8th Central European IUSSI Meeting

BOOK OF ABSTRACTS

Editors:
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27–30 August 2025
Szeged, Hungary

FOREWORD

The 8th Meeting of the Central European Section of the International Union for the Study of Social Insects (IUSSI) takes place between 27 and 30 August 2025. The venue of the conference is the Institute of Biology, University of Szeged, Hungary. The conference website is: http://ce-iussi-meeting-szeged2025.iussi.de/.

The scientific programme includes three plenary talks, 20 oral presentations, three blitz presentations, and five poster presentations. Oral presentations, including discussion, are 15 minutes long, while blitz presentations are 5-minute presentations. These are grouped into five thematic sessions: Ecology and Invasion Biology, Behaviour and Cognition, Colony Organization, Insect-Microbe Interactions, and Chemical Ecology.

In addition to the scientific sessions, the program also includes the General Assembly of the CE Section of the IUSSI, a guided sightseeing tour in Szeged and a post-workshop trip to Tőserdő.

The abstract book contains the conference programme and the schedule of presentations, as well as the abstracts of plenary talks, oral presentations, blitz presentations, and poster presentations. In the schedule of presentations only the presenting author is listed; full author lists are provided with the abstracts.

Organizers: István Elek Maák, Gábor Lőrinczi, Ádám Lőrincz, Fanni Pécsy, Kata Anna Bán, Bonita Ratkai, Kincső Somogyi, Ágota Szabó.

We are grateful to the Institute of Biology, University of Szeged, for funding and hosting the conference.

We thank all participants for their contributions and abstracts, and we wish everyone an enjoyable and inspiring conference!

Szeged, 25 August 2025

The Organizers

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Flexible but simple? Cooperation and tool use in bumblebee cognition

Olli Loukola

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Bumblebees (*Bombus terrestris*) display cognitive abilities that challenge conventional views of insect intelligence. In this presentation, I discuss findings from two recent studies on cooperation and problem-solving.

The first study tested bumblebees in a cooperative task that required two individuals to push a block and simultaneously touch a door to access a reward. Their actions were socially influenced, with individuals adjusting their behaviour and even facing their partners during critical moments, suggesting active coordination.

The second study examined spontaneous problem-solving in a tool-use context. Bees were trained to associate a blue artificial flower with a reward, which was later placed out of reach on the arena ceiling. Bees rolled a ball beneath the flower and climbed on it to access the reward, apparently demonstrating flexible problem-solving. However, perceptual feedback controls complicated this interpretation: bees could roll the ball through a wall opening without seeing the target, and even in the absence of any flower, they still pushed the ball through. This suggests that exploratory behaviour combined with perceptual feedback may explain task success. Yet, because the control did not fully exclude the use of perceptual feedback, it remains unclear whether bees can solve the task without it. This question is the focus of ongoing work.

Together, these findings highlight both the sophistication and the limits of bumblebee cognition, showing how cooperation and problem-solving may emerge from simple mechanisms as well as flexible behaviour.

Developmental plasticity in ants

Eva Schultner

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Ants exhibit spectacular phenotypic variation between and within sexes. The most prominent example is queen-worker dimorphism, which reaches extreme levels in species in which workers completely lack reproductive organs. How queen and worker phenotypes are determined, and how they differentiate over the course of development, has been a central question in ant research for over a century. Nevertheless, until recently progress in understanding the mechanisms underlying these developmental processes has been slow, also because ants rear their brood in piles and larval phenotypes mostly do not diverge until late in development.

In my talk, I will present an overview of our current understanding of queen-worker development in ants, with a special focus on recent advances which promise to revolutionize how we think about this fundamental aspect of ant biology.

Insights into ant social organization and behaviour through the study of *Myrmica* ants

Magdalena Witek

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Myrmica ants have served as model species for research across various disciplines, including chemical communication, population biology, community ecology, and interactions with other organisms. In my presentation, I would like to explore what we can learn about multispecies interactions and the behaviour of social insects by studying *Myrmica* ants.

I will present the chemical and vibroacoustic adaptations of social parasites – such as inquiline queens (*Myrmica karavajevi*) and the larvae of *Phengaris* butterflies – that exploit the multimodal communication systems of *Myrmica* host ants to gain access to and integrate within their societies. It is also fascinating how these host-parasite relationships can be modified by a third partner: a fungus *Rickia wasmannii*. This entomopathogenic fungus not only affects ant behaviour but also influences the division of labour within the colony and age-dependent investment in immune defence.

Finally, I will show how both internal factors (such as colony traits) and external factors can shape collective behaviour, as well as individual and colony-level personality. The widespread presence of *Myrmica* ants in various habitat types and high diversity of different symbionts living inside colonies of this ant genus offer an excellent opportunity for further studies of local adaptations and intriguing species interactions.

Supercolony formation in invasive *Tapinoma magnum* ants

Anna-Lena Bäuchle[1], Volker Nehring[1,*]

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Invasive social insects can rapidly colonise new environments and outcompete local species. A key factor in their dominance is their ability to form vast supercolonies. Individuals collaborate over distances of several kilometres. From a social evolution standpoint, this lack of boundaries between local nests is puzzling because local cheaters should be able to exploit global cooperation. The current spread of the ant species *Tapinoma magnum* in Germany enables us to observe the expansion of an invasive ant species in real time. This will help us to better understand how local invasions turn into large supercolonies.

We collected ants from different locations in the Upper Rhine Valley where *Tapinoma* has become established within the last five years. We investigated the genetic structure of the population. At the same time, we conducted behavioural experiments and analysed nestmate recognition cues to determine whether a supercolony had formed.

Our findings suggest that the Upper Rhine Valley has been invaded by ants of different origins. Some, but not all of the colonies are part of the same supercolony. Using these data, we have reconstructed the possible invasion history and propose different scenarios for how supercolonies can form from separate local invasions.

Behavioural and transcriptomic variation among queens in polygynous colonies of *Stigmatomma* ants

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Investigations of social insect queens are usually restricted to their reproductive role. In ant species with multiple queens per colony (polygyny), queens may not only differ in their reproductive activity, but also in other aspects ranging from the molecular to the behavioural phenotype. However, such variation among nestmate queens remains poorly understood.

Here, we investigated i) whether nestmate queens from polygynous colonies of the ant *Stigmatomma pallipes* differ in activity, behaviour and gene expression, and ii) whether this variation is explained by their mating status and reproductive activity. We used tracking and behavioural scoring to identify two types of queens: low-mobility queens with queen-like behaviour and high-mobility queens with worker-like behaviour.

Dissections of the reproductive tract showed that only one queen per colony was mated and reproductively active. Those mated queens all expressed low-mobility and queen-like behaviour. Transcriptomic analyses revealed that unmated queens have similar brain and fat-body gene expression to workers, while mated queens differed from both unmated queens and workers.

These results elucidate that polygynous colonies of in the ant *S. pallipes* are functionally monogynous, and that unmated queens express worker-like behavioural and transcriptomic profiles. This plasticity within the queen caste raises questions on the functioning and evolution of polygynous colonies in ants.

Argentine ant control has no immediate impact on scavenging and seed removal

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Biological invasions are among the primary drivers of biodiversity loss globally, with invasive ants such as *Linepithema humile* exerting profound impacts on native species and ecosystems. While the effects of invasive ants on native biodiversity are well documented, their influence on ecosystem functioning and the effects of invasive control efforts on these processes remain poorly understood.

We investigated the impacts of *L. humile* on two key ecosystem processes, seed removal and scavenging, and evaluated the immediate effects of invasive ant control on these functions. Using a field manipulation experiment, we established 30 plots (10 m × 10 m) in a Mediterranean area in Sant Cristina d'Aro, Catalonia, divided into three treatments: uninvaded, invaded by *L. humile*, and invaded with *L. humile* subjected to control using biodegradable hydrogel beads. In each plot, we quantified seed removal using four stations, each containing 10 sunflower seeds, and scavenging using four stations with 2 g of dried *Tenebrio molitor* larvae. To prevent vertebrate consumption, all stations were protected. The stations were left in the field for 24 hours, after which we recorded the number of seeds removed and the mass of larvae consumed. Measurements were taken both before the application of the hydrogel beads and 12 days after treatment.

We found that *L. humile* invasions decreased seed removal rates by nearly 50%, but did not affect scavenging rates. Although controlling *L. humile* using biodegradable hydrogels reduced ant foraging activity by 70%, it did not increase seed removal, which remained lower than in uninvaded (control) areas. Similarly, the removal of *Tenebrio* larvae remained unchanged. This research demonstrates that although novel methods appear effective in controlling invasive ant populations, they may not be sufficient to restore key ecosystem functions disrupted by them.

The yeast core microbiome of the oligolectic ground-nesting bee *Andrena vaga*

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Insect-associated microbiomes play a key function in their physiology and response to different stressors, including environmental toxins, plant secondary metabolites and harmful nutrients. In the last decades, the major focus was on the core gut microbiome, mainly bacteria, of (eu)social bees. Research involving other bees and microorganisms was often neglected. Fungi, in particular yeasts from floral nectar and pollen, have recently been investigated and shown to play a role in various aspects of pollinators life, including foraging decision and bee physiology.

Here, we studied several matrices of the early nest stage of the grey-backed mining bee *Andrena vaga* using DNA-metabarcoding and cultivation. We found a highly diverse yeast community among all matrices, with *Triodiomyces crassus* being the only yeast detected on bee eggs. The soil yeast community of the brood chambers showed to be very different from all other matrices, whereas gut and provision were more similar in their composition. Especially in the gut, we found common bee-specific yeasts like *Starmerella bombicola*. Oligolecty is characterized by bees preferring specific food sources, for *A. vaga* it is *Salix* pollen. Potential toxins (e.g., salicin in *Salix*) might be metabolized by the detected yeast community to protect specialist pollinators, which might be first indication for the origin and functionality of oligolecty.

Effects of ethanol consumption on cognitive judgment bias in honeybee

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Ethanol is a toxic, neuroactive compound that naturally occurs in many environments, and foraging honeybees likely encounter it in fermenting floral nectar. While there exist studies on the compound's detrimental effects on honeybee behaviour and learning, it is still not known whether the low, field-realistic concentrations of ethanol influence foraging honeybees' cognitive and behavioural functioning.

In my proposed presentation, I will show how using the cognitive judgment bias (CJB) paradigm, we tested whether acute exposure to a field-realistic ethanol concentration (1%) could induce differences in decision-making under uncertainty, or 'optimistic' or 'pessimistic' bias in honeybee responses. In the study, bees were trained in a classical olfactory conditioning paradigm and then presented with conditioned stimuli and ambiguous odour mixtures to assess decision-making changes. We additionally measured locomotor performance and ethanol absorption via spectrophotometric analysis of haemolymph.

We found no evidence of CJB caused by ethanol, and no impairment of memory or locomotion, despite confirmed ethanol absorption. We interpret the results to suggest that acute exposure to low-dose ethanol does not induce changes in decision-making under uncertainty in honeybees. I will discuss the findings in eco-evo framework, investigating the possibility of evolved resilience of honeybees to ethanol concentrations present in their environment, and from a comparative cognition perspective, highlighting the differences and similarities in ethanol effects on affect and cognition across species.

Beyond the gut: exploring head-associated microbiota in *Cryptotermes* termites using a FAIR metagenomic workflow

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Microbiome research in insects has predominantly focused on the gut, given its central role in digestion and nutrient cycling. However, other body regions, such as the head, which includes salivary glands, mandibles, and neural structures, play key roles in behaviours like communication, foraging, and defence. Despite this functional importance, the microbial communities associated with the head remain largely unexplored across insect taxa, including termites.

In this study, we investigate the head-associated microbiome of two wood-dwelling termite species, *Cryptotermes secundus* and *C. domesticus*, sampled across different colonies. Using a reproducible metagenomic workflow (FAIRYMAGS) implemented in Galaxy, we recovered metagenome-assembled genomes (MAGs) from head-derived termite samples. This approach enabled high-resolution taxonomic and functional profiling, revealing microbial diversity potentially linked to salivary glands, mouthparts, or cuticular surfaces.

Our findings show distinct microbial profiles between species and identify taxa not commonly observed in gut-focused studies. Although it remains unclear whether all of the recovered microbes are resident symbionts or transient environmental associates, the recovered profiles indicate a distinct head-associated microbiota with possible functional relevance. The results raise new questions about the role of non-gut microbes in termite behaviour, health, and social organization. By sharing this workflow and case study, we aim to encourage further research into neglected anatomical niches in social insects.

Ant pupae signal sickness by a complex change in their chemical profile

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Social insects prevent disease spread through cooperative behaviours. While adult ants self-isolate when nearing death, immobile brood cannot leave the colony. Workers therefore maintain colony health by removing sick brood from the nest and disinfecting infected pupae, after cocoon removal, with their antimicrobial poison. This destructive disinfection stops pathogen replication and is triggered by chemical changes in the pupal cuticular hydrocarbons (CHCs). We found these changes are not a passive consequence of infection, but a signal actively produced by the pupae – rather than transferred by workers – and only in the presence of workers.

To identify the chemical basis of this signal, we compared CHC profiles of diseased, signalling pupae with those of healthy, non-signalling pupae using gas chromatography-mass spectrometry (GC-MS). We focused on two unsaturated compounds that were upregulated in signalling pupae: C33:2 (tritriacontadiene) and C33:1 (tritriacontene). To determine structural complexity, we used chemical modifications to identify positional isomers – molecules sharing the same chain length and number of bonds but differing in double bond positions.

Both compounds consisted of multiple positional isomers whose relative abundances changed during signalling: some increased, others decreased, while some remained constant. Therefore, the signal prompting unpacking and destruction represents a complex change in overall abundance and relative ratios of the constituting isomers.

We suggest that such a complicated regulation is key to ensure accurate and targeted communication about disease in the colony, reducing the risk of false positive destructive disinfection of healthy brood. Our findings extend previous research showing that unsaturated hydrocarbons carry rich social information, and further highlight the importance of isomeric diversity. Active chemical signalling of sickness through these complex changes enables rapid and precise colony-level responses to health challenges.

Quality over quantity? *Helianthus annuus* hybrid nutrients shape *Bombus terrestris* colony development

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Floral resource diversity, abundance, and nutritional quality are critical factors for determining the landscape potential to support pollinators. Sunflower, *Helianthus annuus* L., is a globally important oilseed crop with increasing cultivation and appeal due to being bee-friendly. Mass-flowering crops, like sunflowers, may increase the abundance of floral resources for pollinators, but at the cost of reducing the spatial and temporal diversity of resources in agricultural landscapes. Despite this trade-off, not much is known regarding sunflowers realistic impact as a nutritional source, particularly for bees.

We conducted a semi-field study in which *Bombus terrestris* colonies were housed in flight tents to induce foraging on specific Serbian sunflower hybrids (Duško, Konstantin, Romeo, and Kruna). The objective was to assess the impact of these hybrids which differ in pollen and nectar production, on colony development, using the most commonly sold hybrid (Kruna) as a control.

We found that the colonies that foraged on sunflower hybrids selected based on their nectar producing traits (Duško and Romeo), were able to develop more quickly and were significantly heavier two to three weeks after sunflower exposure compared to Kruna. The quantification of floral resources between hybrids were not significantly different. However, Duško and Romeo had pronounced differences in their pollen protein profile shown in a SDS assay. Hybrids also revealed significant differences in there nectar production. Such that Konstantin had significantly less sugar mass per floret after 48 hours compared to 24 hours of undisrupted nectar accumulation, suggesting quicker nectar reabsorption compared to Duško, Romeo, and Kruna.

Overall, these findings offer a novel glimpse into how different sunflower genotypes, with differing nutritional quantity and quality, impacted bumble bee development. Our findings suggest that floral resource traits should be given consideration when selecting sunflower genotypes for agricultural production, as they may offer advantages for neighbouring pollinator communities.

Managing conflicting functions: chemical and physical properties of ant cuticular hydrocarbons

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Global warming dramatically increases the desiccation risk faced by insects. To mitigate water loss, terrestrial insects are covered with a thin, chemically complex layer of cuticular hydrocarbons (CHCs), which in many species also functions as communication signals. Effective protection against desiccation depends on both the chemical composition of this layer and its resulting physical properties.

We investigated how acclimation to constant and fluctuating temperatures, together with habitat-specific thermal variability, influence the CHC layer in two European ant species collected along a continental–maritime climatic gradient, using GC–MS analysis and microrheology.

The viscosity of the CHC layer proved to be heterogeneous, characterized by a bimodal distribution of local viscosities. The higher-viscosity mode was comparable to honey (600–1000 mPa·s), whereas the lower-viscosity mode resembled olive oil (100–150 mPa·s). This bimodality suggests that each mode fulfils a distinct function, enabling the simultaneous execution of otherwise conflicting tasks. Ants acclimated to warm conditions often exhibited significantly higher CHC viscosity, tentatively indicating that CHC composition is adjusted to minimize water loss. Species and populations, however, differed in their acclimatory responses, suggesting that the capacity to adjust depends on native CHC composition and the thermal variability of the habitat. These findings provide new insights into the physical mechanisms underlying the multifunctionality of the CHC layer.

The tale of bees and blossoms… and tailocin-mediated interactions within their microbiomes

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Plants appear to be hot spots for various microorganisms. Favourable conditions, such as nutrient supply and protection against external environmental factors turn particularly flowers into an ideal habitat for bacteria. In general, the plant microbiome consists of both, beneficially associated microorganisms and plant pathogens. Among them, *Erwinia amylovora*, the causative agent of fire blight disease, is a pathogen that colonises the flowers of pome fruit trees, leading to severe agricultural losses in Europe and North America. Bees play a crucial role in spreading bacteria, including *E. amylovora*, through pollination. As pollinators ingest a variety of harmless and pathogenic bacteria during flower visits, their highly conserved bee gut microbiome requires strategies to defend against harmful organisms in order to protect itself and its host.

A well-studied mechanism to mediate interspecific interactions in the digestive system of bees are type VI secretion systems (T6SS) that bacteria use to kill competing bacteria by injecting toxins into neighbouring cells. Another antagonistic system is the use of tailocins – a sub-type of contractile phage tail-like particles (CPTPs) that can be produced by certain bacteria. Unlike T6SS, hundreds of tailocins can be released upon bacterial lysis that kill closely related bacteria by puncturing their membranes, leading to a collapse of the targets' proton motive force. However, tailocin-driven interactions of bee gut bacteria, ingested plant-associated bacteria, and pathogens remain unknown.

Here, we aim to identify natural tailocin-producing antagonists (TPAs) as a biological control strategy against *E. amylovora*, particularly for use in organic farming. We combine bioinformatics and lab approaches to (i) detect tailocin clusters within bacterial genomes, (ii) identify candidates for tailocin induction, and (iii) analyse their potential antagonistic effects via confrontation assays. First preliminary results indicate a highly variable molecular toolbox of such antagonistic systems for different groups of bacteria, including bee pathogens and bee gut symbionts.

Community organization of ants and plants in complex landscapes

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Complex landscapes containing multiple habitat types in close spatial proximity often serve as key targets for biodiversity conservation and provide valuable opportunities to study community assembly and species coexistence mechanisms. Despite their importance in both applied and theoretical contexts, the mechanisms shaping diversity metrics, functional trait distributions, and functional composition across trophic levels in such landscapes remain poorly understood. To address this knowledge gap, we used wood-pastures, one of the most ancient land use forms of Europe, as a model system. These landscapes harbour four distinct habitat types within a relatively small spatial scale: grasslands, large solitary trees, forests, and forest edges.

We sampled three wood-pastures along a 390 km transect in Central and Eastern Europe, focusing on two prominent groups from different trophic levels: ants and plants. We found significant differences in both taxonomic and functional composition for ants and plants across the four habitat types of wood-pastures. However, the mechanisms underlying these patterns differed between the two groups. RLQ and fourth-corner analyses showed that heterogeneity in environmental conditions mainly influenced plant functional traits. In contrast, ant diversity and trait distributions were more strongly associated with vegetation characteristics and habitat structure, and were only indirectly influenced by local microclimate, as revealed by path analyses.

These results suggest that while mapping the increased environmental heterogeneity of wood-pastures, the main mechanisms shaping functional composition and diversity metrics might differ for organisms at different trophic levels (i.e., predominantly environmental filtering for plants and interspecific competition for ants). Consequently, the patterns and peaks of taxonomic and functional diversity do not necessarily coincide across trophic levels in complex landscapes. This highlights that for effective biodiversity conservation in complex landscapes, a holistic approach that considers multiple taxa and trophic levels is essential.

Evolution of invasive supercolonies: new introductions and geographic isolation impact invasiveness of Argentine ants in Europe

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The invasion of exotic species is a major concern in the current globalized scenario, affecting the local ecosystems but also serve as good model for studying evolutionary mechanisms and species adaptations. *Linepithema humile* is one such species forming huge supercolonies ranging thousands of kilometres in the introduced region, attributed to genetic bottleneck. A previous study showed aggressive behaviour between distally located colonies of the same supercolony proposing a mosaic of competitive colonies within the same supercolony. Isolated colonies on their virtue of local adaptive conditions may suggest a possible divergence from the agreed upon supercolony theory. Another key element in understanding the supercolony structure is the period of invasion, post which divergence could occur.

To better understand these effects, we studied colonies of *L. humile* from island and nearby mainland locations separated by a natural barrier in two different locations differing in period of invasion: Northwest Spain, one of the first introductions of the species in Europe and Greece, one of the newer introductions. We performed a four axis metapopulation analysis between island and mainland super colonies: morphometry, genetic relatedness, cuticular hydrocarbons and aggressive behaviour.

We found that one colony from Heraklion, Greece has a very different genetic and cuticular profiles with lethal aggressive interactions with other Greek colonies, suggesting a new supercolony of the species. While, we found no unique divergence patterns between populations of island and mainland, we found that another island population in Greece to have similar cuticular profiles to the main supercolony in NW Spain and genetic profile of all Greek populations except Heraklion to have similarities with the main supercolony. These suggest that the introduction of *L. humile* in Greece was through the island. Our study emphasises that spatial and temporal variations in isolation induces difference among the colonies triggering aggression and destabilising the supercolony structure.

Fitness consequences of an alien fungal infection on an invasive ant species in Europe

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Anthropogenic activities are known to significantly alter environments, with the intentional or unintentional transportation of species potentially leading to biological invasion and emerging infectious diseases. Given the rising incidence of both phenomena, there is a critical need to understand how they interact, influence one another, and affect native biodiversity. Recently, the ectoparasitic fungus *Laboulbenia formicarum* has been observed infecting European populations of the invasive garden ant *Lasius neglectus*. The ecological and evolutionary consequences of this fungal infection for both the invasive species and native ant communities remain largely unknown.

As part of the EMINENT project, I aim to investigate these impacts through an integrative approach combining field surveys, controlled laboratory experiments, and molecular analyses. Here, I present preliminary findings on the effects of *L. formicarum* on the fitness of *L. neglectus* under both natural and experimental conditions. Building on datasets from 2014, comparative field surveys were conducted during the spring seasons of 2024 and 2025 to assess changes in supercolony spatial extent according to their initial infection status. Simultaneously, colony growth is being quantified in standardized microcolony setups by measuring brood production in relation to infection status. These complementary datasets provide first insights into the role of parasitism in modulating the invasive dynamics of *L. neglectus*.

Seasonal variation in host ant aggressiveness elicited by different social parasite types

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Social parasites are social insects that exploit other social insects. In ants, several forms of social parasitism are known, including temporary parasitism, which occurs when host workers are present in the parasite colony only during its foundation, while slave-making involves parasites supplementing their workforce with host workers throughout the colony’s lifetime. Defending against parasites can be costly, and such pressures are expected to drive the evolution of defensive strategies.

Here, we investigated seasonal patterns of aggressiveness in a common host species (*Formica fusca*) towards a temporary social parasite (*Formica polyctena*) and a slave-maker (*Formica sanguinea*). Behavioural tests were conducted across three seasons (spring, summer, and autumn) using host workers from colonies located (1) near *F. polyctena* colonies, (2) near *F. sanguinea* colonies, and (3) in parasite-free control areas.

Hosts from areas with *F. polyctena* were more aggressive towards both parasites in summer than in autumn, and consistently more aggressive towards *F. sanguinea* than *F. polyctena*. Hosts from areas with *F. sanguinea* showed peak aggression towards *F. polyctena* in spring and summer, and towards *F. sanguinea* in summer, and their responses did not differ between parasites across seasons. Hosts from control areas were most aggressive towards both parasites in summer, with similar responses to each.

These findings suggest that seasonal changes in host aggressiveness align with the timing of parasite threats: the colony founding period of *F. polyctena* in spring and early summer, and the slave-raids of *F. sanguinea* in summer. Such seasonal modulation of defensive behaviour may reflect the timing of parasitic and competitive threats, allowing colonies to minimise the costs of defence by increasing aggression only during periods of greatest risk.

Locomotor behavioural variations during the open field test: disentangling the effects of time and exploration

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The open field test (OF) is used to assess locomotion-related traits in a wide range of species. It is used as a tool in numerous research fields, from neurobiology to ecology. Nevertheless, the locomotory behaviour dynamics during the OF are still poorly understood. One main difficulty while interpreting results of this test lies in the effect of novelty. When the test begins, individuals are in an unknown environment, but the more they explore this environment, the more it becomes familiar. Locomotion in a novel environment is considered exploration and differs from activity, which defines locomotion in a known environment. Thus, we expect behavioural changes to occur during the test. If this behavioural switch does happen, the underlying mechanisms and the timing of this switch are important aspects to consider to unify the methodology of OF.

This study aims to characterize locomotion variations during the OF and to provide a method to objectively disentangle exploration from activity. We selected invasive ant (*Linepithema humile*) as our model species due to their high activity level and exploratory behaviour. Ants were individually placed in Petri dishes and recorded for one hour. Using an automatic tracking method, we measured various locomotion parameters and observed how they changed over time and with different OF characteristics (differences in size, enrichment, and temperature). We also tested whether the proportion of area explored may be a better predictor of the moment of transition between exploration and activity than a simple time threshold. Finally, to assess the robustness of our findings, we repeated this experiment with different arthropod species commonly used in OF.

These findings will contribute to a better understanding of the locomotory behaviour and of a widely used behavioural test. They will also provide practical guidance for designing OF with meaningful durations and acclimatization periods.

Adaptations of cuticular hydrocarbons to heat and drought stress in social insects

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All the insects investigated so far have been found to exhibit a complex blend of hydrocarbons (CHCs) on their cuticle. These profiles are often species- and sex-specific, playing a crucial role in intra- and interspecific recognition and communication processes particularly in social insects. Additionally, the CHC layer acts as a protective barrier against desiccation. These profiles are composed of up to 200 different compounds belonging to three substance classes: alkanes, methyl-branched alkanes, and unsaturated hydrocarbons, which have different physicochemical properties. These differences enable the CHC profiles to adapt to environmental conditions such as high temperatures or drought stress by altering the relative composition of compounds with different steric structures.

In this presentation, I compare the CHC profiles of related social insect species across elevational and latitudinal gradients to demonstrate the impact of abiotic factors on CHC selection and profile composition.

Evaluating the risk of biopesticide tank mixtures in social and solitary bees

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Social insects play crucial roles in ecosystems as pollinators, decomposers, and regulators of food webs. Among them, bees are indispensable for both wild plant reproduction and agricultural productivity. However, their populations face increasing threats from multiple anthropogenic pressures including widespread use of pesticides and land management. While biopesticides are often marketed as environmentally friendly alternatives to chemical synthetic ones, risk of tank mixtures consisting of multiple biocontrol agents on bees, remains poorly studied.

This study is part of a broader research project investigating the effects of insecticidal biopesticide mixtures on pollinator health. Here, we examined three bee species with different social structures with a focus on contact exposure. Sublethal and lethal effects of biopesticide tank mixtures were evaluated in the highly eusocial honey bee *Apis mellifera*, the primitively eusocial bumble bee *Bombus terrestris*, and the solitary mason bee *Osmia cornuta*. Standardized exposure tests were conducted under controlled laboratory conditions using adult individuals. The assessment included behavioural impairments, mortality, and species-specific sensitivity to the mixtures.

Exposure to tested tank mixtures resulted in mortality up to 22.4% in honey bees, whereas it remained lower in mason bees (6.3%) and bumblebees (6.7%). The results indicate that honey bees exhibited the highest sensitivity, with up to 78% showing abnormal behaviours compared to less than 10% in the other two species. The observed results highlight the need to consider the interspecific differences when evaluating the potential risks of external stressors such as pesticides on bees. Furthermore, while this study focused on adult contact exposure, future work will include oral exposure routes and exposure of larvae to provide a more comprehensive understanding of risk across developmental stages and exposure pathways.

Distribution of mound building *Formica* species in Romania – a historical perspective

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Mound building *Formica* species (MBF) have a key role in ecosystems. They have serious impact on invertebrate communities, assist seed dispersal, and have a decisive impact on soil properties by e.g. accumulating organic matter in their nests. Their role is also pivotal with regards to pest control in forest habitats. In Romania there are five MBF species known to occur: *F. exsecta*, *F. pratensis*, *F. polyctena*, *Formica rufa*, and *F. truncorum*. Despite the ecological importance of these species, data on their distribution in Romania is scarce.

We now reviewed the existing scientific literature about ants concerning the current territory of Romania starting from the mid-19th century and extracted data on MBF species and their distribution. In addition, new data was also added based on current ongoing country-wide field surveys. Consequently, areas lacking MBF species, thus potentially needing further field surveys were identified.

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI - UEFISCDI, project number 25/2024 COFUND-BIODIVMON-MonitAnt, within PNCDI IV.

Feeding for the heat: nutritional modulation of thermal resistance in ants

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Ants are key components of terrestrial ecosystems, but their limited mobility makes them especially vulnerable to local environmental conditions. Like many organisms, they must cope with rising temperatures caused by global warming and urban heat islands. To deal with heat stress, ants rely on both behavioural and physiological strategies. Physiologically, they upregulate heat shock proteins (HSPs), which help refold damaged proteins, and reinforce their cuticular hydrocarbons (CHCs) layer to limit water loss. A protein-rich diet may promote HSP expression by supplying essential amino acids for protein repair and synthesis, while also supporting CHCs production through improved metabolic and enzymatic function. Carbohydrates, in turn, might help sustain ATP-dependent chaperone activity and can contribute to CHCs biosynthesis, especially when converted into lipids.

In this study, we hypothesised that changes in nutrient availability in human-modified environments could directly affect ants' thermal tolerance. To test this, we analysed the expression of two HSPs (HSP70 and HSP83) and the CHCs profile in *Lasius niger* workers maintained on five artificial designed diets differing in essential amino acid-to-carbohydrate ratios.

Our results show that protein-rich diets generally led to higher expression levels of HSP70 and HSP83. Diet composition also affected CHCs profiles, although the patterns differed between urban and rural populations. In urban ants, the most distinct CHC profiles were observed in response to diets with intermediate essential amino acid-to-carbohydrate (EAA:C) ratios. In contrast, in rural ants, the greatest divergence in CHCs composition occurred in response to the diet with the highest carbohydrate content. These contrasting responses suggest potential evolutionary adaptations in nutrient processing and thermal protection strategies shaped by habitat type.

Latent learning in a tool using ant species

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Ant species from the *Aphaenogaster* genus use debris as tools to collect and transport liquid food to their nests. In our previous work, we formed groups of workers with similar behavioural types and showed that groups of high exploratory workers were more efficient in four ecologically relevant tasks. As a next step, we investigated whether *Aphaenogaster senilis* ants use latent learning during foraging. Latent learning remains hidden at the time of acquisition but manifests later, enabling animals to navigate their environments more efficiently, for example, by improving foraging efficiency.

To test this, we exposed one group of ants from a colony to an empty maze, while another group (control) had no prior exposure. In subsequent trials with food, ants with previous maze experience found the food faster than controls, demonstrating that latent learning enhanced foraging efficiency, particularly in the first two trials. We then characterized maze-solvers and non-solvers for their individual exploratory behaviour and found that solvers were less exploratory than non-solvers, suggesting a possible speed–accuracy trade-off.

These findings indicate that inter-individual variability in foraging strategies may be partly explained by differences in latent learning, and that worker traits may interact to enhance efficiency during foraging and other ecologically relevant tasks.

Service from beyond – direct evidence for cannibalistic necrophagy in ants

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Nitrogen is an essential nutrient for the development and reproduction of most organisms. However, species differ in the sources they exploit and the mechanisms they use to acquire it. In ants, protein requirements are typically met by preying on other arthropods. Nonetheless, these resources can become scarce due to seasonal changes, particularly in temperate or boreal ecosystems. One potential, consistently available nitrogen source is nestmate corpses, consumed through cannibalistic necrophagy, a well-known behaviour in termites but, in ants, it has only been reported indirectly.

In our study, we used a fluorescence-based technique to directly test the occurrence of this behaviour in ants. We provided fluorescently marked food to wood ant workers, and after consumption, their corpses were offered to food-deprived nestmates. Subsequently, we dissected the anesthetised starved nestmates and examined the soft tissues of the crop and midgut under a fluorescence microscope for traces of the marker.

Detecting fluorescence within the digestive tracts of workers provided direct evidence that corpses can indeed serve as a food source in some ant species, helping to meet the colony’s nitrogen requirements. We argue, however, that it is mainly utilized when food availability is scarce. By enabling the recirculation of nitrogen from deceased colony members, necrophagy may play a major role in the ecological and evolutionary success of some ants, mainly those with larger colony size.

Does social immunity come at a cost of immunopathology?

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Immune responses are often destructive in nature as their primary aim is to eliminate pathogens and infected cells from the body. To avoid additional costs and side effects such as also harming the healthy tissue during aggressive anti-pathogen treatment, it is key that immunity measures are targeted precisely to prevent costs of mistargeted “crossfire”. Still, it cannot be completely avoided, for example in the broad and non-specific release of reactive oxygen species or in the misidentification and hence, removal of healthy cells, leading to an additional, indirect cost of the pathogenic infection referred to as immunopathology. Just like immune cells identify and remove virus-infected cells in our bodies, *Lasius* garden ant workers remove brood that suffers fatal fungal infection by destructive disinfection, wherein the ant workers are known to eliminate infected pupae during the asymptomatic pathogenic incubation period in order to control disease spread.

We here determine the immunopathology costs of social immunity, in the form of uninfected pupae also being destroyed, leading to a loss of healthy brood alongside the infected pupae. We predict that the false positive rate of destructive disinfection may depend on disease prevalence. Our study thus, defines how exact ants are at targeting the risks to colony health and whether they may change their risk assessment in case of extensive disease burden to the colony, allowing us to determine yet unexplored possible costs to the colony by the exertion of social immunity.

Keeping the antenna clean: a key feature in social immunity?

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To protect their colony from disease, ants react to incoming pathogens by immediate grooming of contaminated colony members. This response underlies their ability to chemically detect pathogens by an olfactory system that is greatly enriched compared to solitary insects, with the antennae being the most important external structural component. To keep proper antennal function, hymenopterans evolved an intricate organ, the antenna cleaner (strigilis), a comb-like structure that removes particles and secretions, preventing them from accumulating on the antenna. The antenna cleaner allows to remove particles in a broad size range and is necessary for maintaining the structural integrity of the antennal surface, which otherwise becomes covered with a cuticular hydrocarbon layer, impairing their ability to react to alarm pheromones and differentiating between nestmates and non-nestmates. Given this broad effect on their detection ability, we predict that a functional antenna cleaner will also be of importance for ants to detect and react to incoming pathogens – that is, their effective performance of social immunity.

Here we test whether impairing the antenna cleaner function, and thus antennal sensitivity, has an impact on the care behaviour of garden ants towards colony members that were exposed to spores of a pathogenic fungus. By structurally perturbing the antenna cleaner of nestmates through gluing the two main structural components together, we test if malfunctioning of the antenna cleaner may impair the ants’ grooming efficiency, making them “worse helpers”, or also their ability for risk-adjusted self-protection during caregiving, which would in both cases endanger the whole colony by lowering the efficiency of disease-preventing mechanisms.

Nutritional adaptations to urban environments of two synanthropic and co-occurring ant species

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Urban environments are characterized by a reduction in local biodiversity, which limit the availability of food resources for the organisms inhabiting them. This is particularly important for animals with limited mobility, such as ants, as they are unable to exploit distant sources. Resources scarcity in urban areas might also force competition among co-occurring species, potentially influencing species interaction.

In this study, we investigate potential shifts in dietary preferences across ant species as an evolutionary adaptation to either enhance competitiveness or reduce interspecific competition for resources in urban areas. We focus on *Lasius niger* and *Myrmica rubra*, two generalist ant species that share habitats in both urban and rural areas of the Palearctic region. To assess dietary preferences, we conducted a common garden experiment using urban and rural colonies of both species. Subcolonies (two per mother colony) were offered three artificial diets that varied in their ratios of carbohydrates and essential amino acids (EAA). Diet consumption was determined by weighing each type of food before and after exposure to the subcolonies. The feeding activity has been also recorded for further analyses of the foraging and feeding efforts.

Our results show that immediately after collection, *L. niger* exhibited a strong preference for the carbohydrate-rich diet, while *M. rubra* displayed a more generalist feeding pattern. After one week on a standardized artificial diet, both species showed similar dietary profiles. Notably, dietary preferences differed more strongly between species in rural populations, whereas in urban populations, both species consumed all three diets more evenly.

These findings suggest a possible adaptive shift toward broader dietary flexibility in urban habitats. This may reflect a response to nutritional constraints or reduced interspecific competition resulting from biodiversity loss, highlighting the role of nutrition in mediating species coexistence in anthropogenic environments.

Distribution and diagnosis of American foulbrood in honey bee hives

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Honey bees are eusocial insects that are mainly kept by humans for honey production and pollination services. Due to the high density of bees and the homeostatic conditions in the hive that are needed for optimal brood care, it is easy for pathogens to spread within a colony. The most devastating brood disease is American foulbrood (AFB) that is caused by the spore-forming bacterium *Paenibacillus larvae*. An early and reliable diagnosis of *P. larvae* is contributing to stop the disease spread.

Therefore, we developed a lateral flow assay (LFA) that should be used with suspicious honey bee larvae at the hive. Sample material of AFB-infected or suspected of being infected honey bee hives have been collected and tested for the presence of *P. larvae* using either the cultivation method of larvae lysates on Columbia CNA Agar with 5% Sheep Blood, a conventional 16S-rRNA gene-based PCR or the developed LFA. Samples were collected in Germany in 2022 and 2023. Samples (n = 106) were collected either on site or with the assistance of bee inspectors and veterinarians. Using the cultivation method, we could quantify the infection of the material with *P. larvae*. The limits of detection (LOD) have been approximated of colony forming units (CFU) in the tested larvae for PCR (≥1250 CFU/larva) and LFA (31000 CFU/larva).

We found that the cultivation method is the most sensitive detection method and serves as a gold standard followed by the PCR (~77%) and the LFA (~72%). Despite the huge variance in the detection limit between PCR and LFA, similar numbers of positive larvae have been detected. Therefore, the LFA can be considered a great tool for detection and a fast overview of the disease status of honey bee hives using suspicious honey bee larvae.

The role of juvenile hormone in the regulation of caste determination and differentiation in an ant

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Division of reproductive labour between queens and workers is the defining trait of eusocial insects. In ant species with morphological castes, whether an individual develops into a queen or a worker is decided during development, prior to the imaginal moult. Juvenile hormone, which together with ecdysone regulates development across insects, is known to play a key role in caste determination and differentiation across different species of ants. In the ant *Cardiocondyla obscurior*, adult workers completely lack reproductive organs, and the female caste is already determined by late embryogenesis, likely via maternal effects.

We investigated how juvenile hormone affects caste determination and differentiation in this ant by feeding egg-laying queens as well as queen- and worker-destined larvae with a juvenile hormone analogue. First results show that neither caste ratios nor body size or morphology are affected when only larvae are fed with juvenile hormone, consistent with the idea that caste is irreversibly determined by maternal effects during early development. Accordingly, when whole colonies containing egg-laying queens were fed with juvenile hormone, they almost exclusively produced new queens. We are currently testing how the production, morphology and physiology of new queens are affected when only egg-laying queens receive juvenile hormone-supplemented food. Together, the results from these experiments will bring new insight into our understanding of the hormonal regulation of maternal caste determination in ants.

Cost of defence: infection-driven trade-offs between social behaviour and molecular immunity in ants

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Social insects are excellent model organisms for studying immune system functioning, largely due to their exposure to a wide range of pathogens. A potential trade-off may occur between individual and colony-level immune defences, as the energetic investment at each level may vary depending on the specific threats a colony faces. However, the dynamics between these two immunity levels, and the mechanisms underlying potential trade-offs, are still not well understood.

In this study, we investigated both social and individual immune responses in *Formica polyctena* colonies, focusing on phenoloxidase, a key enzyme involved in defence against various pathogens. We measured collective defensive behaviours at the colony level and analysed the molecular immune activity (both active and inactive forms of phenoloxidase) in workers exposed to corpses carrying three types of pathogens: *Rickia wasmannii* hyphae (a *Myrmica* genus specific fungus harmless to *F. polyctena*), *Aegeritella* sp. hyphae (associated with the *Formica* genus), and spores of *Beauveria bassiana*, a generalist entomopathogen, which infects a broad range of insects.

The pathogen-infected corpses triggered varying levels of social defence compared to controls. The strongest social behavioural responses were observed in response to corpses infected with *Aegeritella* sp. and *B. bassiana*. However, a measurable individual immune response was detected only in the *Aegeritella* treatment, likely because *B. bassiana* spores were efficiently removed by hygienic behaviours. These results contribute to a better understanding of how social insects integrate immune responses across individual and collective levels, offering new insights into how personal and social immunity are balanced in complex eusocial systems.

Standardisable 3D-printed ant farms: a universal tool for ant rearing in the laboratory

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Standardized rearing conditions are essential for reproducibility and comparability in laboratory myrmecological research. Although artificial ant nests (formicaria) are commonly used, the biological effects of different nest designs remain underexplored. We developed and tested a set of universal 3D-printed ant farms – Andrásfalvy-S, -M, and -L – designed for broad applicability in laboratory studies. These farms incorporate practical features such as modularity, standardized dimensions, a humidity gradient, removable feeding components, and compatibility with common laboratory equipment (e.g., microscope slides, test tubes). In a controlled experiment, we compared colony development and overwintering success of *Lasius niger* colonies reared in the medium-sized Andrásfalvy-M farms versus traditional test tube nests placed in plastic box arenas (TTPB nests).

Colony growth did not differ significantly between nest types, but time required for maintenance – especially feeding – was significantly lower in the Andrásfalvy-M farms. We further evaluated the applicability of all three farm types by rearing 98 colonies representing 50 different ant species with diverse life history traits. All three farms proved suitable for development and successful overwintering. Andrásfalvy farms were reusable for over two years, provided controlled microclimatic conditions, and allowed gentle colony transfer via connectable modules.

These results demonstrate that the Andrásfalvy farms are effective, flexible, and reproducible tools for ant rearing in the laboratory. Their standardized design can facilitate cross-study comparisons and improve experimental efficiency. The 3D models and setup protocols will be made openly available upon publication of the corresponding manuscript.

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