IUSSI NW European Winter Meeting 2022

Abstract booklet

11:15 - 11:30 **Yannick Wurm, on behalf of Marian Priebe**, Queen Mary University of London
Gene duplication facilitates the evolution of polyphenism in ants

The evolution of superorganismality in ants and subsequent development of distinct phenotypes has provided us with a polyphenism more complex than the ancestral sexual dimorphism. A single genome produces the three discrete castes of workers, queens and males. Our understanding of the processes required to encode such dramatically distinct types of individuals is limited, making ants an excellent system for studying phenotypic innovation.

To understand the transcriptomic architectures underpinning this extreme polyphenism we generated tissue-specific whole transcriptome gene expression data from 19 distinct tissues from all three castes. We identified gene duplications within the genome of the red fire ant *Solenopsis invicta* dating back to the evolution of superorganismality and characterised differences in expression of duplicated genes and gene families across the three castes. We find many examples of duplication and caste-specific subfunctionalisation, with individual genes becoming adapted to the castes in which they are expressed. Our results shed light on how gene duplications allow for a single genome to encode a wide array of distinct castes in the ants and are central to phenotypic innovation in this system.

11:30 - 11:45 **Josie Monaghan***, University of York
A tale of two species (groups): Untangling hybridisation in mound-building red wood ants

Hybridisation between species is common in nature. Recent advances in genome sequencing technology have helped reveal its prevalence across diverse taxa. Mound-building red wood ant *(Formica rufa* group) species play a significant role in forest ecosystems across the Palearctic, and morphological and genetic data have shown extensive hybridisation between these species across Europe. We previously found evidence of unidirectional introgression of mitochondrial haplotypes in the three species extant in Britain. To further explore signals of gene flow, we generated double-digest restriction associated digest (ddRAD) DNA sequence libraries for 123 nests from across the British distributions of *F. rufa*, *F. lugubris* and *F.aquilonia*.
The range of *F. lugubris* overlaps with that of both other species and there are forest locales where two species occur in sympatry. To explore the possibility of current gene flow between species we included samples from both single-species and two-species woodlands. We also included four *F. rufa*, four *F. polyctena*, and four known-hybrid samples from European populations. We generated a total of 135 ddRAD libraries for population structure and gene flow analyses. Principle component analyses (PCA) on 7591 SNPs showed tight species clusters with few intermediates. Estimated co-ancestry analysis supports the species-based clustering of the PCA, however, it also suggests some gene flow between species including a possible F1 hybrid. The co-ancestry results also show various levels of within-species population structure based on geography.
Presented together, our morphological, mitochondrial, and nuclear sequence data provide compelling evidence for current and historical hybridisation between species in British red wood ants.

11:45 - 12:00 **Tomas Kay***, University of Lausanne

Social network position is tightly associated with physiology and behavior in carpenter ants

In social insect colonies workers specialise in different tasks according to their age: younger workers tend to nurse while older workers tend to forage. This has typically been observed by documenting the behaviour of cohorts of age-matched workers at relatively coarse temporal resolutions. Consequently, little is known about the dynamics of individual behavioural change and about how less frequently performed tasks (e.g. cleaning) are integrated into the nurse-to-forager transition. To understand the dynamics of behavioural maturation, we analysed the performance of nursing, foraging and cleaning across four months of automated tracking data from three colonies of *Camponotus fellah*. We found evidence of three seemingly distinct behavioural trajectories: ~43% of workers nursed when young, cleaned at intermediate ages, and foraged when old. However, ~20% of workers transitioned directly from nursing to foraging, and ~37% nursed, and then cleaned, but never transitioned to foraging. The presence of qualitatively different behavioural trajectories among morphologically similar workers is unexpected, and expands our understanding of the regulation of division of labor in insect societies.

12:00 - 12:15 **Laura Campbell***, Durham University

The Evolution of Plant Cultivation by Ants

Outside humans, true agriculture was previously thought to be restricted to social insects farming fungus. However, obligate farming of plants by ants was recently discovered in Fiji, prompting a re-examination of plant cultivation by ants. Here, we generate a database of plant cultivation by ants, identify three main types, and show that these interactions evolved primarily for shelter rather than food. We find that plant cultivation evolved at least 68 times independently for crops (~200 plant species), and 16 times in farmer lineages (~37 ant taxa) in the Neotropics and Asia/Australasia. Because of their high evolutionary replication, and variation in partner dependence, these systems are powerful models to unveil the steps in the evolution and ecology of insect agriculture.

12:15 - 12:30 **Rachael Brown***, University of Bristol

Investigating a body shake behaviour as a potential pathogen alarm signal in Lasius niger

Living in densely populated social groups can provide many fitness benefits to individuals, but also incurs significant costs, such as an increased risk of disease transmission between group members. To counteract this risk, social insects have evolved a series of cooperative disease defence mechanisms which protect them against epidemics. It is vital for colony members to detect a disease threat early so they can mount an effective, rapid collective response. Yet how this information might spread through the colony is still largely unknown. Here, we aim to characterise a whole-body shake behaviour in the ant Lasius niger and test its potential role as a pathogen alarm signal. We measured the frequency of the body shake in different sized groups of workers upon controlled exposure to the entomopathogenic fungus Metarhizium brunneum. Upon meeting a pathogen-exposed worker, unexposed nestmates performed significantly more body shakes than when meeting with a sham-exposed worker. Furthermore, exposed workers performed more body shakes when nestmates were present, regardless of group size. Overall, body shakes may act as a general alarm signal which increases in the presence of a pathogen. Our work may provide new information on how L. niger workers detect infectious threats and potentially signal to nestmates for a rapid, colony-wide response.

14:35 - 14:50 **Linzi Jay Thompson**, University College Dublin
Effects of pesticides on bumblebee queens

Bumblebee queens receive little attention in understanding how pesticides may affect them. Queens are responsible for founding colonies and the production of new queens towards the end of the colonies life, making them important for ensuring stable populations with a diverse genetic pool. During this time, they may be exposed to various pesticides when foraging in the landscape and preparing for diapause. In this experiment we researched how a field realistic dose of either the fungicide prothioconazole, the insecticide acetamiprid or a combination of both effected their ability to survive a 12 week artificial diapause and then initiate a colony. We found that multiple exposures to acetamiprid significantly reduced the longevity of queens and that there was a trend for the insecticide-fungicide mixture to reduce nest initiation. Our results suggest that pesticides can be harmful to queens when they are exposed during their most vulnerable life stages, which at current is overlooked in pesticide risk assessment. Due to the importance of the queen to reproduction it is important that more research is carried out exploring these potential negative effects.

14:50 - 15:05 **Alicja Witwicka***, Queen Mary University of London
Dose-, tissue- and pesticide-specific effects of insecticides on gene expression in Bombus terrestris

Social bees are important insect pollinators of wildflowers and crops, yet their populations are declining because of intensified agriculture and the use of insecticides. Multiple studies reported that neurotoxic insecticides in fact have various sub-lethal effects that hinder pollinator survival. Such findings show that methods used in acute toxicity tests are insufficient to determine the whole spectrum of negative effects of insecticides, and have repeatedly led to restrictions in insecticide usage, challenging the workflows of insecticide manufacturers and regulators. We suggest that the sub-lethal effects of insecticides may be easier to understand if their impacts on pollinator health are measured in a high-resolution manner.
Here, we exposed *Bombus terrestris* bumblebees to acute and chronic doses of three insecticides: Clothianidin, Acetamiprid, and Sulfoxaflor. We find major differences in the effects of acute and chronic exposure to the same insecticide. We also find major differences in the effects of insecticides in brains, Malpighian tubules, and leg muscles. We discuss the implications of the specific findings on our understanding of how the insecticides act and why their effects could have been misevaluated.
Our new findings demonstrate the power of high-resolution molecular methods for disentangling the unintended effects of insecticides. Such “toxicogenomic” approaches, which are widely used in assessments of drugs for humans, are a powerful tool that can accurately assess the effects of insecticides on non-target species, improve insecticide safety assessments, and inform regulatory efforts.

15:05 - 15:20 **Jacob Podesta***, University of York

North, South, East and Nest: How the bearing of artificial canopy gaps affects the distribution of an edge specialist ant in plantation forests

Population distributions of slow dispersing edge specialists are potentially affected by local topography and the land management practices. We use the population expansion of *Formica lugubris* in the North Yorkshire Moors, England, to explore the effects of the bearing of linear features in plantation forest on the dispersal potential and population of wood ants. *F. lugubris* requires both sunlight, from canopy gaps, and trees, as a source of both aphid honeydew and invertebrate prey, and therefore typically occupies forest margins, where both are available. In the plantation forests of the North York Moors, the predominating form of canopy gaps are paths, firebreaks and roads (rides), which are often very straight and uniform in width to accommodate timber harvesting vehicles. In high latitudes, this means that the amount and pattern (spatial and temporal) of light availability is determined by the bearing of the ride and has consequences for the populations of wood ants. Using nearly 10 years of population margins mapping data in conjunction with recent data collection, we attempt to understand how the land management and forestry practices have facilitated or hindered the expansion of these populations, and to what extent the wood ants' current distribution on the North York Moors has been influenced past forestry planning decisions.

16:05 - 16:20 **Nanna Hjort Vidkjaer**, University of Copenhagen

Chemical interactions between the termite fungus crop and antagonistic fungi

Fungus-growing termites (Macrotermitinae) farm a basidiomycete fungus, *Termitomyces*, inside their nest providing nutritious food for the termite host. Despite the termites foraging in pathogen-rich environments, their fungus crop appears remarkable free from antagonistic fungi. Only when the termites are removed or the colony is dying, does the stowaway ascomycete fungus *Pseudoxylaria* appear. *Termitomyces* produce chemical compounds with potential roles in suppressing competitive fungi in the termite nest and *Termitomyces* can inhibit the growth of *Pseudoxylaria* in lab cultures. We hypothesized that natural products produced by the fungal symbiont play a role in defence against *Pseudoxylaria* and other antagonistic fungi and that the production of these compounds is upregulated when *Termitomyces* is challenged by other fungi in co-cultures.

We grew *Termitomyces* and *Pseudoxylaria* in mono- and co-cultures on media offering optimal nutrition and collected plugs for chemical profiling by UHPLC-QTOF-MS/MS. To evaluate how the natural nest conditions influence fungal metabolite production, we also grew the cultures on fungus-comb media and at high CO2 levels characteristic of termite mounds. Our results reveal a complex regulation of *Termitomyces* and *Pseudoxylaria* metabolite production influenced both by co-cultivation and by nest conditions. We are now analysing mass spectrometry imaging data visualizing the spatial metabolite distributions to disentangle which compounds are produced by each fungus during co-cultivation. Uncovering the chemical signalling that governs interactions between *Termitomyces* and *Pseudoxylaria* is important for assessing the role that the crop fungus plays in its own and consequently colony defence.

16:20 - 16:35 **Quentin Willot**, Aarhus University

Thriving in the cold: tolerance to low temperatures and metabolic rate track distribution in ants

Colder climates pose two important challenges for ectotherms. First, they entail the occurrence of cold-extreme episodes and second, they constrain the amount of energy available for development and reproduction. Consequently, increased cold-tolerance is commonly observed in ectotherms found in higher altitude/latitude environments, which may be accompanied by metabolic adaptations. In insects, the occurrence of metabolic cold adaptation to compensate for the effects of lower annual temperatures has received ambiguous support, but might be important for comparatively sessile insects such as ants. In this study, our objectives were thus threefold. First, we (i) aimed at validating the occurrence of metabolic cold adaptation within a single consistent phylogenetical framework made of 13 ant species covering a wide latitudinal distribution in western Europe. Second, we (ii) set to explore the climatic factors driving metabolic cold adaptation using a soil-surface temperature climatic database, and (iii) probe for the potential association of metabolic rate and cold tolerance. Within this phylogenetic framework, we show a clear metabolic compensation effect in higher latitude ant species, with increased metabolic rates at lower temperatures, and a comparatively reduced thermal sensitivity of their respiration rate (Q10-SMR) under warmer conditions. Metabolic rates and Q10-SMR values further track climatic parameters characteristic of colder climates. Adaptation to cooler climates through higher metabolic rates at lower temperatures thus appear to be a key feature of cold adaptation in ants.

16:50 - 17:05 **Tom Bishop**, Cardiff University

Global patterns of convergence in the ants

Does the environment determine the way that ecology and evolution unfolds? If it does, we would expect species and communities in similar environments but different geographic regions to converge in their phenotypes. While there are examples of species-level convergence, the generality of this phenomenon is unclear, and there have been no quantitative tests of global convergence at the community-level. Here, we address this topic in a functionally critical group of insects: the ants. We first quantify the substantial global variation in ant morphology. Then, we use these data to detect patterns of convergence between species and between entire ecological communities. Our results reveal a disconnect between species and community-level convergence. Many ants appear to have converged upon similar morphologies, but these patterns are easily replicated by a randomly evolving null model. At the community level, however, communities on separate continents, but occupying similar environments, tend to converge in their morphological composition. These patterns cannot be replicated by our null modelling and exist despite the deep taxonomic and phylogenetic differences that exist across different continents. In sum: our data reveal how the environment determines the morphology of entire ant communities, but not necessarily of individual species.

17:05 - 17:20 **Christopher Pull**, University of Oxford
Ecology dictates the value of memory for foraging bees

Ecology dictates the value of memory for foraging bees: “Ecological intelligence” hypotheses posit that animal learning and memory evolves to meet the demands posed by foraging, and together with social intelligence and cognitive buffer hypotheses, provide a key framework for understanding cognitive evolution. However, identifying the critical environments where cognitive investment reaps significant benefits has proved challenging. Here, we capitalise upon seasonal variation in forage availability for a social insect model (*Bombus terrestris*) to establish how the benefits of short-term memory vary with resource availability. Through analysis of over 1700 foraging trips carried out over two years, we show that short-term memory predicts foraging efficiency – a key determinant of colony fitness – in plentiful spring foraging conditions, but that this relationship is reversed during the summer floral dearth. Our results suggest that selection for enhanced cognitive abilities is unlikely to be limited to harsh environments where food is hard to find or extract, highlighting instead that the complexity of rich and plentiful environments could be a broad driver in the evolution of certain cognitive traits

17:20 - 17:35 **Richard Gill**, Imperial College London
Seasonal timing of food availability influences bumblebee colony fitness

Wildflower loss from agriculture has dramatically changed the nutritional landscape for insect pollinators, like bumblebees. Whilst the farming of non-flowering crops provides little food, in contrast the farming of mass monocultures can provide a large amount of nectar and pollen. Thus, should concerns over bumblebee fitness in flowering crops landscapes be warranted? One potential issue of bumblebees relying on flowering monocultures is that crop phenology is highly synchronised leading to pulses and gaps of food across a species life cycle. Dependent on whether the flowering species is early or late relative to pollinator emergence times, this creates a 'bloom-and-bust’ or 'bust-and-bloom' food availability scenario that may mismatch with important colony developmental stages. We tested whether bumblebee colony fitness is based not on how much is eaten per se but when it is eaten, by altering ‘when’ during colony development food restrictions are experienced. Colonies experiencing a bloom-bust (high food income followed by relatively low) were able to rear a large workforce and a high number of sexuals (males), but the quality of later emerging males (body mass) was lower relative to colonies experiencing a bust-bloom (low-high). This suggests early mass flowering crops provide a good kick-start to colony growth, but creates an over-dependency on food income, leaving colonies experiencing a bloom-bust scenario to trade-off male mass for number. Moreover, higher colony initial mass exaggerated diet treatment impacts, showing that colony size at the time when flowering crops bloom (proxy of seasonal timing of colony founding) determines colony growth trajectories.

Flash talks:

1. **Mahika K. Dixit***, Imperial College London
   Surprising consistency of insect pollinator body-size through time, despite differences in responses among life-history groups.

Considering current rates of biodiversity loss and habitat modification, understanding the long-term trends in key taxa is important for protecting these taxa and their associated ecosystem processes, especially under anthropogenic stressors like climate change. Trends in body size are of particular interest in pollinators due to the links between pollinator body size and ecosystem functioning. Here we quantify body size trends in two key insect pollinator groups over the past century using multiple museum collections, thus circumventing shifting baselines. We use inter-tegular distance (distance between the points where the forewings attach to the body) as a proxy for body size in landmarked images of 6 species of UK bumblebee and 9 species of UK butterfly. Remarkably, we find that body size has remained relatively constant across the century and in response to temperature in these taxa, suggesting that environmental change is not the major determinant of body size change in these taxa. We do, nonetheless, find that some species of both taxa show weak trends with temperature and temporal trends in two species of butterfly, suggesting small and species-specific body size change. Different bumblebee castes and butterfly life stages also showed differences in response. Ultimately, these findings suggest that the responses of pollinator body size to environmental change are more subtle and species-specific than previously thought, meaning that broad meta-analysis studies may miss such trends. Regardless, these results overall suggest that the future of insect pollinators under anthropogenic climate change may not be as universally dire as previously thought.

2. **Gabriel Hernandez***, Queen Mary University of London
   Multiple optima - evidence of balancing selection in the genome of the fire ant

Red fire ants are fascinating superorganisms with diverse phenotypes. Each colony contains individuals from three castes, queens, males, and workers that differ dramatically in morphology, physiology, behaviour, and lifespan. Furthermore, this species has two distinct social forms with single-queen colonies which have higher fitness in new habitats, and multiple-queen colonies that can outcompete single-queen colonies in dense habitats. How can a single genome encode such divergent phenotypes, where the genome seems to be under simultaneous selection for multiple optima?
In some cases, the solution lies in phenotype-specific gene expression. However, we also hypothesize that some fire ant genes are under balancing selection, i.e. where selection simultaneously favours multiple divergent alleles. To test whether this type of intragenomic conflict occurs in the ants, we analysed whole genome sequences of 200 haploid male *Solenopsis invicta* fire ants collected from across the native range of this model species. We indeed found dozens of genes with strong signatures of balancing selection. These genes contribute to multiple processes including immunity, neural development, and circulation. Our findings also reveal examples of caste intragenomic conflict can be resolved. Overall, our results contribute to understanding how different castes, social forms, and immune pressures are encoded in the genome, and the diversity of evolutionary pressures superorganismal evolution must respond to.

3. **Yonghe Zhou***, Queen Mary University of London
   Title TBC

Animals are assumed to follow a strategy of energy maximisation, and therefore should choose feeding options based on the offered energy intake rates. However, both humans and monkeys' choices are better explained by their subjective evaluations of sensory food qualities rather than by energy content. Bumblebees (*Bombus terrestris*) are classical models for economic decision making and are faced with floral choices with various sensory properties. However, it remains unclear whether their choices are driven by energy maximisation irrespective of the perceived values of options. Here we show that when trained with flowers offering similar energy intake rates, bumblebees developed preferences for the sweeter option. In contrast, with two options that differed in energy intake rates, bees had no preference between options because sweetness and resistance had been balanced. Further, decision dynamics during training indicate that bumblebees simultaneously evaluate sweetness and resistance but not energy gain. These results indicate that instead of energy maximisation, bumblebees' food preferences are jointly affected by their subjective evaluation of nectar sweetness and resistance. Our findings suggest that perceived value of sensory food qualities as a driving force for food preferences is shared by evolutionarily distant species.

4. **Aoife Cantwell-Jones***, Imperial College London
   Mapping individuals in multi-dimensional trait space predicts shared roles in plant-bumblebee networks

Bumblebees play a central role in many plant-pollinator systems, helping underpin ecosystem stability. With these systems being impacted by high rates of environmental change, it is vital we understand the mechanisms that structure bee interactions with plants and subsequent competition. To date, using traits to predict interactions has received mixed results. A possible explanation is that species-level interactions are typically modelled using mean trait values, ignoring variation in those traits. Indeed, central to bumblebee biology is large intraspecific (functional) trait variation, meaning that, to truly understand the foraging role of any given species, we must determine where all individuals from a species fit in functional-trait space. Using individual-level interaction and trait data from an Arctic montane ecosystem, we investigate how bumblebee intraspecific variation and degree of spread in functional-trait space affect dietary breadth and floral-resource sharing with other species. Using hypervolumes, we ask whether 1) intraspecific variation mediates dietary breadth and foraging specialisation, and 2) the degree to which bee species (and castes) overlap in trait space shapes their similarity in foraging role (using motif analysis). We find bee species (and castes) with larger trait variation interact with more plant species, and that bees overlapping more in trait space have more similar foraging roles. Overall, our results show high-resolution trait data and intraspecific variation are integral to developing predictive frameworks of plant-pollinator interactions under environmental change.

5. **Thomas Roberts-McEwen***, University of Portsmouth
   Superhero spiders: How group-living arachnids could be uniquely powerful biological control agents

Group-living spiders may be uniquely suited for controlling flying insect pests, as their high tolerance for conspecifics and low levels of cannibalism result in large, predator dense capture webs. We tested the ability of the facultatively communal spider, *Cyrtophora citricola*, to control the tomato leaf miner, *Tuta absoluta* – a major pest of tomato crops worldwide – in lab settings. Using small experimental colonies with spiders of a range of body sizes, we tested whether prey capture success was affected by spider body size, and whether prey capture differed between *T. absoluta*, easily-caught flightless fruit flies, *Drosophila hydei,* and larger black soldier flies, *Hermetia illucens*. We found that larger spiders generally caught more prey, that prey capture success was similar for *T. absoluta* and easy-caught fruit flies, while black soldier flies were rarely caught, suggesting that spiders are good candidates for biological pest control of *T. absoluta*. We further investigated the seasonal variations in web sizes in southern Spain and found that the spiders would likely be most effective biological control agents in the planting and growing season in Spring. Finally, we show that *C. citricola* in Spain have >50% infection rates of an egg predatory wasp, *Philolema palanichamyi*, which may need to be controlled to maximise the effectiveness of the spiders. Together, these results suggest that group-living spiders could successfully mitigate damage caused by aerial pest arthropods. However, community ecology must be considered when implementing any biological control strategies involving *C. citricola*, to avoid population damage and reduced pest control efficacy.

6. **Thomas Oliver***, Bangor University & Rothamsted Research
   How do you solve a problem like insect telemetry?

Insect telemetry involves the use of tracking technology to understand invertebrate movement and behaviour. The use of such technologies have been successful in visualising the numerous natural behaviours and real time impacts environmental stressors have on wild and managed invertebrate species. The technique contains a number of weaknesses, however. Current techniques either rely on heavy tags, which may impact the flight behaviour of the insect, and therefore produce inaccurate data. Other techniques utilise a lighter, passive tag used in tandem with a secondary emitter and receiver, often requiring a significant investment of money and specialist knowledge to operate and maintain. So, how do you solve a problem like insect telemetry?

7. **Ana Cuesta-Maté***, University of Copenhagen
   Social bee microbiomes are the missing link in the origin of human fermented foods

Lactic acid bacteria and acetic acid bacteria are generally considered beneficial microbes for humans and used to produce several fermented foods and beverages. Humans have historically domesticated these bacteria to obtain the ferments that we consume, but the question remains on how these microbes got there in the first place. Fermented food researchers find it difficult to sample these bacteria in natural habitats, but our work shows that some of these bacteria are found in the bee gut and environment, the beehive, royal jelly, bee bread, and nectar of flowers and fruits social bees visit. Lactobacillus, Bacillus and Fructobacillus associated with social bees are closely related to the fermenting food microbes of wine, cheese or buttermilk. Similarly, Acetobacteraceae typically found in the bee environment are closely related to microbes of kefir, kombucha or vinegar. Could social bees be responsible for the original inoculation of these microbes into the food substrates that then will transform into the ferments that we consume? Furthermore, for the establishment of spontaneous fermentation of food, the reinoculation of the same microbes is necessary, as the microbiome of social bees is highly conserved. There could be, however, anthropological intervention in the transmission of these microbes into the fermented food, one new perspective to look into the domestication of bees. In this project, we will compare the genomes from bee-associated and food-associated microbes and explore the possible human and bee routes of transmission.

8. **Hannah Wolmuth-Gordon***, Royal Holloway University of London
   High temperature reduces bumblebee gut parasite infectivity

To survive, all organisms must withstand changing environmental conditions. Temperature varies throughout the day, with the seasons and between climates. How individuals respond to temperature affects their interactions with one another. Changes to the interaction between parasites and their hosts can have a large effect on disease dynamics. The gut parasite, *Crithidia bombi*, is transmitted between bumblebees via flowers and therefore, is exposed to different environmental temperatures prior to infection. We investigated whether temperature exposure affects the parasite's infectivity. Prior to inoculation, *C. bombi* was incubated at 10, 20, 30, 40 or 50°C for either 10 or 60 minutes. Prevalence and infection intensity were measured in bees one week later. Prevalence but not infection intensity was significantly affected by incubation length and the interaction between incubation temperature and length. The parasite was unable to infect hosts after being exposed to 50°C for 60 minutes. Furthermore, *C. bombi* cells excreted from infected individuals were significantly more likely to look burst and unhealthy after being exposed to extreme temperatures, such as 10 and 50°C. These results highlight that temperature can have a substantial effect on disease transmission. Parasite infectivity may vary between climates, the seasons and with climate change. Here, high temperatures curtail infectivity and therefore, in the future more frequent heatwaves are likely to impact *C. bombi* transmission. The results emphasise the importance of investigating the effect of environmental conditions from the perspective of both the host and the parasite to accurately determine the effects of environmental changes on infection dynamics.

9. **Adriano Wanderlingh***, University of Bristol
Studying collective disease defence via automatic recognition of sanitary behaviours in Lasius niger

The gregarious lifestyle of the eusocial insects exposes them to greater risk of pathogen transmission. Consequently, they have evolved a variety of cooperative behavioural defences against disease. Division of labour is crucial in reducing pathogen spread because contacts between groups of workers specialised upon different tasks are reduced, thus conferring 'organisational immunity'. The effectiveness of organisational immunity has been predicted to increase with colony size because task specialisation is thought to be more pronounced in large social groups. If true, this would reduce the need for additional sanitary measures in large colonies. However, these predictions have not been tested experimentally due to the difficulty of obtaining exhaustive records of the interactions and behaviours of all individuals within large colonies. Here, we use automated individual tracking and supervised machine learning to study division of labour and sanitary behaviours in colonies of the ant *Lasius niger*. We investigate how colony size influences social organisation, and how this, in turn, affects disease transmission dynamics and the incidence of sanitary behaviours in pathogen-exposed colonies. Our study uncovers links between group size, social organisation and sanitary behaviours providing further insight on how social insects modulate their disease defence strategies according to colony size.

10. **Luke Leckie***, University of Bristol
Architectural Immunity?

Dense living, frequent social contacts and high relatedness of individuals are predicted to make eusocial insects particularly susceptible to infectious diseases. However, natural ant colonies rarely experience epidemics owing to a suite of collective disease defence mechanisms conferring social immunity. These include behaviours which limit the occurrence and spread of disease inside ant nests, such as maintenance of waste chambers and modulation of social networks. Nests architecture is a prominent feature of social insect societies that shape, and are shaped by, colony collective behaviours. Specific nest architectures may influence colony interaction networks and serve to enhance or limit the spread of disease. However, it is yet unknown whether ant colonies might actively alter their nest architecture to decrease epidemic risk when faced with an infectious disease. In this study, we tested this hypothesis by introducing pathogen-exposed workers into nest-building *Lasius niger* colonies. Using X-ray micro-computed tomography, three-dimensional nest architecture was imaged and nest spatial networks were created. Individual digging behaviour, nest architecture and networks were then compared to those of sham-exposed colonies. We predict nest networks in exposed colonies to have lower connectedness, higher compartmentation and more entrances than in sham-exposed colonies. Furthermore, we expect a trade-off between digging and social care may lead to individuals investing less in digging in exposed colonies. Modulation of digging behaviour and nest architecture may be a component of social immunity and benefit colony epidemic outcomes in a pathogen-rich environment.

11. **Daniel Schläppi**, University of Bristol

Do black garden ants, Lasius niger *(Hymenoptera: Formicidae), avoid virus infected food?*

Social insects have evolved a diverse array of sanitary strategies to counteract the heightened risk of disease transmission associated with living in dense groups. Avoidance of prey items contaminated with pathogenic fungi is an early line of disease defence displayed by some social insects. However, discrimination against food sources contaminated with more cryptic infectious agents has not been reported so far. In this study, we investigated whether black garden ants Lasius niger avoid food infected with the Acute bee paralysis virus (ABPV). The ABPV is commonly detected in European honey bees (*Apis mellifera*) and has confirmed pathogenicity in *L. niger* after foodborne cross-species transmission. In a laboratory experiment *L. niger* foragers were presented with a binary choice between artificially ABPV-infected honeybee pupae and control pupae. In contrast to our expectations, we found no apparent avoidance of contaminated food. Instead, the initial recruitment of foragers was significantly greater for ABPV-infected food sources, suggesting that infected food sources are exploited more strongly than healthy sources. These results suggest that ABPV might evade the first line of disease defence in *L. niger* and could potentially initiate epidemics in this alternative host species.

*Student presentation

Posters:

Ayman Asiri, Cardiff University
Title TBC*

Honeybees are one of the world's most important pollinators, but they are under threat worldwide by the emergence and persistence of infectious diseases. European foulbrood (EFB), caused by *Melisococcus plutonius*, is a highly virulent honeybee brood disease. Early EFB detection would allow beekeepers to treat infected hives earlier, thereby improving prognosis. Infection has been shown to change the volatile organic compound (VOC) profiles in several human and livestock systems. We investigated changes in the VOC profiles of honeybee brood inoculated with the causative agent of EFB. Over a six day period, headspace VOCs from 48 EFB infected larvae and 48 uninfected larvae were collected daily and analysed using TD-GC-MS-TOF. Preliminary results suggest that the VOC profiles shift over time, as well as in response to infection. We identify a potential non-invasive method of detecting EFB in hives, which may be used by beekeepers to detect and respond to disease faster. Olfactory cues, such as VOCs, may also play an important role in triggering hygienic behaviour by signalling infected brood for removal by hygienic workers. Our future studies will use behavioural assays to assess whether the identified VOCs are a mechanism underlying hygienic behaviour.

Maximilian Bolder, Johannes Gutenberg University of Mainz
Title TBC*

The ontogeny of a biological system is the developmental process that produces the self-assembly and specialization of its components from a single unit. While extensive research efforts have focused on the ontogeny of multicellular organisms, building up the entire field of developmental biology, there are few experimental investigations of the ontogeny of insect societies. In the majority of social insect species, queens found their colonies independently, and during this stage express a variety of behaviors, including brood care. Only when the first workers have been produced do the queens become specialized in egg production. Recent research revealed that established queens of two ant species revert to expressing brood care upon experimental removal of their workers. This reversibility of the queen's behavioral specialization revealed the importance of worker presence in initiating and maintaining the queen's specialization in egg production. Preliminary experiments suggest that this reversibility may be found in multiple species of ants, but queens of some species appear to perform very little brood care irrespective of the social environment. We plan to investigate the reasons for this variability in queen behavioral specialization inside the ant phylogeny. So far, we have analyzed the behavior of some species in the subfamilies Formicinae, Myrmicinae and Dolichoderinae, and are actively seeking collaborators to broaden the spectrum of ant species to be investigated. Collecting data across species with distinct specificities would allow us to trace the evolutionary history of queen behavioral specialization and would help us better understand the division of labor in insect societies.

Liz Bates, Newquay University Centre

How the differences between individuals contribute to bumblebee colony flexibility

Groups of cooperative organisms, including humans, benefit from the contributions of both specialist and generalist individuals. Group responses to resource or task demand emerge from the specific actions of individuals within the group. Bumble bee colonies need to respond to resource demands for both pollen and nectar for colony growth and reproduction. Bumble bee workers have been shown to display differences in foraging preferences, for these essential resources and in activity level. This study investigated the responses of *Bombus terrestris* individuals to resource demand by subjecting colonies to different pollen and sucrose storage scenarios, considering their individual forage preferences and activity levels. The sucrose sensitivity and olfactory learning ability of individuals was then compared along a spectrum of foraging preference (between pollen and sucrose foraging) using the proboscis extension reflex paradigm. This study highlights the potential value of foraging preferences in bumble bee colonies but also the flexibility these colonies have with a small foraging workforce.

Owen Corbett, University College London

Aggression-mediated queen succession in tropical Polistes *wasps**

In tropical *Polistes* wasp species, a period of intense aggressive conflict occurs following queen loss until a new queen is established. During this period, an individual's investment in aggressive competition versus nest maintenance and brood provisioning represents a tradeoff between continued indirect fitness and potential direct fitness. Using data from manipulation experiments in two species, I have characterise the individual- and nest-level changes in behaviour and social network structure associated with queen loss and succession, and identify the behavioural and physiological mechanisms which determine new queen identity. I investigate the impact of the age and social rank of an individual on their investment in co-operative provisioning versus aggression during the process of succession, and in their capacity to develop reproductively in response to queen loss. By characterising competitors, caretakers, and future queens, and the processes by which colony function is maintained through periods of massive social upheaval, we can gain a better understanding of how reproductive conflict is resolved in societies where workers retain their potency and a queen's reign is not limited by seasonal colony decline.

Tesni Houlston, University of Aberdeen

*Does disturbance of the magnetic field affect locomotor activity in the buff-tailed bumble bee (*Bombus terrestris*)?**

Magnetosense, the ability to navigate using the Earth's magnetic field as a compass, is possessed by many organisms. However, the magnetic field can be disturbed by geomagnetic storms or anthropogenic factors such as electrical lines, train networks and radio transmitters. These disturbances have been shown to disorientate animals and have been linked to a decline in forager return rates in honeybees. Due to the potential effect of magnetic field disturbances on pollinator populations, this study aimed to investigate whether the buff-tailed bumblebee, *Bombus terrestris*, is affected by magnetic field disturbances. A behavioural assay assessing the activity of bees in a novel environment in search of a food reward was used to address this interdisciplinary question. We show that bumblebees can detect the magnetic field, and our findings suggest that exposure to disrupted magnetic fields could negatively affect foraging ability. However, further research is needed to confirm this and understand the extent of this effect in a natural setting. To assess how the bumblebees might be detecting the magnetic fields, the expression of CRY-1, a gene linked to magnetosense in other animals, was assessed by RT-qPCR. There was no association between CRY-1 gene expression and magnetosense, providing indirect support for a magnetite-based mechanism in bumblebees. This study found that magnetic field disruptions may need to be considered in future pollinator conservation plans, identified key areas for future research and highlighted the value of an interdisciplinary approach to the study of magnetosense.

Natalie Imirzian, Imperial College London
Title TBC

Leaf-cutter ant colonies have workers which vary substantially in size, with body mass of sister ants ranging from less than 1 mg to over 80 mg. The adaptive value of a large size range remains unclear, but size and task preference correlate, suggesting certain worker sizes are specialised for specific roles in the colony. We investigated whether body size changes are accompanied by size-independent shape changes using morphometric analysis of high-resolution µCT-scans of *Atta vollenweideri* workers. We focused on mandible and head shape, due to their relevance across the size range: larger workers need a strong bite force for leaf cutting and defence behaviours, while smaller workers must delicately handle brood and fungal material. Morphometric analysis typically involves linear dimensions, but such a dimensional reduction risks missing important information on morphological specialization. Alternatively, 3D changes can be computed from landmark points, which introduces observer bias and reduces the resolution of observed changes. We instead compute the deformation of the complete 3D ambient space to create a shape atlas, which maps each surface mesh to a mean shape. Our analysis identifies small-scale shape variation thus emphasizing the differences between morphs. We find that worker mandibles and head capsules vary not only in size, but also in shape. Both body parts take two distinct morphologies, clustered with size: one for the smallest workers (<1mg) and one for the rest. The clustering of shapes coincides with task preferences, suggesting adaptation to different tasks within the colony workforce.

Rajbir Kaur, University of Bristol
How honeybees fare during a historical heat wave in the UK

In 2022, the UK faced its most intense and frequent heat waves, breaking records of hundreds of years, with temperatures reaching 40 degrees Celsius (104 F) in some regions. The increased frequency of heat waves is now the recent addition to the long list of negative impacts we pose to the environment as a species. While the heat wave severely impacted humans in terms of health and economy, its impact on non-human species can only be studied using opportunistic data from that period. As part of an ongoing project, we had 27 beehives kept in 9 locations in the southwest of England during the heat wave period. Our records include colonies' weight data taken every hour using an automatic weighing balance. We estimated two important colony-associated parameters: 1. colony's foraging activity and 2. net weight gain/loss for each day. An analysis comparing colony's foraging activity and net weight during the heat wave vs. non-heat wave periods (before and after) will help us understand the effect of the heatwave on bee foraging activity. In addition, waggle dance frequency and nectar concentrations measured from the incoming foragers provide us with more insight into the impact on foraging resources. Currently, the above-mentioned datasets are under analysis with aim to provide valuable information about the impact of future heatwaves on honeybee foraging activity.

Ruyan Li, University of Copenhagen

Functional study on an uncharacterized gene involved in caste differentiation in the pharaoh ant, Monomorium pharaonis *

The transition from solitary organisms to social life is one of the major transitions in evolution. Eusocial hymenopterans (ants, some bees, and some wasps) best exemplify the transition for their reproductive division of labor between queen caste and worker caste. However, the molecular mechanisms mediating caste differentiation during development is largely unknown. After analyzing >1400 whole-genome transcriptomes of two ant species, *M. pharaonis* and *A. echinatior*, we identify an uncharacterized gene (ID: 39887) with caste-differentiated expression in all post-embryonic developmental stages. Using *M. pharaonis* as a model organism, we find that 39887 gene is transcribed in larval fat body through HCR RNA-FISH. Immunostaining results suggest that 39887 protein is secreted to many organs including brain. Vivo-Morpholino-based knockdown of 39887 gene in the last stage of queen larvae results in several worker-biased traits when they developed into pupae, including smaller thorax, wings, compound eyes and ocelli. Treat worker larvae with juvenile hormone analog significantly increases the transcription level of 39887 gene. These results show that 39887 gene plays an important role in regulating development of queens and workers, in a way mediated by juvenile hormone.

Florent Masson, University of Bristol

Deciphering the interplay between individual and collective immunity

Contagious pathogens are a threat to animal societies where individuals are in close contact with one another. In response to the heightened risk of disease transmission, eusocial insects have evolved collective defence mechanisms conferring 'social immunity' to the colony. The efficiency of social defences raises a major evolutionary question: Does social immunity relax the need to invest in individual immunity? The hypothesis of an immune erosion in social insects has been backed-up by comparative genomics studies, but it has received little experimental support due to the lack of molecular tools in these models. In this project, we propose to develop appropriate tools to test this hypothesis experimentally in the black garden ant Lasius niger. Preliminary results show that all key genes of the insect canonical immune pathways are present in L. niger genome, which suggests immune competence. By developing an RNA interference approach to generate immunocompromised ants, we aim to first establish whether these canonical pathways have retained their full functionality in ants. Second, by combining automated individual tracking and targeted RNAi-mediated immunosuppression, we aim to investigate the interaction between individual and social immunity at the colony level. We predict that social immunity should relax the need for individuals benefitting from high social immune protection (e.g. the queen and nurses) to invest in individual immunity.

Jaya Robinson, University of Oxford
*Superorganism immune memory in ant colonies**

The effect of sociality on disease transmission is a key evolutionary debate. For animals, a more social lifestyle results in frequent, close contact with conspecifics - increasing the likelihood of pathogenic infection; however sociality also enables stronger cooperation in anti-pathogen behaviours. Social insects provide a particularly pertinent case study in this debate, as their colonies of highly related, frequently interacting individuals, results in conditions ideal for pathogen invasion. However, social insects also demonstrate a suite of behaviours that reduce the detrimental effects of pathogens, including social network plasticity, elimination of contaminated waste, and grooming of infected nestmates (allogrooming). The effects of prior experience on these collective defences have not yet been explored in depth, despite prior studies which hint at their presence. This project aims to address this gap, investigating the effects of experience in *Temnothorax* ant colonies. We will do this by repeatedly presenting ants with infected larvae over 12 days, measuring the duration, frequency, and recruitment speed of allogrooming at the start and end of the experimental period, and comparing changes in performance to ants exposed to uninfected larvae. We will also investigate the longevity and pathogen-specificity of these effects, and determine whether grooming by experienced ants reduces fungal spore numbers and increases larvae survival time. Overall, this project aims to demonstrate a novel behavioural mechanism in social insects - drawing parallels with mechanisms at other biological scales - and use this to further elucidate the role of pathogens and immune systems in shaping major evolutionary transitions.

Jacques Liam Salin, University of Copenhagen
The soldier's role in social immunity defense for Macrotermes bellicosus

Social insect colonies, including termites, have been predicted to be prone to infectious diseases; yet they have very robust social immunity defences that help prevent infections from establishing. Previous studies have found that the soldier caste in wood-dwelling termite species has antimicrobial compounds in their saliva that are potentially involved in social immunity. Mound-building termites have yet to be studied in this regard, and the objectives of this MSc project is, therefore, to investigate if termite soldiers of *Macrotermes bellicosus* play such a role . Our goal is to answer two main questions, 1. Do soldiers participate in social immunity? and 2. Does exposure to infected nestmates alter soldier antifungal compounds? We intend to test our first question by using LT-50 experiments where *Metarhizium robertsii*-infected worker termites are grouped to allow for direct, indirect, or no interactions with soldiers. Over the course of several days, termite mortality will be recorded and compared across the three groups. Soldiers will after the LT-50 experiment be dissected into head, abdomen, and gut to subsequently test for antifungal activity against *M. robertsii* in zone of inhibition assays, comparing exposed and non-exposed soldiers. The significance of these results may give a better understanding of the role of the soldier caste in social immunity in mound-building termites.

Filip Turza, Jagiellonian University
*The impact of body size on rescue actions in monomorphic antRs**

The relationship between body size and behavior of ants is explored more in polymorphic species than in those with narrow size variation, the so-called monomorphic ants. Behavioral variation connected to body size remains understudied in these latter ants. Here, we studied a form of altruism called rescue, which is a risky activity in which the altruist attempts to help another individual facing danger. In a behavioral experiment in the field, we tested the behavior of differently-sized workers in the context of rescue occurring between nestmates when one of them is ensnared. As our study model, we chose the monomorphic ant Formica cinerea, characterized by large and unexplained behavioral variation in rescue activity. Our research aimed to find out whether rescuers characterized by smaller or larger body sizes differ in providing help and whether the body size of the victims affects their behavior during ensnarement. Our data showed that smaller workers were somewhat more specialized in risky rescue actions than larger individuals. Additionally, smaller workers were more active in distress. We discussed the results in terms of the value of differently-sized workers for the colony. These findings broaden our understanding of the importance of body size in all ants and highlight the need to study individual differences in behavior among social insects, even those without broad size variation between individuals.

Yongqiang Wu, University of Bristol
*Do food stores affect immune gene expression and abdominal fat reserves in honeybees?**

Honeybees (*Apis mellifera*) are important pollinators and they face many anthropogenic challenges that affect the external environment of colonies, such as the quality and distribution of food sources. Food source availability is likely to affect hive food stores and individual nutritional stress. We tested if the amount of food stored in the hive would affect the expression in immune genes and the amount of abdominal fat acids. We manipulated the quantity of honey stored in hives and quantified the expression of seven immune genes (using qPCR) which have been linked to immune responses and colony overwintering survival. We also predicted that hive honey reserves affect the fat storage of individual bees. We quantified fatty acids from bee abdomen using GC-MS. We found that bees in starved colonies showed a higher expression of the immune gene defensin1, which has been linked to overwinter survival. We found no changes in fatty acid 5 days after manipulating food stores. Our results show that the amount of honey stored in hives affects the expression of one of our tested immune genes, but did not affect abdominal fat content. Our results suggest that food source availability might impact bee immunity and, thereby, the health of colonies.

Olivia Walthaus, Imperial College London
Title TBC*

Leaf-cutter ants cut fragments from fruits and leaves to feed a fungus used as a crop. Their colonies consist of workers which vary vastly in size, potentially increasing foraging efficiency. A crucial part of a leaf-cutter foraging trip is the mechanical interaction between ant mandible and plant, defined by two key forces: the maximum force the ant can apply (bite force), and the force required to fracture the plant fragment (cutting force). The bite force is determined by the physiology and anatomy of the bite apparatus; the cutting force, in turn, depends on the tissue's structural and mechanical properties, and the mandible geometry. How do these two forces compare?

To address this question, we used a custom-built fibre optic force setup to measure the force required to cut a range of natural and synthetic substrates with both worker mandibles and razorblades. Mandible cutting forces were typically lower than those of razorblades, demonstrating extreme mandible sharpness. However, peak mandible cutting forces can be more than 1000x the body weight of a typical worker. To assess if ants can generate forces of this magnitude, we measured bite forces using a custom-built rig. Leaf-cutter ants generate exceptionally large bite forces, corresponding to almost 3000x their body weight. We argue that the evolution of this remarkable bite performance was driven by the need to cut plant fragments during foraging.

Peter Xu, University of York

*Unstable Environm-ants: Does environmental predictability affect food risk-mitigation in ants?**

Social insect societies develop in environmental conditions which are always in flux. These changes can be either predictable or unpredictable, and social insects must be prepared to adjust to both. Resource distribution, and in particular, food availability is important in determining many aspects of a social insect's life history and behaviour. Multiple strategies have evolved within humans and/or the wider animal kingdom to minimise the risks of acquiring food in an unpredictable environment. These include: diversification/generalism, storage, mobility/nomadism/migration and resource exchange/polydomy. We test the hypothesis that environmental factors affecting food resource predictability influences which of these strategies evolves, using a meta-analysis of the model clade Formicidae, which displays all five of these behaviours. We also aim to understand the evolutionary history of these strategies using ancestral state reconstruction methods. We predict that within ants the distribution of behaviours between predictable and unpredictable environments will follow trends seen in other non-ant species. In environments showing long-term predictability, food storage behaviours will be more prevalent, while nomadism and generalism will be favoured under long-term unpredictability. Food exchange behaviour is less often observed in non-human animals, with less consensus on the circumstances it occurs, and thus it is unclear whether we would expect the behaviour to be more common under predictable or unpredictable environments.

***Student Poster**