no reproduction. Studies like this are crucial to deepen our understanding of economically relevant insects, such as BSF, and to optimize breeding for broader applications.

Interkingdom-interactions in black soldier fly oviposition

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As the world population grows and food and feed demand increases, rearing insects as nutrient source gains increasing attention. *Hermetia illucens*, the black soldier fly (BSF), emerged as a promising species for large-scale rearing. However, industrial insect farming faces a bottleneck, as large quantities of fertilized eggs are needed. This study focused on the influence of insect-microbe interactions to investigate oviposition attraction using egg-laying bioassays combined with semiochemical, microbiological, and genetic analyses. Microbial volatile organic compounds (mVOCs), chemical signals released by microbes, influence the oviposition behavior of female BSF. Thus, it is important to cultivate, identify, and study the microbes that produce cues attractive for BSF oviposition. Attraction experiments with various organic substrates revealed a preference of BSF females for fresh chicken manure. Therefore, our aim was to characterize the microbiome of chicken manure and link mVOCs attractive to BSF females to the microbes producing them. Since microbial cultivability on artificial nutrient media is very limited as these rarely meet microbial needs for specific nutrients and growth conditions, a dual cultivation approach and metabarcoding was used to characterize the microbiome. Standard on-plate cultivation was compared with the iChip device that allows the microbial inoculum to be incubated in its natural environment. The isolated microbes were cultured in a series of mini diffusion chambers sealed by a semi-permeable membrane, providing them with natural nutrients and growth factors, while isolating them from other microbes. After growth in the iChip, previously unculturable microbes were transferred to agar plates, and the mVOCs they produced were analyzed using gas chromatography coupled to mass spectrometry (GC-MS). The iChip resulted in a higher yield of cultivable microbes than standard on-plate cultivation. This study shows that the iChip can provide access to previously uncultivable microbial diversity that can play a key role in understanding insect-microbe communication pathways and signals.

Characterization of Gut Microbiota in *Chironomus riparius* Under Natural and Laboratory Conditions

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The gut microbiota of the freshwater insect *Chironomus riparius* is crucial for the organism's adaptation to environmental stress and holds promise for applications in pollution remediation. In this study, a preliminary characterization of the bacterial communities within the gut microbiome of *C. riparius* larvae was conducted, comparing samples from natural habitat with those from laboratory conditions. Isolation and molecular analysis revealed that *Bacillus* (including 13 species, such as *B. subtilis* and *B. thuringiensis*) and *Paenibacillus* were predominant in samples from natural habitat, whereas *Peribacillus frigoritolerans*, *Lysinibacillus fusiformis*, and *Gottfriedia solisilvae* were more characteristic of laboratory samples. Five bacterial species, including *Metabacillus idriensis*, *Peribacillus simplex*, *Neobacillus cucumis*, *Bacillus thuringiensis/toyonensis*, and *Fictibacillus phosphorivorans*, were identified in both environments. Natural habitat samples exhibited higher alpha and beta diversity than laboratory cultured larvae, likely due to the complex ecological factors in natural environments compared to the stability of laboratory conditions. Differential abundance analysis identified unique bacterial species within each sample type, emphasizing the influence of specific environmental conditions on microbiome composition. These results highlight the importance of characterizing microbiota at the time of collection from natural habitats, followed by subsequent analysis after laboratory acclimation to monitor shifts over time. Future studies should investigate the mechanisms underlying these microbiome shifts and their potential impact on *C. riparius* adaptability, offering insights for microbial applications in environmental protection. The authors would like to thank all those involved in the COST Action CA22140 – Improved Knowledge Transfer for Sustainable Insect Breeding (Insect-IMP) for the support in disseminating the results of the presented study.

Session 5

Theatre 2

Dietary Protein Source and Level Influence the Free Amino Acid Composition of Black Soldier Fly Larvae (BSFL)

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A balanced amino acid profile in animal diets is important to ensure efficient growth and protein utilisation. When insects are produced as animal feed, their amino acid composition can be a key aspect for their application and market value. In the case of black soldier fly larvae (BSFL), the rearing substrate has been shown to have a minimal influence on the total amino acid composition of a BSFL strain. However, substrate amino acid limitations could influence the free amino acid fraction in the larvae, which affect their availability when used as feed components. To provide more insights, a factorial experiment was performed in which apple pulp (a low-protein component) was mixed with three different peptones (meat, casein or soy) to formulate isoproteomic substrates differing in their protein source and subsequent amino acid composition. These diets were provided to BSFL at two total protein levels (3.87% or 1.33% /DM). The growth of larvae was influenced by both factors (protein source and protein level). When the total amino acids were analysed, a significant reduction of most amino acids in BSFL grown on the low protein diets was observed, however, this was not the case for all amino acids in the BSFL biomass such as Ornithine. The "protein type" did not influence the contents of all analysed total amino acids, and significant differences were found only in the free amino acids fraction. The source of protein influenced the BSFL content of the free Asparagine, Methionine, Tyrosine, Histidine, Cysteine, in addition to the free derivatives Hydroxylysine and Hydroxyproline. The results provide insights into the influence of the amino acid profile of the diets on the composition of free amino acids in the BSFL produced.

High-throughput phenotyping of Black Soldier Flies – A survey of challenges and needs in the industryI. Fodor¹, A. Bouwman¹, A. Chauhan², E. Ellen¹, T. Veldkamp¹, S. Naser El Deen¹¹ Wageningen University and Research, Wageningen Livestock Research, De Elst 1., 6708 WD Wageningen, Netherlands, ² Wageningen University and Research, Wageningen Food & Biobased Research, Stippeneng 4., 6708 WE Wageningen, Netherlands

Sensor-based information is essential for monitoring, phenotyping, and decision-making in animal farming. However, differences in taxonomy, size, and management have prevented insect rearing from benefiting fully from the advancements made in precision farming in other livestock species. As a result, the insect farming industry still relies heavily on manual processes to obtain quantitative data from the animals. Collaboration between academia and industry could help bridge this gap, but this requires a deep understanding of the industry's specific needs. We conducted interviews with Black Soldier Fly breeding and producing companies in different countries between October and December 2024, to learn about (1) the challenges they face when it comes to phenotyping Black Soldier Flies across different life stages, and (2) how could high-throughput, non-invasive, sensor-based phenotyping support their company. Companies were at different levels of implementing sensor-based measurements for phenotyping and monitoring, but the vast majority of collecting quantitative information still relied on manual processes. During the interviews, counting the number of individuals (e.g., larvae, pupae, flies) was one of the most prominent needs at this stage for companies. Most companies were very positive about the potential implementation of high-throughput phenotyping methods, and – depending on their circumstances – would be willing to buy or co-develop such tools. In summary, high-throughput phenotyping could greatly support Black Soldier Fly breeding and production, and our work's input can help steer research, development, and innovation to meet actual industry needs.

Session 5

Theatre 4

Automated Recording Approaches reveal Novel Traits in the Honeybee (*Apis Mellifera*)D. Gupta², G. Petersen^{1,2}, P. Fennessy², P. Dearden³¹ LAVES Institute for Apiculture, Herzogin-Eleonore-Allee 5, 29221 Celle, Germany, ² AbacusBio Ltd, 446 Moray Place, 9016 Dunedin, New Zealand, ³ University of Otago, Department of Biochemistry, 915 Cumberland Street, 9016 Dunedin, New Zealand

Human understanding of honeybee biology remains limited despite decades of dedicated research as well as centuries of interest from natural philosophers. Recruiting and hoarding behaviours and their impact on the division of labour especially remain well described, but poorly understood, partly because they are difficult to fully observe without disturbing colony behaviours. One way to decipher some of the layers of behaviour impacting on colony performance is through the use of remote sensing technologies. Several remote monitoring systems are available for colonies of the European Honeybee, *Apis mellifera*, ranging from simple hive scales to more sophisticated systems including measures of colony activity and health. Using a continuous monitoring system for colony weight as well as IN activity (No of bees entering the hive over a 10-minute interval) and OUT activity (No of bees leaving the hive), we monitored 200 beehives in New Zealand. Local maxima were identified in the OUT activity, indicating that a spike in flight activity occurred at this time point. Overall colony activity was classified into predominantly early activity, predominantly late activity, and activity occurring in two phases, indicating that foraging efforts had been reduced over a mid-day period. “Early” or “late” patterns were far more likely to occur in colonies which had previously shown the same pattern, indicating that these were based on a genetic foundation. Two-phased activity patterns, indicating foragers were recruited back into within-hive work when nectar flow was abundant, were more common during times that resulted in higher hive weight gain and thus higher demand for worker bees to move food deep into the hive. Independent of the strength of nectar flow, colonies with a two-phased activity pattern were found to be more successful in terms of weight gain. Susceptibility to the “pull” from bees attempting to recruit foragers back into the hive thus seems to be a factor in the expression of nectar and pollen hoarding behaviours. This could be investigated further using the same technology.

A case study of selective breeding in the Biological Control industry - Improving mass rearing of a predatory mite, phenotyping, selecting and identifying molecular markers

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The Biological Control industry involves mass-rearing insects and mites that are natural enemies of agricultural pests, using them to control these pests in agricultural fields. Effective mass rearing is critical, though genetic improvements in this process remain underdeveloped. Spider mites are major plant pests globally, and Biological Control has been used against them since the 1960s. A key natural enemy is the predatory mite *Phytoseiulus persimilis*, which is highly effective in the field but difficult to mass rear. This is because it only reproduces on spider mites, which require the cultivation of plants in large quantities, complicating the rearing system and limiting product formulations. To address this, we worked to develop a mass-rearing protocol for *P. persimilis* using an alternative diet. This involved surveying various diets and selective breeding of the predator. We collected wild *P. persimilis* populations, combined them to create a base population, and then selected for those able to thrive on the new diet. We phenotyped the original commercial population, the base population, and the selected population at both the individual level (measuring daily oviposition) and the population level (measuring growth rate). The selected population exhibited improved traits in both areas. We then searched for genetic markers distinguishing the selected population from others. Using Next Generation Sequencing (NGS), we sequenced a pool of several hundred predatory mites and assembled a draft genome. Comparing the sequences of mites from different populations to this genome identified 34 Single Nucleotide Polymorphisms (SNPs) that differed between the selected population, better suited to the alternative diet, and the others. The new rearing system, using the selected population and alternative diet, is now commercially available worldwide under the brand BioPersi+ © and is protected by patents.

Session 5

Theatre 6

The adaptive design of breeding objectives of Black Soldiers Flies (*Hermetia illucens*): - an example from one of the earliest European farms and processors

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Mass Black Soldier Fly (BSF) breeding, rearing, and processing is still under development and largely based on assumptions, rather than hard-proven facts and decades of experience. Consequently, the entire value and upcycling chain involving BSF is volatile and subject to frequent changes, directly impacting the definition and ongoing adaptation of breeding objectives. Starting up as a classic garage-sized operation in 2018, Grinsect has become the first licensed insect protein and fat manufacturer in Hungary (2020) and with it, one of the first ones in the European Union (EU). Because of the lack of deep sector-wide knowledge, a painful try-and-error investments needed to be done throughout the whole supply chain, from breeding, rearing, and processing, up to marketing and logistics. Additionally, the assumption was, that BSF farming needs to be large-scale to produce a protein-rich feed with a competitive price. This meant, in practice, that breeding needed to be efficient, processing needed to be developed to maximize the value of the raw material, and costs needed to be kept low. Therefore, breeding objectives in the early beginning were basic. The goal was to keep the BSF population as sustainably healthy as possible and make them produce in local conditions with the lowest possible cost. As the company developed, an additional objective was added. This added objective was marketability. This performance was measured in the sense of being ready for larvae harvest on the 15th day of life, with the most valuable nutritional content possible. Putting it in an example and numbers, the partially defeated BSF insect meal needed to have a minimum of 55% crude protein and a maximum of 15% fat. To sum up, the design of Grinsect's BSF breeding objectives is in some kind of reverse approach. The reason for this is, that is, that it reacts to local feed possibilities and market needs in addition to the legislative background. These variables are still not stable, thus breeding objectives need to be adapted constantly up to today.

Evaluation of selective breeding programme designs for black soldier fly larvae body weightL. A. De Castro Lara¹, M. Martinez Castellero¹, I. Pocrnic¹, T. De Paula Oliveira¹, J. Obšteter¹¹ University of Edinburgh, The Roslin Institute, Easter Bush, EH259RG Edinburgh, United Kingdom

This talk will present our work on evaluating different designs of a selective breeding programme for black soldier fly larvae body weight using stochastic simulations. The black soldier fly is a vigorous insect with high fecundity and fast growth with important group dynamics. The large number of offspring, the short generation interval, and the substantial genetic diversity indicate a great potential for selective breeding of the black soldier fly. While genomic resources and population genetics research of BSF is progressing, research on selective breeding is still in early stages. Here we used stochastic simulation built with AlphaSimR to simulate ancestral history of black soldier fly, domestication and early breeding, followed by a set of possible scenarios. In the scenarios, we have varied phenotyping and selection intensity, the availability of sex information, and crossing method, and different statistical approaches to analyse the available data. The results show that expectedly phenotyping and selection intensity increase genetic gains. However, due to sexual dimorphism in body weight of larvae, care must be taken in increasing selection intensity to prevent selecting exclusively female larvae. In terms of the availability of sex information and crossing method, there was not much difference in short-term genetic gain, which were impressive, but availability of sex information and control of crossing enabled better management of genetic diversity and hence larger long-term genetic gains. Different methods to analyse the available data give different accuracies of selection, but the differences are less important than the design of a breeding programme. In summary, selective breeding in black soldier fly is expected to generate large short-term genetic gains, which can continue for many generations, particularly with continued investment in data and information to support optimal breeding decisions.

Genetic parameters for honey production in a recently created Buckfast bee breeding nucleus in FranceJ. D'Ambrosio¹, R. Azémar², B. Poirot², D. D. Decante³, A. Decourtye³, M. Sourdioux⁴, B. Basso⁵, F. Phocas⁶, P. Haffray¹¹ SYSAAF, Aquaculture and Bee breeding, Station LPGP/INRAE, Campus de Beaulieu, 35042 Rennes, France,² L'Abeille Ligérienne, 10 Rue Henri Bessemer, 17140 Lagord, France, ³ ITSAP, Domaine Saint Paul, Site Agroparc, 228 route de l'Aérodrome, 84914 Avignon, France, ⁴ SYSAAF, UMR BOA, Centre INRAE Val de Loire, 37380 Nouzilly, France, ⁵ INRAE, UMT-Prade, Domaine Saint Paul, Site Agroparc, 228 route de l'Aérodrome, 84914 Avignon, France, ⁶ INRAE, GABI, Domaine de Vilvert, 78350 Jouy-en-Josas, France

We report genetic parameters and estimated breeding values (EBVs) for honey production in a small-sized Buckfast bee breeding recently established by professional beekeepers (L'Abeille Ligérienne, Lagord, France). Virgin queens were mated naturally or in island mating station or inseminated with drone-producing colonies with queens from selected dams. 910 queens were phenotyped from 2017 to 2023 (17 apiaries, 8 beekeepers). Significant year, apiary, beekeeper effects and pedigree structure indicated the need to increase genetic ties between apiaries and improve contributions of parents across apiaries. Heritability (standard error) for Workers (WM) and Queen (QM) Models were 0.34 (0.16) and 0.18 (0.09). The colony ranking did not change according to the model used (Pearson correlations 0.93 to 0.98). The colony model, which partitions direct genetic effect (workers) and a maternal genetic effect (queen), didn't converge. Selection the 10% best queens on pedigree-BLUP EBVs led to increases in selection response of 18% and 35% compared to selection on phenotypes only corrected for environmental effects. The colony rankings between EBVs and colony performances corrected for year and apiary were consistent (correlations of 0.71 with WM and 0.80 with QM). Heritability were in the same range as reported by previous authors. The non-optimized set up of the breeding scheme design was able to capture some additive genetic variance. These preliminary results are encouraging to improve the design of the breeding program. An improvement of the genetic evaluation is expected with the 2024 and 2025 year-classes for which apiaries will be genetically better connected. This work was supported by the French National CASDAR support (GNEDP0924005735).

Framework and Design of the FBN Breeding Program for Enhanced Efficiency in Black Soldier Fly (*Hermetia illucens*)

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Black soldier fly (BSF) larvae efficiently convert feed and food by-products and biomass side streams into protein-rich biomass, thus contributing to circular agriculture and improved sustainability of livestock feeding. Current commercial breeding programs primarily use phenotypic selection, which focuses on observable traits like body weight and biomass yield. While effective, phenotypic selection often lacks precision in accounting for the genetic and environmental factors influencing key traits. We address these limitations by applying between-family selection, using genetic data to improve precision in selecting for feed conversion efficiency, larval biomass yield, and survival rates. A base population will be developed by mating genetically distinct BSF strains over multiple generations to promote genetic recombination and enhance diversity. From this population, it is aimed to establish a nested family design, creating full-sib families with half-sib relationships across families. Detailed pedigree tracking will be used to monitor genetic relationships and minimize inbreeding. Each family will be divided into three subgroups, which will be reared under controlled environmental conditions, including standardized lighting, temperature, and humidity, while being fed a consistent Gainesville diet to ensure comparable nutrient supply. This approach will minimize environmental and technical variability, enabling accurate and reliable performance evaluations. The collected data will be analysed to estimate heritabilities, repeatabilities, and genetic correlations for the target traits. These genetic parameter estimates will guide selection decisions, optimizing genetic gains across generations. As the project is still in progress, this presentation will focus on the design of our breeding program. It is expected to significantly improve sustainable protein production, support circular waste management, and enhance the scalability and efficiency of BSF breeding programs for commercial applications.

Strong potential for selection for larger larval size in the black soldier fly (*Hermetia illucens*)

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Using selective breeding to increase the size of larvae is a potential strategy to make insect production more efficient. Based on observations in other insect species, we hypothesized that larval size in black soldier flies (*Hermetia illucens* L.; Diptera: Stratiomyidae) is moderately heritable and should, therefore, respond to selection. We estimated the additive genetic variation for larval size assessed in three ways: individual larval surface area (ISA), group surface area (GSA), and group weight (GW). A full-/half-sib design was used, and a total of 92 sires mated successfully with 169 females (1.84 females per male). For each female, on the day of egg-hatching, two cups were prepared with a sufficient amount of feed, and fifty larvae were transferred to each cup. On day 12 after egg hatching, from each cup thirty larvae were selected randomly, and ISA was recorded (9 486 larvae from 317 full-sib groups). For each full-sib group, the GW was recorded as the collective weight of the thirty larvae (317 records), and the GSA was the aggregated ISA of the thirty larvae (317 records). The data were analyzed using sire-dam models including the population average and batch (3 batches) as fixed effects, and sire, dam and cup as random effects. The results showed a high heritability for ISA (0.40), with a moderate effect of the common environment (0.21). For GSA and GW, moderate-to-high heritabilities were observed (0.30 and -0.39). The results show that there is great potential for black soldier fly breeders to select for bigger larval size.

Innovative phenotyping systems to advance selective breeding in black soldier fly*G. Gebreyesus*¹¹ Aarhus University, Center for Quantitative Genetics and Genomics, C. F. Møllers Allé 3, bld. 1130, 8000 Aarhus, Denmark

Selective breeding holds immense promise for improving the quantity and quality of Black Soldier Fly (BSF) products. However, its implementation has been limited, largely due to challenges in phenotyping key traits, which stem from the small size of BSF larvae and their high-density rearing systems. Recent advancements in imaging technologies and artificial intelligence (AI) present transformative opportunities to overcome these hurdles. While the potential of computer vision (CV) and AI in insect farming is increasingly acknowledged, much of the existing research focuses on pest monitoring and taxonomy, often neglecting the specific demands of BSF production. Unlike traditional farming, integrating these technologies into BSF production requires dedicated research and development due to the unique biological and production characteristics of the species. Our recent studies demonstrate the potential of deep learning-based image analysis for phenotyping BSF larvae. These methods enable the measurement of growth traits, identification of life stages, and classification of larval sex. Additionally, multispectral imaging shows promise for predicting larval protein and lipid content, critical parameters for improving BSF farming efficiency. Beyond phenotyping, digital tools powered by AI have the potential to revolutionize other aspects of insect farming, such as climate control and optimizing harvest timing through data-driven decision-making. These advancements highlight the pivotal role of innovative phenotyping systems in unlocking the full potential of selective breeding, ultimately contributing to the scalability and sustainability of BSF farming and the broader insect farming industry.

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